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Stem Cell

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Stem Cell Technology Research Literatures

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Abstract: Stem cells are derived from embryonic and non-embryonic tissues. Most stem cell studies are for animal stem cells and plants have also stem cell. Stem cells were discovered in 1981 from early mouse embryos. Stem cells have the potential to develop into all different cell types in the living body. Stem cell is a body repair system. When a stem cell divides it can be still a stem cell or become adult cell, such as a brain cell. Stem cells are unspecialized cells and can renew themselves by cell division, and stem cells can also differentiate to adult cells with special functions. Stem cells replace the old cells and repair the damaged tissues. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. This article introduces recent research reports as references in the related studies.
[Herbert M. **Stem Cell Technology Research Literatures.** Stem Cell. 2023;14(1):1-128] ISSN:1945-4570 (print); ISSN: 1945-4732 (online).http://www.sciencepub.net/stem. marsscj140123.01. 01.doi:10.7537/marsscj140123.01.

Key words: stem cell; technology; life; research; literature

Introduction

The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. This article introduces recent research reports as references in the related studies.

The following introduces recent reports as references in the related studies.

Aarseth, B. L., et al. (2021). "Formation and functionalization of Ge-nanoparticles in ZnO." Nanotechnology **32**(50).

Semiconductor nanocrystals are often proposed as a viable route to improve solar energy conversion in photovoltaics and photoelectrochemical systems. Embedding the nanocrystals in, e.g. a transparent and conducting electrode of a solar cell will promote the photon absorption and subsequent transfer of the generated charge carriers from the nanocrystal, and thereby enhance the function of the electrode. This can be accomplished by embedding a semiconducting nanocrystal with a small bandgap in a transparent conducting oxide (TCO), which is commonly utilized as electrode in new generation solar cells. Here, we demonstrate the incorporation, formation, and functionalization of germanium (Ge) nanocrystals in zinc oxide utilizing ion implantation, where post implantation annealing at 800 degrees C results in diamond cubic Ge nanocrystals with sizes between 2 and 20 nm. Photoluminecence spectra show a distinct emission around 0.7 eV arising from the Ge nanocrystals, and with additional emission features up to 1.15 eV due to quantum confinement, demonstrating a novel functionalization and tunability of the TCO electrode.

Agarwal, R., et al. (2015). "Delivery and tracking of quantum dot peptide bioconjugates in an intact developing avian brain." ACS Chem Neurosci **6**(3): 494-504.

Luminescent semiconductor approximately 9.5 nm nanoparticles (quantum dots: QDs) have intrinsic physiochemical and optical properties which enable us to begin to understand the mechanisms of nanoparticle mediated chemical/drug delivery. Here, we demonstrate the ability of CdSe/ZnS core/shell QDs surface functionalized with a zwitterionic compact ligand to deliver a cell-penetrating lipopeptide to the developing chick embryo brain without any apparent toxicity. Functionalized QDs were conjugated to the palmitoylated peptide WGDap(Palmitoyl)VKIKKP9GGH6, previously shown to uniquely facilitate endosomal escape, and microinjected into the embryonic chick spinal cord canal at embryo day 4 (E4). We were subsequently able to follow the labeling of spinal cord extension into the ventricles, migratory neuroblasts, maturing brain cells, and complex structures such as the choroid plexus. QD intensity extended throughout the brain, and peaked between E8 and E11 when fluorescence was concentrated in the choroid plexus before declining to

hatching (E21/P0). We observed no abnormalities in embryonic patterning or embryo survival, and mRNA in situ hybridization confirmed that, at key developmental stages, the expression pattern of genes associated with different brain cell types (brain lipid binding protein, Sox-2, proteolipid protein and Class III-beta-Tubulin) all showed a normal labeling pattern and intensity. Our findings suggest that we can use chemically modified QDs to identify and track neural stem cells as they migrate, that the choroid plexus clears these injected QDs/nanoparticles from the brain after E15, and that they can deliver drugs and peptides to the developing brain.

Akhan, E., et al. (2015). "Nanoparticle labeling of bone marrow-derived rat mesenchymal stem cells: their use in differentiation and tracking." Biomed Res Int **2015**: 298430.

Mesenchymal stem cells (MSCs) are promising candidates for cellular therapies due to their ability to migrate to damaged tissue without inducing immune reaction. Many techniques have been developed to trace MSCs and their differentiation efficacy; however, all of these methods have limitations. Conjugated polymer based water-dispersible nanoparticles (CPN) represent a new class of probes because they offer high brightness, improved photostability, high fluorescent quantum yield, and noncytotoxicity comparing to conventional dyes and quantum dots. We aimed to use this tool for tracing MSCs' fate in vitro and in vivo. MSC marker expression, survival, and differentiation capacity were assessed upon CPN treatment. Our results showed that after CPN labeling, MSC markers did not change and significant number of cells were found to be viable as revealed by MTT. Fluorescent signals were retained for 3 weeks after they were differentiated into osteocytes, adipocytes, and chondrocytes in vitro. We also showed that the labeled MSCs migrated to the site of injury and retained their labels in an in vivo liver regeneration model. The utilization of nanoparticle could be a promising tool for the tracking of MSCs in vivo and in vitro and therefore can be a useful tool to understand differentiation and homing mechanisms of MSCs.

Alansary, D., et al. (2020). "Detecting single ORAI1 proteins within the plasma membrane reveals higher-order channel complexes." J Cell Sci **133**(1).

ORAI1 proteins form highly selective Ca(2+) channels in the plasma membrane. Crystallographic data point towards a hexameric stoichiometry of ORAI1 channels, whereas optical methods postulated ORAI1 channels to reside as dimers at rest, and other data suggests that they have a tetrameric configuration. Here, liquid-phase scanning transmission electron microscopy (STEM) and quantum dot (QD) labeling was utilized to study the conformation of ORAI1 proteins at rest. To address the question of whether ORAI1 was present as a dimer, experiments were designed using single ORAI1 monomers and covalently linked ORAI1 dimers with either one or two label-binding positions. The microscopic data was statistically analyzed via the pair correlation function. Label pairs were found in all cases, even for concatenated dimers with one label-binding position, which is only possible if a significant fraction of ORAI1 was assembled in larger order oligomers than dimers, binding at least two QDs. This interpretation of the data was consistent with Blue Native PAGE analysis showing that ORAI1 is mainly present as a complex of an apparent molecular mass larger than that calculated for a dimer.

Alexander, A., et al. (2019). "Amalgamation of Stem Cells with Nanotechnology: A Unique Therapeutic Approach." Curr Stem Cell Res Ther **14**(2): 83-92.

In the last few years, the stem cell therapy has gained much popularity among researchers and scientists of biomedical field. It became an effective and alternative approach for the treatment of various physiological conditions (like accidental injuries, burn damage, organ failure, bone marrow transfusion, etc.) and chronic disorders (diabetes, cancer, neurodegenerative disorders, periodontal diseases, etc.). Due to the unique ability of cellular differentiation and regeneration, stem cell therapy serves as the last hope for various incurable conditions and severe damages. The amalgamation of stem cell therapy with nanotechnology brings new prospects to the stem cell research, as it improves the specificity of the treatment and controls the stem cell proliferation and differentiation. In this review article, we have discussed various nanocarrier systems such as carbon nanotubes, quantum dots, nanofibers, nanoparticles, nanodiamonds, nanoparticle scaffold, etc. utilized for the delivery of stem cell inside the body.

Apostolos, A. J., et al. (2020). "Facile Synthesis and Metabolic Incorporation of m-DAP Bioisosteres Into Cell Walls of Live Bacteria." ACS Chem Biol **15**(11): 2966-2975.

Bacterial cell walls contain peptidoglycan (PG), a scaffold that provides proper rigidity to resist lysis from internal osmotic pressure and a barrier to protect cells against external stressors. It consists of repeating sugar units with a linkage to a stem peptide that becomes cross-linked by cell wall transpeptidases (TP). While synthetic PG fragments containing l-lysine in the third position on the stem peptide are easier to access, those with meso-diaminopimelic acid (m-DAP) pose a severe synthetic challenge. Herein, we describe a solid phase synthetic scheme based on widely available building blocks to assemble meso-cystine (m-CYT), which mimics key structural features of m-DAP. To

demonstrate proper mimicry of m-DAP, cell wall probes were synthesized with m-CYT in place of m-DAP and evaluated for their metabolic processing in live bacterial cells. We found that m-CYT-based cell wall probes were properly processed by TPs in various bacterial species that endogenously contain m-DAP in their PG. Additionally, we have used hybrid quantum mechanical/molecular mechanical (QM/MM) and molecular dynamics (MD) simulations to explore the influence of m-DAP analogs on the PG cross-linking. The results showed that the cross-linking mechanism of transpeptidases occurred through a concerted process. We anticipate that this strategy, which is based on the use of inexpensive and commercially available building blocks, can be widely adopted to provide greater accessibility of PG mimics for m-DAP containing organisms.

Arumugam, P. and J. M. Song (2016). "Quantitative evaluation of ABC transporter-mediated drug resistance based on the determination of the anticancer activity of camptothecin against breast cancer stem cells using TIRF." Integr Biol (Camb) **8**(6): 704-711.

Elevated expression of drug efflux pumps such as multidrug resistant protein-1 (MDR1/ABCB1) and multidrug resistance associated protein-1 (MRP1/ABCC1) in cancer stem cells (CSCs) among a bulky tumor cell population was attributed to drug resistance. For the first time, we have quantitatively evaluated the cytotoxic profile of camptothecin (CPT) against the CSC. In the present study, a Qdot based total internal reflection fluorescence (TIRF) detection system effectively interpreted that drug resistance to CPT was reduced in the CSC under ABCB1 inhibited conditions. This study revealed that quantitative finding of the EC50 value for apoptosis and necrosis in correlation with the ABC inhibitor and CSC population using TIRF could provide more details of the anti-cancer efficacy of chemotherapeutic agents.

Asgari, V., et al. (2019). "The Story of Nanoparticles in Differentiation of Stem Cells into Neural Cells." Neurochem Res **44**(12): 2695-2707.

Stem cells have been long looked at as possible therapeutic vehicles in regenerative medicine largely due to their multi-lineage differentiation potential and paracrine actions. Therefore, development of new procedures for the differentiation of stem cells into different cell types holds great potential for opening new opportunities in regenerative medicine. In addition to various methods for inducing stem cell differentiation, the utilization of nanomaterials for differentiation of stem cells has recently received considerable attention and has become a potential tool for such purpose. Multiple lines of evidence revealed that nanomaterial-based scaffolds, inorganic nanoparticles (NPs), and biodegradable polymers have led to significant progress in regulation of stem cell differentiation. Several studies indicated that different NPs including selenium, gold, graphene quantum dots (QDs) and silica could be employed for the regulation of differentiation of stem cells such as human mesenchymal stem cells (hMSCs). In addition, magnetic core-shell NPs could be applied for the regulation of neural stem cell (NSC) differentiation. Taken together, these findings suggested that NPs are potential candidates which could be utilized for the differentiation of stem cells into various cell types such as neural cells. Herein, we summarized the application of NPs for differentiation of stem cells into various cells in particular neural cells.

Askenasy, N. (2006). "From the atom to the cell: Is the cat alive? Quantum mechanics and stem cell plasticity as deja vu." Stem Cells Dev **15**(4): 488-491.

The concepts submitted by quantum mechanics fascinated the scientific community during the first half of the 20(th) century. The second half was dominated by biology, culminating in the sequencing of the human genome and the study of stem cells. Although the anticipated revolution of cellular therapies in medicine is in its infancy, the conceptual debate over stem cell plasticity shares similarities with evolution of the quantum theory. Are there notions and modes of thinking that stem cell biologists should adopt from the evolution in the interpretation of the laws of physics?

Atchudan, R., et al. (2022). "Tunable fluorescent carbon dots from biowaste as fluorescence ink and imaging human normal and cancer cells." Environ Res **204**(Pt D): 112365.

Growing global biowaste and its environmental issues challenge the need for converting biowastes into a beneficial product. Among the biowaste, here kiwi fruit (Actinidia Deliciosa) peels are considered for the preparation of carbon dots (CDs). Using a green one-pot hydrothermal-carbonization method, kiwi fruit peels were effectively converted into valuable kiwi fruit peel carbon dots (KFP-CDs). The morphology, physio-chemical and optical properties of as-synthesized KFP-CDs were analyzed using various analytical techniques such as X-ray powder diffraction, Raman spectroscopy, attenuated total reflection-Fourier transform infrared spectroscopy, field emission scanning electron microscopy, high-resolution transmission electron microscopy, X-ray photoelectron spectroscopy, Ultraviolet-visible, and fluorescence spectroscopy. The KFP-CDs revealed a homogeneous spherical shape, monodispersed with an average size of 5 nm. The characterization confirms that KFP-CDs have functional groups such as -CN, -COOH, and -OH which are responsible for the easy dispersion of KFP-CDs in aqueous media. Without any preprocessing, KFP-CDs

exhibit strong fluorescence upon exposure to UV light. Further, KFP-CDs displayed excitation-dependent fluorescence emission with a good quantum yield of about 18%. Thus by considering the excellent properties of KFP-CDs, KFP-CDs were used as fluorescent ink for drawing and writing without any capping/passivation agent. The pictures and words were instantaneously viewed when exposed to UV light. In addition, KFP-CDs tested for cell imaging in four human cell lines (normal and cancer cells) bestowed excellent biocompatibility and low cytotoxicity, which is important for the safe and long-term development of cellular imaging. The findings imply that KFP-CDs can be utilized as a cell labeling agent for mesenchymal stem cells, breast cancer, and thyroid cancer cells in vitro imaging. Thus, these observations revealed that investigating sustainable resource-based CDs can open up new avenues for tackling environmental issues.

Avots, A., et al. (2002). "Plasticity of hematopoietic stem cells and cellular memory." Immunol Rev **187**: 9-21.

Stem cell systems represent an effective and powerful approach for tissue development and regeneration of diverse tissue types. Common and defining features of these exceptional cells are the capacity for self-renewal and the potential for differentiation into multiple mature cell types. Recently, surprising new observations have indicated that stem cells isolated from one adult tissue can also give rise to mature cells of other cell lineages, irrespective of classical germ layer designations. This discovery has resulted in quantum leaps in both scientific knowledge and the potential applications of stem cells. The new findings contradict central dogmas of commitment and differentiation of stem and progenitor cells. However, the true potential of somatic stem cells is just emerging and the new findings have to be defined more fully and integrated into a unifying model of stem cell potential and behavior. Here we analyze the developmental potential of hematopoietic stem cells of mouse and man following their injection into the murine preimplantation blastocyst, an environment that allows the development of all cell lineages. In addition, we discuss the emerging lines of evidence of the developmental plasticity of hematopoietic and other somatic stem cells and consider how cellular memory of transcriptional states is established and may be potentially involved in this phenomenon.

Ayoubi, M., et al. (2017). "Biochemical mechanisms of dose-dependent cytotoxicity and ROS-mediated apoptosis induced by lead sulfide/graphene oxide quantum dots for potential bioimaging applications." Sci Rep **7**(1): 12896.

Colloidal quantum dots (CQD) have attracted considerable attention for biomedical diagnosis and imaging as well as biochemical analysis and stem cell tracking. In this study, quasi core/shell lead sulfide/reduced graphene oxide CQD with near infrared emission (1100 nm) were prepared for potential bioimaging applications. The nanocrystals had an average diameter of ~4 nm, a hydrodynamic size of ~8 nm, and a high quantum efficiency of 28%. Toxicity assay of the hybrid CQD in the cultured human mononuclear blood cells does not show cytotoxicity up to 200 microg/ml. At high concentrations, damage to mitochondrial activity and mitochondrial membrane potential (MMP) due to the formation of uncontrollable amounts of intracellular oxygen radicals (ROS) was observed. Cell membrane and Lysosome damage or a transition in mitochondrial permeability were also noticed. Understanding of cell-nanoparticle interaction at the molecular level is useful for the development of new fluorophores for biomedical imaging.

Aziz, A. N., et al. (2018). "Laevifins A-G, clerodane diterpenoids from the Bark of Croton oblongus Burm.f." Phytochemistry **156**: 193-200.

A phytochemical investigation of the stem barks of the Malaysian Croton oblongus Burm.f. (Syn. Croton laevifolius Blume) (Euphorbiaceae) yielded seven previously undescribed ent-neo-clerodane diterpenoids, laevifins A - G and the known crovatin (3). Structures were established by a combination of spectroscopic methods including HRESIMS, NMR spectroscopy and X-ray crystallography. The absolute configuration of crovatin and laevifins A-G was established by comparison of experimental ECD and theoretical TDDFT ECD calculated spectra. This is the first report on the occurrence of the sesquiterpenoid cryptomeridiol in a Croton species. In vitro cytotoxicity assays on laevifins A, B and G showed moderate activities against the MCF-7 cancer cell line (IC50 102, 115 and 106muM, respectively) while beta-amyrin and acetyl aleuritolic acid showed good anti-inflammatory activity on the LPS-induced NF-kappaB translocation inhibition in RAW 264.7cells assay with IC50 values of 23.5 and 35.4mug/mL, respectively.

Bagalkot, V., et al. (2007). "Quantum dot-aptamer conjugates for synchronous cancer imaging, therapy, and sensing of drug delivery based on bi-fluorescence resonance energy transfer." Nano Lett **7**(10): 3065-3070.

We report a novel quantum dot (QD)-aptamer(Apt)-doxorubicin (Dox) conjugate [QD-Apt(Dox)] as a targeted cancer imaging, therapy, and sensing system. By functionalizing the surface of fluorescent QD with the A10 RNA aptamer, which recognizes the extracellular domain of the prostate specific membrane antigen (PSMA), we developed a

targeted QD imaging system (QD-Apt) that is capable of differential uptake and imaging of prostate cancer cells that express the PSMA protein. The intercalation of Dox, a widely used antineoplastic anthracycline drug with fluorescent properties, in the double-stranded stem of the A10 aptamer results in a targeted QD-Apt(Dox) conjugate with reversible self-quenching properties based on a Bi-FRET mechanism. A donor-acceptor model fluorescence resonance energy transfer (FRET) between QD and Dox and a donor-quencher model FRET between Dox and aptamer result when Dox intercalated within the A10 aptamer. This simple multifunctional nanoparticle system can deliver Dox to the targeted prostate cancer cells and sense the delivery of Dox by activating the fluorescence of QD, which concurrently images the cancer cells. We demonstrate the specificity and sensitivity of this nanoparticle conjugate as a cancer imaging, therapy and sensing system in vitro.

Bankoti, K., et al. (2020). "Carbon nanodot decorated acellular dermal matrix hydrogel augments chronic wound closure." J Mater Chem B **8**(40): 9277-9294.

Impaired skin regeneration in chronic wounds like in diabetes corresponds to high oxidative stress, poor angiogenesis and insufficient collagen hyperplasia. Therefore, a multifaceted strategy for treatment is required to address critical issues associated with chronic wound healing. Fascinating application of nanomaterials in chronic wounds is still limited; hence, in the present work bioactive solubilized decellularized dermal matrix (sADM) was employed to form a hydrogel with chitosan (CTS) at physiological pH/temperature and modified with reactive oxygen species (ROS) scavenging carbon nanodots (ND). A detailed in vitro investigation found that the ND modified bioactive hydrogel (CsADMND) is suitable for human amniotic membrane derived stem cell (hAMSC) delivery. Also, CsADMND was observed to possess a good ROS scavenging property, hemocompatibility and pro-angiogenic potential as demonstrated by 2,2-diphenyl-1-picrylhydrazyl (DPPH), haemolysis and chick chorioallantoic membrane (CAM) assay, respectively. The hybrid hydrogel promoted migration of cells in vitro in scratch assay owing to its antioxidant potential and the presence of bioactive moieties. Further, its efficacy in healing full thickness (FT) chronic wounds was evaluated in a streptozotocin (STZ) induced diabetic model. The CsADMND hydrogel after association with hAMSCs led to stimulation of early angiogenesis, superior collagen deposition, rapid wound closure, complete reepithelialisation, and formation of distinct organized dermal epidermal junctions (DEJ) post 21 days of healing. These results suggest that the hAMSC laden CsADMND hydrogel may serve as a promising therapeutic strategy for the management of chronic wounds.

Barat, B., et al. (2009). "Cys-diabody quantum dot conjugates (immunoQdots) for cancer marker detection." Bioconjug Chem **20**(8): 1474-1481.

The present work demonstrates the use of small bivalent engineered antibody fragments, cys-diabodies, for biological modification of nanoscale particles such as quantum dots (Qdots) for detection of target antigens. Novel bioconjugated quantum dots known as immunoQdots (iQdots) were developed by thiol-specific oriented coupling of tumor specific cys-diabodies, at a position away from the antigen binding site to amino PEG CdSe/ZnS Qdots. Initially, amino PEG Qdot 655 were coupled with reduced anti-HER2 cys-diabody by amine-sulfhydryl-reactive linker [N-epsilon-maleimidocaproyloxy] succinimide ester (EMCS) to produce anti-HER2 iQdot 655. Spectral characterization of the conjugate revealed that the spectrum was symmetrical and essentially identical to unconjugated Qdot. Specific receptor binding activity of anti-HER2 iQdot 655 was confirmed by flow cytometry on HER2 positive and negative cells. Immunofluorescence results showed homogeneous surface labeling of the cell membrane with Qdot 655 conjugate. In addition, cys-diabodies specific for HER2, as well as prostate stem cell antigen (PSCA), were conjugated successfully with amino PEG Qdot 800. All of these iQdots retain the photoluminescence properties of the unconjugated Qdot 800 as well as the antigen binding specificity of the cys-diabody as demonstrated by flow cytometry. Simultaneous detection of two tumor antigens on LNCaP/PSCA prostate cancer cells (which express PSCA and HER2) in culture was possible using two iQdots, anti-HER2 iQdot 655 and anti-PSCA iQdot 800. Thus, these iQdots are potentially useful as optical probes for sensitive, multiplexed detection of surface markers on tumor cells. The present thiol-specific conjugation method demonstrates a general approach for site-specific oriented coupling of cys-diabodies to a wide variety of nanoparticles without disturbing the antigen binding site and maintaining small size compared to intact antibody.

Barckhausen, C., et al. (2016). "GMP-Compliant Expansion of Clinical-Grade Human Mesenchymal Stromal/Stem Cells Using a Closed Hollow Fiber Bioreactor." Methods Mol Biol **1416**: 389-412.

This chapter describes a method for GMP-compliant expansion of human mesenchymal stromal/stem cells (hMSC) from bone marrow aspirates, using the Quantum((R)) Cell Expansion System from Terumo BCT. The Quantum system is a functionally closed, automated hollow fiber bioreactor system designed to reproducibly grow cells in either GMP or

research laboratory environments. The chapter includes protocols for preparation of media, setup of the Quantum system, coating of the hollow fiber bioreactor, as well as loading, feeding, and harvesting of cells. We suggest a panel of quality controls for the starting material, the interim product, as well as the final product.

Barnett, J. M., et al. (2013). "Imaging of endothelial progenitor cell subpopulations in angiogenesis using quantum dot nanocrystals." Methods Mol Biol **1026**: 45-56.

Over the last decade, research has identified a class of bone marrow-derived circulating stem cells, termed endothelial progenitor cells (EPCs), that are capable of homing to vascular lesions in the eye and contributing to pathological ocular neovascularization (NV). In preclinical and biological studies, EPCs are - frequently identified and tracked using a intracellularly loaded fluorescent tracer, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbo cyanine perchlorate-labeled acetylated LDL (DiI-acLDL). However, this method is limited by photobleaching and insufficient quantum efficiency for long-term imaging applications. We have developed a method for conjugation of high quantum efficiency, photostable, and multispectral quantum dot nanocrystals (QD) to acLDL for long-term tracking of EPCs with improved signal-to-noise ratios. Specifically, we conjugated QD to acLDL (QD-acLDL) and used this conjugated fluorophore to label a specific CD34(+) subpopulation of EPCs isolated from rat bone marrow. We then utilized this method to track CD34(+) EPCs in a rat model of laser-induced choroidal neovascularization (LCNV) to evaluate its potential for tracking EPCs in ocular angiogenesis, a critical pathologic feature of several blinding conditions.

Basieva, I., et al. (2011). "Quantum-like interference effect in gene expression: glucose-lactose destructive interference." Syst Synth Biol **5**(1-2): 59-68.

In this note we illustrate on a few examples of cells and proteins behavior that microscopic biological systems can exhibit a complex probabilistic behavior which cannot be described by classical probabilistic dynamics. These examples support authors conjecture that behavior of microscopic biological systems can be described by quantum-like models, i.e., models inspired by quantum-mechanics. At the same time we do not couple quantum-like behavior with quantum physical processes in bio-systems. We present arguments that such a behavior can be induced by information complexity of even smallest bio-systems, their adaptivity to context changes. Although our examples of the quantum-like behavior are rather simple (lactose-glucose interference in E. coli growth, interference effect for differentiation of tooth stem cell induced by the presence of mesenchymal cell, interference in behavior of PrP(C) and PrP(Sc) prions), these examples may stimulate the interest in systems biology to quantum-like models of adaptive dynamics and lead to more complex examples of nonclassical probabilistic behavior in molecular biology.

Bengtsson, F., et al. (2020). "Mechanisms behind species-specific water economy responses to water level drawdown in peat mosses." Ann Bot **126**(2): 219-230.

BACKGROUND AND AIMS: The ecosystem engineers Sphagnum (peat mosses) are responsible for sequestering a large proportion of carbon in northern peatlands. Species may respond differently to hydrological changes, and water level changes may lead to vegetation shifts in peatlands, causing them to revert from sinks to sources of carbon. We aimed to compare species-specific responses to water level drawdown within Sphagnum, and investigate which traits affect water economy in this genus. METHODS: In a mesocosm experiment, we investigated how water level drawdown affected water content (WC) in the photosynthetically active apex of the moss and maximum quantum yield of photosystem II (i.e. Fv/Fm) of 13 Sphagnum species. Structural traits were measured, and eight anatomical traits were quantified from scanning electron microscopy micrographs. KEY RESULTS: Mixed-effects models indicated that at high water level, large leaves were the most influential predictor of high WC, and at low water level WC was higher in species growing drier in the field, with larger hyaline cell pore sizes and total pore areas associated with higher WC. Higher stem and peat bulk density increased WC, while capitulum mass per area and numerical shoot density did not. We observed a clear positive relationship between Fv/Fm and WC in wet-growing species. CONCLUSIONS: While we found that most hummock species had a relatively high water loss resistance, we propose that some species are able to maintain a high WC at drawdown by storing large amounts of water at a high water level. Our result showing that leaf traits are important warrants further research using advanced morphometric methods. As climate change may lead to more frequent droughts and thereby water level drawdowns in peatlands, a mechanistic understanding of species-specific traits and responses is crucial for predicting future changes in these systems.

Bhirde, A., et al. (2011). "Nanoparticles for cell labeling." Nanoscale **3**(1): 142-153.

Cell based therapeutics are emerging as powerful regimens. To better understand the migration and proliferation mechanisms of implanted cells, a means to track cells in living subjects is essential, and to achieve that, a number of cell labeling techniques have been developed. Nanoparticles, with their superior

physical properties, have become the materials of choice in many investigations along this line. Owing to inherent magnetic, optical or acoustic attributes, these nanoparticles can be detected by corresponding imaging modalities in living subjects at a high spatial and temporal resolution. These features allow implanted cells to be separated from host cells; and have advantages over traditional histological methods, as they permit non-invasive, real-time tracking in vivo. This review attempts to give a summary of progress in using nanotechnology to monitor cell trafficking. We will focus on direct cell labeling techniques, in which cells ingest nanoparticles that bear traceable signals, such as iron oxide or quantum dots. Ferritin and MagA reporter genes that can package endogenous iron or iron supplement into iron oxide nanoparticles will also be discussed.

Biava, P. M., et al. (2019). "Stem Cell Differentiation Stage Factors and their Role in Triggering Symmetry Breaking Processes during Cancer Development: A Quantum Field Theory Model for Reprogramming Cancer Cells to Healthy Phenotypes." Curr Med Chem **26**(6): 988-1001.

A long history of research has pursued the use of embryonic factors isolated during cell differentiation processes for the express purpose of transforming cancer cells back to healthy phenotypes. Recent results have clarified that the substances present at different stages of cell differentiation-which we call stem cell differentiation stage factors (SCDSFs)-are proteins with low molecular weight and nucleic acids that regulate genomic expression. The present review summarizes how these substances, taken at different stages of cellular maturation, are able to retard proliferation of many human tumor cell lines and thereby reprogram cancer cells to healthy phenotypes. The model presented here is a quantum field theory (QFT) model in which SCDSFs are able to trigger symmetry breaking processes during cancer development. These symmetry breaking processes, which lie at the root of many phenomena in elementary particle physics and condensed matter physics, govern the phase transitions of totipotent cells to higher degrees of diversity and order, resulting in cell differentiation. In cancers, which share many genomic and metabolic similarities with embryonic stem cells, stimulated redifferentiation often signifies the phenotypic reversion back to health and nonproliferation. In addition to acting on key components of the cellular cycle, SCDSFs are able to reprogram cancer cells by delicately influencing the cancer microenvironment, modulating the electrochemistry and thus the collective electrodynamic behaviors between dipole networks in biomacromolecules and the interstitial water field. Coherent effects in biological water, which are derived from a dissipative QFT framework, may offer new diagnostic and therapeutic targets at a systemic level, before tumor instantiation occurs in specific tissues or organs. Thus, by including the environment as an essential component of our model, we may push the prevailing paradigm of mutation-driven oncogenesis toward a closer description of reality.

Bigildeev, A. E., et al. (2017). "[The effects of interleukin-1 beta and gamma-quantum braking radiation on mesenchymal progenitor cells]." Mol Biol (Mosk) **51**(3): 447-459.

In murine bone-marrow stromal microenvironment cells and in human multipotent mesenchymal stromal cells (MMSCs), proinflammatory cytokine interleukin-1 beta (IL-1beta) serves as a growth factor. In murine bone tissue, IL-1beta expression increases in vivo after irradiation. Here, we have presented our evaluation of the effects of exogenous IL-1beta on the expression of NF-kB transcription factors in human MMSCs and stromal layer cells of murine long-term bone marrow cultures (LTBMCs). The cytokine signaling pathway was also activated in murine LTBMC by braking electron radiation in doses of 3-12 Gy. The level of expression of genes that code for IL-1beta, IL-1beta type-I receptor and NF-kB and IKK protein families have been studied at different time points post exposure. In both human and murine stromal cells, exogenous IL-1beta led to an increase in the level of expression of its own gene, while levels of expression of NF-kB and IKK gene families were not substantially changed. Nevertheless, in human cells, a significant correlation between levels of expression of IL-1beta and all NF-kB family genes was detected. It points to a similarity in IL-1beta signal pathways in mesenchymal and hematopoietic cells, where the posttranslational modifications of NF-kB transcription factors play a major role. The irradiation of murine LTBMC resulted in a transient increase in the expression of genes that code NF-kB transcription factors and IL-1beta. These results indicate an important role of Rel, Rela, Relb, and Nfkb2 genes in the induction of IL-1beta signal pathway in murine stromal cells. An increase in IL-1beta expression after the irradiation of stromal cells may be related to both the induction of inflammation due to massive cell death and to a profound stimulation of the expression of this proinflammatory cytokine expression.

Bignone, P. A., et al. (2013). "Identification of human embryonic progenitor cell targeting peptides using phage display." PLoS One **8**(3): e58200.

Human pluripotent stem (hPS) cells are capable of differentiation into derivatives of all three primary embryonic germ layers and can self-renew indefinitely. They therefore offer a potentially scalable source of replacement cells to treat a variety of degenerative

diseases. The ability to reprogram adult cells to induced pluripotent stem (iPS) cells has now enabled the possibility of patient-specific hPS cells as a source of cells for disease modeling, drug discovery, and potentially, cell replacement therapies. While reprogramming technology has dramatically increased the availability of normal and diseased hPS cell lines for basic research, a major bottleneck is the critical unmet need for more efficient methods of deriving well-defined cell populations from hPS cells. Phage display is a powerful method for selecting affinity ligands that could be used for identifying and potentially purifying a variety of cell types derived from hPS cells. However, identification of specific progenitor cell-binding peptides using phage display may be hindered by the large cellular heterogeneity present in differentiating hPS cell populations. We therefore tested the hypothesis that peptides selected for their ability to bind a clonal cell line derived from hPS cells would bind early progenitor cell types emerging from differentiating hPS cells. The human embryonic stem (hES) cell-derived embryonic progenitor cell line, W10, was used and cell-targeting peptides were identified. Competition studies demonstrated specificity of peptide binding to the target cell surface. Efficient peptide targeted cell labeling was accomplished using multivalent peptide-quantum dot complexes as detected by fluorescence microscopy and flow cytometry. The cell-binding peptides were selective for differentiated hPS cells, had little or no binding on pluripotent cells, but preferential binding to certain embryonic progenitor cell lines and early endodermal hPS cell derivatives. Taken together these data suggest that selection of phage display libraries against a clonal progenitor stem cell population can be used to identify progenitor stem cell targeting peptides. The peptides may be useful for monitoring hPS cell differentiation and for the development of cell enrichment procedures to improve the efficiency of directed differentiation toward clinically relevant human cell types.

Bignone, P. A., et al. (2016). "Selection of Phage Display Peptides Targeting Human Pluripotent Stem Cell-Derived Progenitor Cell Lines." Methods Mol Biol **1357**: 269-283.

The ability of human pluripotent stem cells (hPS) to both self-renew and differentiate into virtually any cell type makes them a promising source of cells for cell-based regenerative therapies. However, stem cell identity, purity, and scalability remain formidable challenges that need to be overcome for translation of pluripotent stem cell research into clinical applications. Directed differentiation from hPS cells is inefficient and residual contamination with pluripotent cells that have the potential to form tumors remains problematic. The derivation of scalable (self-renewing) embryonic progenitor stem cell lines offers a solution because they are well defined and clonally pure. Clonally pure progenitor stem cell lines also provide a means for identifying cell surface targeting reagents that are useful for identification, tracking, and repeated derivation of the corresponding progenitor stem cell types from additional hPS cell sources. Such stem cell targeting reagents can then be applied to the manufacture of genetically diverse banks of human embryonic progenitor cell lines for drug screening, disease modeling, and cell therapy. Here we present methods to identify human embryonic progenitor stem cell targeting peptides by selection of phage display libraries on clonal embryonic progenitor cell lines and demonstrate their use for targeting quantum dots (Qdots) for stem cell labeling.

Blach, P., et al. (2020). "Graphene Enclosure of Chemically Fixed Mammalian Cells for Liquid-Phase Electron Microscopy." J Vis Exp(163).

A protocol is described for investigating the human epidermal growth factor receptor 2 (HER2) in the intact plasma membrane of breast cancer cells using scanning transmission electron microscopy (STEM). Cells of the mammalian breast cancer cell line SKBR3 were grown on silicon microchips with silicon nitride (SiN) windows. Cells were chemically fixed, and HER2 proteins were labeled with quantum dot nanoparticles (QDs), using a two-step biotin-streptavidin binding protocol. The cells were coated with multilayer graphene to maintain a hydrated state, and to protect them from electron beam damage during STEM. To examine the stability of the samples under electron beam irradiation, a dose series experiment was performed. Graphene-coated and non-coated samples were compared. Beam induced damage, in the form of bright artifacts, appeared for some non-coated samples at increased electron dose D, while no artifacts appeared on coated samples.

Braydich-Stolle, L., et al. (2005). "In vitro cytotoxicity of nanoparticles in mammalian germline stem cells." Toxicol Sci **88**(2): 412-419.

Gametogenesis is a complex biological process that is particularly sensitive to environmental insults such as chemicals. Many chemicals have a negative impact on the germline, either by directly affecting the germ cells, or indirectly through their action on the somatic nursing cells. Ultimately, these effects can inhibit fertility, and they may have negative consequences for the development of the offspring. Recently, nanomaterials such as nanotubes, nanowires, fullerene derivatives (buckyballs), and quantum dots have received enormous national attention in the creation of new types of analytical tools for biotechnology and the life sciences. Despite the wide

application of nanomaterials, there is a serious lack of information concerning their impact on human health and the environment. Thus, there are limited studies available on toxicity of nanoparticles for risk assessment of nanomaterials. The purpose of this study was to assess the suitability of a mouse spermatogonial stem cell line as a model to assess nanotoxicity in the male germline in vitro. The effects of different types of nanoparticles on these cells were evaluated by light microscopy, and by cell proliferation and standard cytotoxicity assays. Our results demonstrate a concentration-dependent toxicity for all types of particles tested, whereas the corresponding soluble salts had no significant effect. Silver nanoparticles were the most toxic while molybdenum trioxide (MoO(3)) nanoparticles were the least toxic. Our results suggest that this cell line provides a valuable model with which to assess the cytotoxicity of nanoparticles in the germ line in vitro.

Burk, J., et al. (2017). "Characterisation and intracellular labelling of mesenchymal stromal cells derived from synovial fluid of horses and sheep." Vet J **222**: 1-8.

Multipotent mesenchymal stromal cells (MSCs) derived from synovial fluid (SF) are considered to be a promising cell type for therapeutic applications in joint disease. However, despite their potential relevance for clinical and experimental studies, there is insufficient knowledge about SF-derived MSCs isolated from horses and sheep. In this study, cells were recovered from healthy SF and bone marrow (BM) of sheep, and from healthy and osteoarthritic SF of horses. Ovine SF-MSCs were used to assess the efficiency of intracellular labelling with quantum dots (QDs). Colony forming units, generation times, trilineage differentiation potential and expression of CD73, CD90 and CD105 at mRNA level were assessed. QD labelling was efficient, with >98% positive cells directly after labelling at 10 nmol/L and >95% positive cells directly after labelling at 2 nmol/L. The label decreased over 7 days of culture, with more persistence at the higher labelling concentration. No significant differences in proliferation were observed. All MSCs had trilineage differentiation potential, but adipogenesis was more distinct in equine samples and chondrogenesis was most pronounced in ovine SF-MSCs. CD73, CD90 and CD105 were expressed in equine and ovine MSCs.

Busca, A., et al. (2010). "Iron overload in patients receiving allogeneic hematopoietic stem cell transplantation: quantification of iron burden by a superconducting quantum interference device (SQUID) and therapeutic effectiveness of phlebotomy." Biol Blood Marrow Transplant **16**(1): 115-122.

Iron overload (IO) is a known adverse prognostic factor in patients who undergo allogeneic hematopoietic stem cell transplantation (HSCT) for thalassemia and appears to play a similar role in patients with other hematologic disorders. The estimation of IO is based primarily on serum ferritin level; however, many confounding factors can result in ferritin overestimation, especially in HSCT recipients. The aim of the present study was to quantify IO after HSCT using a superconducting quantum interference device (SQUID), and to evaluate the impact of IO on hepatic function and infections. In addition, the feasibility of iron depletion was investigated. A total of 102 consecutive allogeneic HSCT recipients admitted to our outpatient department between December 2005, and December 2007, were analyzed. Primary diagnosis included acute leukemia/myelodysplastic syndrome in 61% of cases. Assessment of IO after HSCT included serum ferritin; in those with hyperferritinemia (ferritin>1000 ng/mL), liver iron concentration (LIC) was evaluated by SQUID magnetic susceptometry. Iron removal therapy was offered to patients with moderate IO (LIC 1000-2000 microg Fe/g wet weight [ww]) or severe IO (LIC >2,000 microg Fe/g ww). Fifty-seven patients had a ferritin level <1000 ng/mL: the median time between HSCT and assessment of ferritin level was 1006 days (range, 93-5239 days), significantly different from the median time of 183 days (range, 78-2957 days) in the 45 patients with a ferritin level >1000 ng/mL. Out of 42 patients evaluated by SQUID, 29 had moderate to severe IO (median LIC value, 1493 microg Fe/g ww [range, 1030-3253]). In a multivariate analysis, a significant correlation was found between a ferritin level >1000 ng/mL and the presence of at least one abnormal liver function test (LFT) ORo=6.8; 95% CI=2.2-20.6). In addition, the rate of proven/probable invasive fungal disease was significantly higher in the patients with hyperferritinemia (13% vs 0%; P=.006). Nineteen of the 24 patients considered eligible for iron-depletion therapy underwent regular phlebotomy; 13 completed the program in a median of 287 days (range, 92-779 days), reaching the target of a ferritin level<500 ng/mL; LIC was significantly reduced (median, 1419 microg Fe/g ww to 625 microg Fe/g ww; P < .001) in 8 of the 9 patients who were revaluated by SQUID at the end of the iron-depletion program. In conclusion, the measurement of LIC obtained by SQUID documented the presence of moderate/severe IO in 69% of the patients with a high ferritin level. Our data showed that in HSCT recipients, high ferritin level is an independent risk factor for abnormal LFTs, and IO may be considered a potential risk factor for fungal infections. A phlebotomy program may be feasible in two-thirds of the patients who might benefit from iron depletion.

Cai, H., et al. (2020). "Sulfonated glycosaminoglycan bioinspired carbon dots for effective cellular labelling and promotion of the differentiation of mesenchymal stem cells." J Mater Chem B **8**(26): 5655-5666.

Although carbon dots (CDs) have been synthesized and applied in a variety of biological fields, such as disease diagnosis and gene/drug delivery, the exploration of facile bioinspired synthesis and applications of CDs is still of great significance. Particularly, recent increasing research has clearly confirmed that nanomaterials can affect a series of physiological behaviors and functions of mesenchymal stem cells (MSCs) (e.g., differentiation and pluripotency). Therefore, it is very important to develop multifunctional nanomaterials to simultaneously realize the cellular labelling and regulation of MSC behaviors in practical applications. Herein, sulfonated glycosaminoglycan-bioinspired CDs as bi-functional nanomaterials were ingeniously designed for cellular imaging and promoting the differentiation of rat bone MSCs (rBMSCs) in different culture media, which simultaneously met the two fundamental requirements in the field of MSC-based treatments (e.g., precisely directing the differentiation of MSCs and effective cellular labeling). These bifunctional CDs were successfully prepared via one-pot hydrothermal synthesis by using d-glucosamine hydrochloride (GA.HCl) and sodium p-styrenesulfonate (NaSS) as the reactants. The synthesized CDs with a uniform particle size (around 4 nm) dispersed well in aqueous solutions and exhibited remarkable fluorescence stability under different conditions. Additionally, cell viability and proliferation results demonstrated that the CDs possessed good biocompatibility, having negligible effects on the self-renewal potential of rBMSCs. The as-prepared CDs presented a cytoplasmatic distribution after being ingested by rBMSCs; thus, they are particularly suitable for cellular imaging. More importantly, the addition of CDs to osteogenic and chondrogenic induction media (OIM and CIM), respectively, was capable of effectively promoting the osteogenic and chondrogenic differentiation of rBMSCs due to the generation of reactive oxygen species (ROS) while having no influence on their pluripotency. In brief, this study not only implements a cellular labeling method based on CDs that were synthesized by a biomimicking strategy, but also paves a new way to regulate the differentiation of MSCs by designing multifunctional nanomaterials; this will enable the extensive development of facile synthesis methods and new applications of CDs and will also provide some research foundations for MSC-based fields.

Cai, X., et al. (2016). "Organic Nanoparticles with Aggregation-Induced Emission for Bone Marrow Stromal Cell Tracking in a Rat PTI Model." Small **12**(47): 6576-6585.

Stem-cell based therapy is an emerging therapeutic approach for ischemic stroke treatment. Bone marrow stromal cells (BMSCs) are in common use as a cell source for stem cell therapy and show promising therapeutic outcomes for stroke treatment. One challenge is to develop a reliable tracking strategy to monitor the fate of BMSCs and assess their therapeutic effects in order to improve the success rate of such treatment. Herein, TPEEP, a fluorogen with aggregation-induced emission characteristics and near-infrared emission are designed and synthesized and further fabricated into organic nanoparticles (NPs). The obtained NPs show high fluorescence quantum yield, low cytotoxicity with good physical and photostability, which display excellent tracking performance of BMSCs in vitro and in vivo. Using a rat photothrombotic ischemia model as an example, the NP-labeled BMSCs are able to migrate to the stroke lesion site to yield bright red fluorescence. Immunofluorescence staining shows that the NP labeling does not affect the normal function of BMSCs, proving their good biocompatibility in vivo. These merits make TPEEP NP a potential cell tracker to evaluate the fate of BMSCs in cell therapy.

Carlson-Stevermer, J., et al. (2017). "Assembly of CRISPR ribonucleoproteins with biotinylated oligonucleotides via an RNA aptamer for precise gene editing." Nat Commun **8**(1): 1711.

Writing specific DNA sequences into the human genome is challenging with non-viral gene-editing reagents, since most of the edited sequences contain various imprecise insertions or deletions. We developed a modular RNA aptamer-streptavidin strategy, termed S1mplex, to complex CRISPR-Cas9 ribonucleoproteins with a nucleic acid donor template, as well as other biotinylated molecules such as quantum dots. In human cells, tailored S1mplexes increase the ratio of precisely edited to imprecisely edited alleles up to 18-fold higher than standard gene-editing methods, and enrich cell populations containing multiplexed precise edits up to 42-fold. These advances with versatile, preassembled reagents could greatly reduce the time and cost of in vitro or ex vivo gene-editing applications in precision medicine and drug discovery and aid in the development of increased and serial dosing regimens for somatic gene editing in vivo.

Castanheira, P., et al. (2009). "DAPI diffusion after intravitreal injection of mesenchymal stem cells in the injured retina of rats." Cell Transplant **18**(4): 423-431.

To evaluate DAPI (4',6-diamidino-2-phenylindole) as a nuclear tracer of stem cell migration and incorporation it was observed the pattern of retinal integration and differentiation of mesenchymal stem cells (MSCs) injected into the vitreous cavity of rat eyes with retinal injury. For this purpose adult rat retinas were submitted to laser damage followed by transplantation of DAPI-labeled BM-MSCs grafts and double-labeled DAPI and quantum dot-labeled BM-MSCs. To assess a

possible DAPI diffusion as well as the integration and differentiation of DAPI-labeled BM-MSCs in laser-injured retina, host retinas were evaluated 8 weeks after injury/transplantation. It was demonstrated that, 8 weeks after the transplant, most of the retinal cells in all neural retinal presented nuclear DAPI labeling, specifically in the outer nuclear layer (ONL), inner nuclear layer (INL), and ganglion cell layer (GCL). Meanwhile, at this point, most of the double-labeled BM-MSCs (DAPI and quantum dot) remained in the vitreous cavity and no retinal cells presented the quantum dot marker. Based on these evidences we concluded that DAPI diffused to adjacent retinal cells while the nanocrystals remained labeling only the transplanted BM-MSCs. Therefore, DAPI is not a useful marker for stem cells in vivo tracing experiments because the DAPI released from dying cells in moment of the transplant are taken up by host cells in the tissue.

Cavalier-Smith, T. (2002). "The neomuran origin of archaebacteria, the negibacterial root of the universal tree and bacterial megaclassification." Int J Syst Evol Microbiol **52**(Pt 1): 7-76.

Prokaryotes constitute a single kingdom, Bacteria, here divided into two new subkingdoms: Negibacteria, with a cell envelope of two distinct genetic membranes, and Unibacteria, comprising the new phyla Archaebacteria and Posibacteria, with only one. Other new bacterial taxa are established in a revised higher-level classification that recognizes only eight phyla and 29 classes. Morphological, palaeontological and molecular data are integrated into a unified picture of large-scale bacterial cell evolution despite occasional lateral gene transfers. Archaebacteria and eukaryotes comprise the clade neomura, with many common characters, notably obligately co-translational secretion of N-linked glycoproteins, signal recognition particle with 7S RNA and translation-arrest domain, protein-spliced tRNA introns, eight-subunit chaperonin, prefoldin, core histones, small nucleolar ribonucleoproteins (snoRNPs), exosomes and similar replication, repair, transcription and translation machinery. Eubacteria (posibacteria and negibacteria) are paraphyletic, neomura having arisen from Posibacteria within the new subphylum Actinobacteria (possibly from the new class Arabobacteria, from which eukaryotic cholesterol biosynthesis probably came). Replacement of eubacterial peptidoglycan by glycoproteins and adaptation to thermophily are the keys to neomuran origins. All 19 common neomuran character suites probably arose essentially simultaneously during the radical modification of an actinobacterium. At least 11 were arguably adaptations to thermophily. Most unique archaebacterial characters (prenyl ether lipids; flagellar shaft of glycoprotein, not flagellin; DNA-binding protein lob; specially modified tRNA; absence of Hsp90) were subsequent secondary adaptations to hyperthermophily and/or hyperacidity. The insertional origin of protein-spliced tRNA introns and an insertion in proton-pumping ATPase also support the origin of neomura from eubacteria. Molecular co-evolution between histones and DNA-handling proteins, and in novel protein initiation and secretion machineries, caused quantum evolutionary shifts in their properties in stem neomura. Proteasomes probably arose in the immediate common ancestor of neomura and Actinobacteria. Major gene losses (e.g. peptidoglycan synthesis, hsp90, secA) and genomic reduction were central to the origin of archaebacteria. Ancestral archaebacteria were probably heterotrophic, anaerobic, sulphur-dependent hyperthermoacidophiles; methanogenesis and halophily are secondarily derived. Multiple lateral gene transfers from eubacteria helped secondary archaebacterial adaptations to mesophily and genome re-expansion. The origin from a drastically altered actinobacterium of neomura, and the immediately subsequent simultaneous origins of archaebacteria and eukaryotes, are the most extreme and important cases of quantum evolution since cells began. All three strikingly exemplify De Beer's principle of mosaic evolution: the fact that, during major evolutionary transformations, some organismal characters are highly innovative and change remarkably swiftly, whereas others are largely static, remaining conservatively ancestral in nature. This phenotypic mosaicism creates character distributions among taxa that are puzzling to those mistakenly expecting uniform evolutionary rates among characters and lineages. The mixture of novel (neomuran or archaebacterial) and ancestral eubacteria-like characters in archaebacteria primarily reflects such vertical mosaic evolution, not chimaeric evolution by lateral gene transfer. No symbiogenesis occurred. Quantum evolution of the basic neomuran characters, and between sister paralogues in gene duplication trees, makes many sequence trees exaggerate greatly the apparent age of archaebacteria. Fossil evidence is compelling for the extreme antiquity of eubacteria [over 3500 million years (My)] but, like their eukaryote sisters, archaebacteria probably arose only 850 My ago. Negibacteria are the most ancient, radiating rapidly into six phyla. Evidence from molecular sequences, ultrastructure, evolution of photosynthesis, envelope structure and chemistry and motility mechanisms fits the view that the cenancestral cell was a photosynthetic negibacterium, specifically an anaerobic green non-sulphur bacterium, and that the universal tree is rooted at the divergence between sulphur and non-sulphur green bacteria. The negibacterial outer membrane was lost once only in the history of life, when Posibacteria arose about 2800 My ago after their ancestors diverged from Cyanobacteria.

Cepeda-Perez, E., et al. (2016). "SERS and integrative imaging upon internalization of quantum dots into human oral epithelial cells." J Biophotonics **9**(7): 683-693.

CdTe quantum dots (QDs) are widely used in bio-applications due to their size and highly efficient optical properties. However internalization mechanisms thereof for the variety of freshly extracted, not cultivated human cells and their specific molecular interactions remains an open topic for discussion. In this study, we assess the internalization mechanism of CdTe quantum dots (3.3 nm) capped with thioglycolic acid using non cultivated oral epithelial cells obtained from healthy donors. Naked gold nanoparticles (40 nm) were successfully used as nanosensors for surface-enhanced Raman spectroscopy to efficiently identify characteristic Raman peaks, providing new evidence indicating that the first interactions of these QDs with epithelial cells occurred preferentially with aromatic rings and amine groups of amino acid residues and glycans from trans-membrane proteins and cytoskeleton. Using an integrative combination of advanced imaging techniques, including ultra-high resolution SEM, high resolution STEM coupled with EDX spectroscopy together with the results obtained by Raman spectroscopy, it was determined that thioglycolic acid capped CdTe QDs are efficiently internalized into freshly extracted oral epithelial cells only by facilitated diffusion, distributed into cytoplasm and even within the cell nucleus in three minutes.

Chall, S., et al. (2014). "An efficient, Schiff-base derivative for selective fluorescence sensing of Zn(2)(+) ions: quantum chemical calculation appended by real sample application and cell imaging study." Org Biomol Chem **12**(33): 6447-6456.

Since zinc ions (Zn(2+)) are involved in numerous biological phenomena and go through subsequent interactions with zinc-binding proteins, we have attempted a sensitive fluorescence based detection of this second most abundant metal ion using an engineered and synthesized Schiff-base ligand, namely 2,4-bis((Z)-2-(1-(pyridin-2-yl)ethylidene)hydrazinyl)pyrimidine (PyHP). The ligand exhibits a zinc-induced fluorescence response when investigated in a MeOH-buffer (10 mM HEPES, pH = 7) (4 : 1) solvent mixture. The presence of zinc ions (lambda(ex) = 410 nm, quantum yield, varphi = 0.20) causes approximately 45 fold fluorescence enhancement at 489 nm. Formation of the metal-ligand complex was ascertained by (1)H NMR and mass spectra analysis. 1 : 1 binding affinity was ascertained according to Job's plot. Apart from this, theoretical interpretation of the experimental outcome was also obtained by applying density functional theory (DFT) to the PyHP-Zn(2+) complex formation. The practical applicability of the ligand has been tested in bacterial cells as well as in mammalian cell imaging and also by measuring and comparing the amount of Zn(2+) in some real samples such as liquid milk, tomato juice, banana stem juice and commercial fruit juice.

Chan, Y. Y., et al. (2010). "Anti-inflammatory principles from the stem and root barks of Citrus medica." Chem Pharm Bull (Tokyo) **58**(1): 61-65.

Bioassay-guided investigation of the anti-inflammatory principles from the stem and root barks of Citrus medica L. var. sarcodactylis SWINGLE has led to the isolation of a new coumarin, namely citrumedin-B (1) and thirty known compounds. The anti-inflammatory components were xanthyletin (2), nordentatin (3), atalantoflavon (4) and lonchocarpol A (5) which displayed potent nitric oxide (NO)-reducing activity in microglial cells. The structure of this new compound was completely elucidated using a combination of 2D NMR techniques (correlation spectroscopy (COSY), nuclear Overhauser effect spectroscopy (NOESY), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC)) and HR-electrospray ionization (ESI)-MS analyses. The known compounds were identified by comparison of their spectroscopic and physical data with those reported in the literature. These results can be inferred from the treatment of allergic response and inflammatory properties of Citrus medica L. var. sarcodactylis SWINGLE in traditional Chinese medicine.

Chang, J. C., et al. (2008). "The use of peptide-delivery to protect human adipose-derived adult stem cells from damage caused by the internalization of quantum dots." Biomaterials **29**(7): 925-936.

Label of human bone mesenchymal stem cells with CdSe/ZnS quantum dots (QDs) had been demonstrated to impair cell functions and activities. In the present study, QDs delivered by two different routes, Pep-1-labeled QDs (LQ) and PolyFect transfected QDs (TQ), were utilized to assess the effects of delivery mechanisms on various cellular responses of the QDs-internalized human adipose-derived adult stem cells (hADAS). Examination of labeled cells by flow cytometry and laser scanning confocal microscopy showed that LQ had higher fluorescence intensity due to the cluster formation and their distribution in cytoplasma while TQ were preferentially accumulated at peri-nuclear regions. The fluorescence intensity of the LQ group was still higher than that of the TQ group at 28 days after labeling, though cellular LQ were partitioned after initial cell division. Pep-1 but not PolyFect delivery facilitated QDs to escape from lysosome degradation. Pep-1 delivery of QDs rescued the cells from the negative effects caused by the

internalized QDs on cell proliferation and on the expressions of CD29 and CD105 as well as osteogenic and chondrogenic-associated lineage markers. The same effect was also observed in the expression of alkaline phosphatase activity, calcium deposition and secretion of chondrogenic matrices (GAG and collagen type II) in micromass culture. These indicated that Pep-1-delivered QDs may serve appropriately to track the hADAS employed in cell therapy/tissue engineering applications. The results also suggested that the endo-/lysosome degradation of QDs may depend on different surface coatings and critically influence the differentiation of hADAS.

Chazelas, P., et al. (2021). "Oxidative Stress Evaluation in Ischemia Reperfusion Models: Characteristics, Limits and Perspectives." Int J Mol Sci **22**(5).

Ischemia reperfusion injury is a complex process consisting of a seemingly chaotic but actually organized and compartmentalized shutdown of cell function, of which oxidative stress is a key component. Studying oxidative stress, which results in an imbalance between reactive oxygen species (ROS) production and antioxidant defense activity, is a multi-faceted issue, particularly considering the double function of ROS, assuming roles as physiological intracellular signals and as mediators of cellular component damage. Herein, we propose a comprehensive overview of the tools available to explore oxidative stress, particularly in the study of ischemia reperfusion. Applying chemistry as well as biology, we present the different models currently developed to study oxidative stress, spanning the vitro and the silico, discussing the advantages and the drawbacks of each set-up, including the issues relating to the use of in vitro hypoxia as a surrogate for ischemia. Having identified the limitations of historical models, we shall study new paradigms, including the use of stem cell-derived organoids, as a bridge between the in vitro and the in vivo comprising 3D intercellular interactions in vivo and versatile pathway investigations in vitro. We shall conclude this review by distancing ourselves from "wet" biology and reviewing the in silico, computer-based, mathematical modeling, and numerical simulation options: (a) molecular modeling with quantum chemistry and molecular dynamic algorithms, which facilitates the study of molecule-to-molecule interactions, and the integration of a compound in a dynamic environment (the plasma membrane...); (b) integrative systemic models, which can include many facets of complex mechanisms such as oxidative stress or ischemia reperfusion and help to formulate integrated predictions and to enhance understanding of dynamic interaction between pathways.

Chen, A. A., et al. (2010). "Multiplexed, high-throughput analysis of 3D microtissue suspensions." Integr Biol (Camb) **2**(10): 517-527.

Three-dimensional (3D) tissue models have significantly improved our understanding of structure/function relationships and promise to lead to new advances in regenerative medicine. However, despite the expanding diversity of 3D tissue fabrication methods, approaches for functional assessment have been relatively limited. Here, we describe the fabrication of microtissue (mu-tissue) suspensions and their quantitative evaluation with techniques capable of analyzing large sample numbers and performing multiplexed parallel analysis. We applied this platform to 3D mu-tissues representing multiple stages of liver development and disease including: embryonic stem cells, bipotential hepatic progenitors, mature hepatocytes, and hepatoma cells photoencapsulated in polyethylene glycol hydrogels. Multiparametric mu-tissue cytometry enabled quantitation of fluorescent reporter expression within populations of intact mu-tissues (n>/= 10(2)-10(3)) and sorting-based enrichment of subsets for subsequent studies. Further, 3D mu-tissues could be implanted in vivo, respond to systemic stimuli, retrieved and quantitatively assessed. In order to facilitate multiplexed 'pooled' experimentation, fluorescent labeling strategies were developed and utilized to investigate the impact of mu-tissue composition and exposure to soluble factors. In particular, examination of drug/gene interactions on collections of 3D hepatoma mu-tissues indicated synergistic influence of doxorubicin and siRNA knockdown of the anti-apoptotic gene BCL-XL. Collectively, these studies highlight the broad utility of mu-tissue suspensions as an enabling approach for high n, populational analysis of 3D tissue biology in vitro and in vivo.

Chen, B., et al. (2021). "Comparison of Ferroptosis-Inhibitory Mechanisms between Ferrostatin-1 and Dietary Stilbenes (Piceatannol and Astringin)." Molecules **26**(4).

Synthetic arylamines and dietary phytophenolics could inhibit ferroptosis, a recently discovered regulated cell death process. However, no study indicates whether their inhibitory mechanisms are inherently different. Herein, the ferroptosis-inhibitory mechanisms of selected ferrostatin-1 (Fer-1) and two dietary stilbenes (piceatannol and astringin) were compared. Cellular assays suggested that the ferroptosis-inhibitory and electron-transfer potential levels decreased as follows: Fer-1 >> piceatannol > astringin; however, the hydrogen-donating potential had an order different from that observed by the antioxidant experiments and quantum chemistry calculations. Quantum calculations suggested that Fer-1 has a much

lower ionization potential than the two stilbenes, and the aromatic N-atoms were surrounded by the largest electron clouds. By comparison, the C4'O-H groups in the two stilbenes exhibited the lowest bond disassociation enthalpies. Finally, the three were found to produce corresponding dimer peaks through ultra-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry analysis. In conclusion, Fer-1 mainly depends on the electron transfer of aromatic N-atoms to construct a redox recycle. However, piceatannol and astringin preferentially donate hydrogen atoms at the 4'-OH position to mediate the conventional antioxidant mechanism that inhibits ferroptosis, and to ultimately form dimers. These results suggest that dietary phytophenols may be safer ferroptosis inhibitors for balancing normal and ferroptotic cells than arylamines with high electron-transfer potential.

Chen, C., et al. (2014). "[Enrichment and functional characterization of cancer stem cells from squamous cell carcinoma of head and neck by suspension culture]." Zhonghua Zhong Liu Za Zhi **36**(1): 17-22.

OBJECTIVE: Initiation, growth, recurrence, and metastasis of head and neck squamous cell carcinoma (HNSCC) have been related to the cancer stem cells (CSC) that can be identified by their aldehyde-dehydrogenase-isoform-1 (ALDH-1) activity. In this study, we try to prove that suspension culture can enrich ALDH-1 high expression cells within HNSCC cell lines and the enriched cells possess cancer stem cell properties. METHODS: Cells from five HNSCC cell lines were cultured in ultra-low attachment plates in serum-free Quantum 263 medium supplemented with 10 ng/ml EGF and 10 ng/ml bFGF, and ALDH-1 expression level was evaluated by ALDEFLUOR assay. ALDH-1 high expression cells were separated by FACS sorting, and their phenotypical and functional properties were characterized. RESULTS: Spheroids can be formed from all five HNSCC cell lines (UD-SCC1, UT-SCC22, UM-SCC11B, UT-SCC9 and UT-SCC24A) under anchorage independent culture condition. The proportion of ALDH1 high expression cells were highly increased in speroids derived cells (SDCs) compared with their monolayers ($P < 0.05$). The clones formed by ALDH1 high expression cells on average contained 197 (197 +/- 47) cells compared with 33 (33 +/- 16) cells in clones generated from ALDH1 low expression cells ($P < 0.01$). Single ALDH1 high expression cell could significantly better regenerate a spheroid (UT-SCC9: 17.1%, UD-SCC1:19.3%), whereas under the same conditions single ALDH1 low expression cells regenerated only in one case a spheroid ($P < 0.01$). SDCs from all five tested cell lines also showed a significantly increased invasion capacity ($P < 0.05$). We also found that the mRNA levels of Oct-4, Sox2, and Nanog were all significantly increased in the SDC. The reactive oxygen species (ROS) levels in SDCs from UD-SCC1 and UT-SCC24A were significantly increased compared with their monolayer counterpart [(26.3 +/- 4.9)% vs (8.6 +/- 1.7)% and (72.1 +/- 6.1)% vs (23.7 +/- 7.5)%, $P < 0.05$)]. CONCLUSION: Cancer stem cells can be enriched by suspension culture, which may be of importance in investigation of their contribution to therapy resistance, tumor recurrence and metastasis.

Chen, D., et al. (2017). "Bright Polymer Dots Tracking Stem Cell Engraftment and Migration to Injured Mouse Liver." Theranostics **7**(7): 1820-1834.

Stem cell therapy holds promise for treatment of intractable diseases and injured organs. For clinical translation, it is pivotal to understand the homing, engraftment, and differentiation processes of stem cells in a living body. Here we report near-infrared (NIR) fluorescent semiconductor polymer dots (Pdots) for bright labeling and tracking of human mesenchymal stem cells (MSCs). The Pdots exhibit narrow-band emission at 775 nm with a quantum yield of 22%, among the highest value for various NIR probes. The Pdots together with a cell penetrating peptide are able to track stem cells over two weeks without disturbing their multipotent properties, as confirmed by the analyses on cell proliferation, differentiation, stem-cell markers, and immunophenotyping. The in vivo cell tracking was demonstrated in a liver-resection mouse model, which indicated that the Pdot-labeled MSCs after tail-vein transplantation were initially trapped in lung, gradually migrated to the injured liver, and then proliferated into cell clusters. Liver-function analysis and histological examination revealed that the inflammation induced by liver resection was apparently decreased after stem cell transplantation. With the bright labeling, superior biocompatibility, and long-term tracking performance, the Pdot probes are promising for stem cell research and regenerative medicine.

Chen, D., et al. (2018). "Purification of Semiconducting Polymer Dots by Size Exclusion Chromatography Prior to Cytotoxicity Assay and Stem Cell Labeling." Anal Chem **90**(9): 5569-5575.

Semiconducting polymer dots (Pdots) as fluorescent probes have shown promising applications because of their excellent optical properties. However, apparent differences were observed in cytotoxicity assays, which might originate from impurities introduced in polymer synthesis or nanoparticle preparation. A simple gel-filtration-based purification method was used to address this issue. Purified Pdots displayed obviously decreased cytotoxicity as compared with the same batch of unpurified Pdots. The purified Pdots were further examined in a cytotoxicity study on mesenchymal stem cells (MSCs), which are very

sensitive to exogenous probes. The results indicated that purified Pdots did not affect the proliferation ability of MSCs, while unpurified Pdots could have obvious cytotoxicity. In addition, the purified Pdots did not show cytotoxicity even after 6 months of storage. Our results demonstrated that gel filtration is an effective method for obtaining Pdots with minimal cytotoxicity, which are more suitable for biological applications.

Chen, F., et al. (2019). "Silicon Carbide Nanoparticles as a Photoacoustic and Photoluminescent Dual-Imaging Contrast Agent for Long-Term Cell Tracking." Nanoscale Adv **1**(9): 3514-3520.

Silicon carbide nanoparticles (SiCNPs) are durable, physically resilient, chemically inert, and biocompatible. Silicon carbide particles smaller than 10 nm show photoluminescence due to quantum confinement effects and have been reported in imaging different cell lines. To further explore the potential of silicon carbide nanomaterials in cell imaging, we studied the photoluminescence and photoacoustic properties of three SiCNPs of approximately 30, 80, and 620 nm. All these SiCNPs show photoluminescence and photoacoustic signals; and the 620 nm silicon carbide nanoparticles (SiCNP620) show the highest photoluminescence and photoacoustic intensity. The SiCNP620 are biocompatible with good cell labeling capacity. They could image mesenchymal stem cells in vitro for more than 20 days via photoluminescence even when the cells were differentiated into adipocytes and osteocytes. The same SiCNP620 could also produce photoacoustic signals and track stem cells in vivo for over 14 days.

Chen, G., et al. (2018). "Revealing the Fate of Transplanted Stem Cells In Vivo with a Novel Optical Imaging Strategy." Small **14**(3).

Stem-cell-based regenerative medicine holds great promise in clinical practices. However, the fate of stem cells after transplantation, including the distribution, viability, and the cell clearance, is not fully understood, which is critical to understand the process and the underlying mechanism of regeneration for better therapeutic effects. Herein, we develop a dual-labeling strategy to in situ visualize the fate of transplanted stem cells in vivo by combining the exogenous near-infrared fluorescence imaging in the second window (NIR-II) and endogenous red bioluminescence imaging (BLI). The NIR-II fluorescence of Ag2 S quantum dots is employed to dynamically monitor the trafficking and distribution of all transplanted stem cells in vivo due to its deep tissue penetration and high spatiotemporal resolution, while BLI of red-emitting firefly luciferase (RfLuc) identifies the living stem cells after transplantation in vivo because only the living stem cells express RfLuc. This facile strategy allows for in situ visualization of the dynamic trafficking of stem cells in vivo and the quantitative evaluation of cell translocation and viability with high temporal and spatial resolution, and thus reports the fate of transplanted stem cells and how the living stem cells help, regeneration, for an instance, of a mouse with acute liver failure.

Chen, G., et al. (2015). "In vivo real-time visualization of mesenchymal stem cells tropism for cutaneous regeneration using NIR-II fluorescence imaging." Biomaterials **53**: 265-273.

Mesenchymal stem cells (MSCs) have shown great potential for cutaneous wound regeneration in clinical practice. However, the in vivo homing behavior of intravenously transplanted MSCs to the wounds is still poorly understood. In this work, fluorescence imaging with Ag2S quantum dots (QDs) in the second near-infrared (NIR-II) window was performed to visualize the dynamic homing behavior of transplanted human mesenchymal stem cells (hMSCs) to a cutaneous wound in mice. Benefiting from the desirable spatial and temporal resolution of Ag2S QDs-based NIR-II imaging, for the first time, the migration of hMSCs to the wound was dynamically visualized in vivo. By transplanting a blank collagen scaffold in the wound to help the healing, it was found that hMSCs were slowly recruited at the wound after intravenous injection and were predominantly accumulated around the edge of wound. This resulted in poor healing effects in terms of slow wound closure and thin thickness of the regenerated skin. In contrast, for the wound treated by the collagen scaffold loaded with stromal cell derived factor-1alpha (SDF-1alpha), more hMSCs were recruited at the wound within a much shorter time and were homogenously distributed across the whole wound area, which enhances the re-epithelialization, the neovascularization, and accelerates the wound healing.

Chen, G., et al. (2018). "Recent Advances in Tracking the Transplanted Stem Cells Using Near-Infrared Fluorescent Nanoprobes: Turning from the First to the Second Near-Infrared Window." Adv Healthc Mater **7**(20): e1800497.

Stem cell-based regenerative medicine has attracted tremendous attention for its great potential to treat numerous incurable diseases. Tracking and understanding the fate and regenerative capabilities of transplanted stem cells is vital for improving the safety and therapeutic efficacy of stem cell-based therapy, therefore accelerating the clinical application of stem cells. Fluorescent nanoparticles (NPs) have been widely used for in vivo tracking of the transplanted stem cells. Among these fluorescent NPs, near-infrared (NIR) NPs have greatly improved the sensitivity, tissue penetration depth, spatial and temporal resolutions of the fluorescence imaging-based stem cell tracking

technologies due to the reduced absorption, scattering, and autofluorescence of NIR fluorescence in tissues. Here, this review summarizes the recent studies regarding the tracking of transplanted stem cells using NIR NPs and emphasizes the recent advances of fluorescence imaging in the second NIR window (NIR-II, 1000-1700 nm). Furthermore, the challenges and future prospects of the NIR NP-based technologies are also discussed.

Chen, H., et al. (2007). "Altered membrane dynamics of quantum dot-conjugated integrins during osteogenic differentiation of human bone marrow derived progenitor cells." Biophys J **92**(4): 1399-1408.

Functionalized quantum dots offer several advantages for tracking the motion of individual molecules on the cell surface, including selective binding, precise optical identification of cell surface molecules, and detailed examination of the molecular motion without photobleaching. We have used quantum dots conjugated with integrin antibodies and performed studies to quantitatively demonstrate changes in the integrin dynamics during osteogenic differentiation of human bone marrow derived progenitor cells (BMPCs). Consistent with the unusually strong BMPC adhesion previously observed, integrins on the surface of undifferentiated BMPC were found in clusters and the lateral diffusion was slow (e.g., approximately 10(-11) cm2/s). At times as early as those after a 3-day incubation in the osteogenic differentiation media, the integrin diffusion coefficients increased by an order of magnitude, and the integrin dynamics became indistinguishable from that measured on the surface of terminally differentiated human osteoblasts. Furthermore, microfilaments in BMPCs consisted of atypically thick bundles of stress fibers that were responsible for restricting the integrin lateral mobility. Studies using laser optical tweezers showed that, unlike fully differentiated osteoblasts, the BMPC cytoskeleton is weakly associated with its cell membrane. Based on these findings, it appears likely that the altered integrin dynamics is correlated with BMPC differentiation and that the integrin lateral mobility is restricted by direct links to microfilaments.

Chen, H., et al. (2016). "Application prospective of nanoprobes with MRI and FI dual-modality imaging on breast cancer stem cells in tumor." J Nanobiotechnology **14**(1): 52.

Breast cancer (BC) is a serious disease to threat lives of women. Numerous studies have proved that BC originates from cancer stem cells (CSCs). But at present, no one approach can quickly and simply identify breast cancer stem cells (BCSCs) in solid tumor. Nanotechnology is probably able to realize this goal. But in study process, scientists find it seems that nanomaterials with one modality, such as magnetic resonance imaging (MRI) or fluorescence imaging (FI), have their own advantages and drawbacks. They cannot meet practical requirements in clinic. The nanoprobe combined MRI with FI modality is a promising tool to accurately detect desired cells with low amount in tissue. In this work, we briefly describe the MRI and FI development history, analyze advantages and disadvantages of nanomaterials with single modality in cancer cell detection. Then the application development of nanomaterials with dual-modality in cancer field is discussed. Finally, the obstacles and prospective of dual-modal nanoparticles in detection field of BCSCs are also pointed out in order to speed up clinical applications of nanoprobes.

Chen, J., et al. (2010). "Quantum dot labeling based on near-field optical imaging of CD44 molecules." Micron **41**(3): 198-202.

The lateral organization of membrane proteins and lipids domains has a direct impact on many cellular processes, but generally these domains are too small to be resolved by diffraction-limited resolution of fluorescence microscopy. Here, we use quantum dot (QD) labeling based on near-field optical imaging, to study the nanoscale organization of hyaluronan receptor CD44 molecules of fixed mesenchymal stem cells (MSCs) in air, with a optical resolution down to 50 nm. The photostability and high luminance of QD evidently improve the signal-to-noise ratio and reproducibility of near-field optical data. Importantly, the blinking-intensity analysis was proposed to identify single QD, providing a calibration to relate intensity to numbers of antibody for the first time. Additionally, the fluorescence-topographic imaging enables us to investigate the topographic location pattern. Our results demonstrate that CD44 molecules on MSCs are enriched into nanosized domain and they predominantly locate on the peak of the membrane protrusions, which may contribute to clarify the underlying mechanism of functions ascribed to these molecules.

Chen, J., et al. (2017). "Porphyra polysaccharide-derived carbon dots for non-viral co-delivery of different gene combinations and neuronal differentiation of ectodermal mesenchymal stem cells." Nanoscale **9**(30): 10820-10831.

In this study, multifunctional fluorescent carbon dots (CDs) were synthesized using a one-pot hydrothermal carbonization reaction, with the naturally-occurring porphyra polysaccharide (PPS) serving as a single carbon source for the first time and ethylenediamine (Ed) acting as the surface passivation agent. The resulting CDs enjoyed a high quantum yield (56.3%), excitation-dependent fluorescence, small size (<10 nm), spherical shape, uniform distribution, positive

surface charge, low cytotoxicity and excellent ability to condense macromolecular plasmid DNA. The synthesized CDs were employed for neuronal induction from ectodermal mesenchymal stem cells for the first time via highly efficient non-viral gene delivery. The optimal combination of factors (Ascl1 and Brn2) was selected from seven different combinations out of Ascl1, Brn2 and Sox2 according to the expression of neuronal markers (Tuj1, Map2 and Tau). The results of qRT-PCR demonstrated that the CDs possessed a significantly higher transfection efficiency than the commercially available transfection reagents PEI (25 kDa) and Lipofectamine2000. Moreover, the CDs/pDNA nanoparticles exhibited more efficient neuronal differentiation of the EMSCs than the AT-RA-containing induction medium. Furthermore, the CDs/pDNA nanoparticles could enter cells via both caveolae- and clathrin-mediated endocytosis. Taken together, the natural polysaccharide PPS-derived CDs enriched the current application of CDs by employing the CDs as a novel non-viral gene carrier for neuronal differentiation of adult stem cells, which held great promise in tissue engineering and bioimaging.

Chen, J., et al. (2021). "Synthesis of biocompatible and highly fluorescent N-doped silicon quantum dots from wheat straw and ionic liquids for heavy metal detection and cell imaging." Sci Total Environ **765**: 142754.

Silane-based precursors for the synthesis of water-dispersible silicon quantum dots (SiQDs) present harmful effects on both researchers and the environment, due to their high toxicity. Though waste wheat straw is an abundant source of natural silicon, its application towards the synthesis of biocompatible SiQDs for metal detection has not yet been explored. In this study, N-doped SiQDs demonstrating uniform spherical morphologies, excellent water dispersity and strong fluorescence emission with a quantum yield of 28.9% were facilely synthesized by using wheat straw (WS) as silicon source and allyl-3-methylimidazolium chloride (AMIMCl) as nitrogen source. The wheat straw based SiQDs (WS-SiQDs) showed linear fluorescence quenching ((F0-F)/F) with Cr(VI) and Fe(III) concentration in the range of 0-6 x 10(-4) M. Following immobilization on hydrophilic silica hydrogels, WS-SiQDs@silica hydrogels demonstrated enhanced fluorescence emission which can selectively detect Cr(VI) and Fe (III) to the limits of 142 and 175 nM, respectively. Moreover, cell imaging results reflected that WS-SiQDs can penetrate the membranes of dental pulp stem cells and react with the nucleuses of the stem cells. The stem cells maintained high viability under the conditions of 24 h incubation and SiQD concentration below 50 mg.L(-1), thus indicating low cytotoxicity of WS-SiQDs. The as-prepared SiQDs demonstrated notable structural and fluorescent properties, therefore representing promising biocompatible fluorescent nanomaterials for metal detection and cell imaging.

Chen, X., et al. (2016). "Grafted c-kit(+)/SSEA1(-) eye-wall progenitor cells delay retinal degeneration in mice by regulating neural plasticity and forming new graft-to-host synapses." Stem Cell Res Ther **7**(1): 191.

BACKGROUND: Despite diverse pathogenesis, the common pathological change observed in age-related macular degeneration and in most hereditary retinal degeneration (RD) diseases is photoreceptor loss. Photoreceptor replacement by cell transplantation may be a feasible treatment for RD. The major obstacles to clinical translation of stem cell-based cell therapy in RD remain the difficulty of obtaining sufficient quantities of appropriate and safe donor cells and the poor integration of grafted stem cell-derived photoreceptors into the remaining retinal circuitry. METHODS: Eye-wall c-kit(+)/stage-specific embryonic antigen 1 (SSEA1)(-) cells were isolated via fluorescence-activated cell sorting, and their self-renewal and differentiation potential were detected by immunochemistry and flow cytometry in vitro. After labeling with quantum nanocrystal dots and transplantation into the subretinal space of rd1 RD mice, differentiation and synapse formation by daughter cells of the eye-wall c-kit(+)/SSEA1(-) cells were evaluated by immunochemistry and western blotting. Morphological changes of the inner retina of rd1 mice after cell transplantation were demonstrated by immunochemistry. Retinal function of rd1 mice that received cell grafts was tested via flash electroretinograms and the light/dark transition test. RESULTS: Eye-wall c-kit(+)/SSEA1(-) cells were self-renewing and clonogenic, and they retained their proliferative potential through more than 20 passages. Additionally, eye-wall c-kit(+)/SSEA1(-) cells were capable of differentiating into multiple retinal cell types including photoreceptors, bipolar cells, horizontal cells, amacrine cells, Muller cells, and retinal pigment epithelium cells and of transdifferentiating into smooth muscle cells and endothelial cells in vitro. The levels of synaptophysin and postsynaptic density-95 in the retinas of eye-wall c-kit(+)/SSEA1(-) cell-transplanted rd1 mice were significantly increased at 4 weeks post transplantation. The c-kit(+)/SSEA1(-) cells were capable of differentiating into functional photoreceptors that formed new synaptic connections with recipient retinas in rd1 mice. Transplantation also partially corrected the abnormalities of inner retina of rd1 mice. At 4 and 8 weeks post transplantation, the rd1 mice that received c-kit(+)/SSEA1(-) cells showed significant increases in a-wave and b-wave amplitude and the percentage of time spent in the dark area. CONCLUSIONS: Grafted c-kit(+)/SSEA1(-) cells

restored the retinal function of rd1 mice via regulating neural plasticity and forming new graft-to-host synapses.

Chen, Y. (2012). "Quantum dots for labeling live cells." Methods Mol Biol **906**: 193-198.

Quantum dots (QDs) are semiconductor nanocrystals with unique photo-physical properties. QDs are brightly fluorescent and photostable, and therefore represent a novel class of fluorescence probes for bio-imaging and cell monitoring. There are different techniques for labeling cells with QDs. Moreover, there is evidence that optical properties of the QD can be changed as a result of interaction with live cells. This manuscript describes a simple method for labeling of rat endothelial progenitor cells using ODs and for characterization of their optical properties.

Chen, Y. Y., et al. (2021). "Cytotoxicity and cell imaging of six types of carbon nanodots prepared through carbonization and hydrothermal processing of natural plant materials." RSC Adv **11**(27): 16661-16674.

In this study we prepared six types of carbon nanodots (CNDs) from natural plant materials - through carbonization of two species of bamboo (Bamboo-I, Bamboo-II) and one type of wood (Wood), and through hydrothermal processing of the stem and root of the herb Mahonia oiwakensis Hayata (MO) and of the agricultural waste of two species of pineapple root (PA, PB). The resulting CNDs were spherical with dimensions on the nanoscale (3-7 nm); furthermore, CND-Bamboo I, CND-Wood, CND-Bamboo II, CND-MO, CND-PA, and CND-PB displayed fluorescence quantum yields of 9.63, 12.34, 0.90, 10.86, 0.35, and 0.71%, respectively. X-ray diffraction revealed that the carbon nanostructures possessed somewhat ordered and disordered lattices, as evidenced by broad signals at values of 2theta between 20 and 30 degrees . CND-Bamboo I, CND-Wood, and CND-Bamboo II were obtained in yields of 2-3%; CND-MO, CND-PA, and CND-PB were obtained in yields of 17.64, 9.36, and 22.47%, respectively. Cytotoxicity assays for mouse macrophage RAW264.7 cells treated with the six types of CNDs and a commercial sample of Ag nanoparticles (NPs) revealed that each of our CNDs provided a cell viability of 90% at 2000 mug mL(-1), whereas it was only 20% after treatment with the Ag NPs at 62.5 mug mL(-1). The six types of CNDs also displayed low cytotoxicity toward human keratinocyte HacaT cells, human MCF-7 breast cancer cells, and HT-29 colon adenocarcinoma cells when treated at 500 mug mL(-1). Moreover, confocal microscopic cell imaging revealed that the fluorescent CND-Bamboo I particles were located on the MCF-7 cell membrane and inside the cells after treatment for 6 and 24 h, respectively. We have thoroughly investigated the photoluminescence properties and carbon nanostructures of these highly dispersed CNDs. Because of the facile green synthesis of these six types of CNDs and their sourcing from abundant natural plants, herbs, and agriculture waste, these materials provide a cost-effective method, with low cytotoxicity and stable fluorescence, for biolabeling and for developing cell nanocarriers.

Cheng, A. K., et al. (2009). "Aptamer-based detection of epithelial tumor marker mucin 1 with quantum dot-based fluorescence readout." Anal Chem **81**(15): 6130-6139.

Mucin 1 (MUC1) is a glycoprotein expressed on most epithelial cell surfaces, which has been confirmed as a useful biomarker for the diagnosis of early cancers. In this paper, we report an aptamer-based, quantitative detection protocol for MUC1 using a 3-component DNA hybridization system with quantum dot (QD)-labeling: in the absence of MUC1 peptides, strong fluorescence is observed upon mixing the three specially designed DNA strands (quencher, QD-labeled reporter, and the MUC1 aptamer stem); in the presence of MUC1 peptides, a successive decrease in fluorescence intensity is detected since the MUC1 peptide binds to the aptamer strand in such a way to allow the quencher and fluorescence reporter to be brought into close proximity (which leads to the occurrence of fluorescence resonance energy transfer, FRET, between the quencher and QD). The detection limit for MUC1 with this novel approach is in the nanomolar (nM) level, and a linear response can be established for the approximate range found in blood serum. This study also provided further insight into the aptamer/analyte binding site/mode for MUC1, which augments the possibility of improving this detection methodology for the early diagnosis of different types of epithelial cancers of large populations.

Cheng, F., et al. (2009). "Quantum-dot-based technology for sensitive and stable detection of prostate stem cell antigen expression in human transitional cell carcinoma." Int J Biol Markers **24**(4): 271-276.

Quantum dots (QDs) as a biological labeling material for medical applications need to be evaluated for the sensitivity and stability of their fluorescence. Comparison of QD-based immunolabeling and commonly used immunohistochemical staining in detecting the expression of prostate stem cell antigen (PSCA) in bladder tumor tissues revealed that the two methods had similar sensitivity in the differential display of PSCA expression correlated with tumor stage and grade (kappa=0.92, $p<0.001$). In addition, the intensity of QD fluorescence remained stable for at least 10 days after conjugation to the PSCA protein and nearly 96% of the positive expression in samples lasted for one month.

Chernov, A. S., et al. (2018). "Influence of wideband visible light with an padding red component on the functional state of mice embryos and embryonic stem cells." J Photochem Photobiol B **188**: 77-86.

It is known that visible light, including sunlight and laboratory lighting, adversely affect the development of embryos in vitro. In with article we present a technology for the synthesis of composite screens, capable to photoconvert UV and a part of the blue spectrum into red light with the maximum ~630nm. It is established that the application of such transformed light with an evident red component raises the chances of embryos to survive and protects embryonic stem cells. To create photoconversion screens, the CdZn/Se quantum dots were obtained, the average size being about 7nm. When the quantum dots are excited by electromagnetic waves of the UV and blue spectral range, photoluminescence is observed. The average photon energy for photoluminescence is of the order of 2eV. On the basis of CdZn/Se quantum dots and methylphenylsiloxane polymer, light-transforming composite screens were made. In case of the light-transforming composite screen, the UV component disappeared from the energy spectrum, and the intensity of the blue region of the spectrum was reduced. On the contrary, in the red region (lambdamax=630nm) one can see a little more than two-fold increase of intensity. It is shown that when exposed to 2-cell embryos by transformed light, the proportion of normally developing embryos increases by 20%, the number of dead embryos decreases twice, and number of dead and apoptotic cells was lower in blastocysts, what's decreased by 70%, as compared to the control group. When blastocysts are transferred to the feeder substrate, colonies of embryonic stem cells are formed. Cells obtained from blastocysts irradiated with transformed visible light are in a normal state in 90% of cases and did not change expression levels, biochemistry and morphology for at least 20 passages. It is assumed that the data obtained can be used for the design of systems of efficient cultivation of embryonic cells for tissue engineering and cell therapy.

Chetty, S. S., et al. (2019). "Noninvasive Tracking and Regenerative Capabilities of Transplanted Human Umbilical Cord-Derived Mesenchymal Stem Cells Labeled with I-III-IV Semiconducting Nanocrystals in Liver-Injured Living Mice." ACS Appl Mater Interfaces **11**(9): 8763-8778.

Acute liver injury is a critical syndrome ascribed to prevalent death of hepatocytes and imperatively requires liver transplantation. Such a methodology is certainly hampered due to the deficit of healthy donors. In this regard, stem cell-based regenerative therapies are attractive in repairing injured tissues and organs for medical applications. However, it is crucial to understand the migration, engraftment, and regeneration capabilities of transplanted stem cells in the living animal models. For the first time, we demonstrate rapid labeling of umbilical cord-derived mesenchymal stem cells (MSCs) with near-infrared (NIR)-fluorescent CuInS2-ZnS nanocrystals (CIZS-NCs) to develop innovative nanobioconjugates (MSCs-CIZS-NBCs) that exhibit 98% labeling efficiency. Before nanobioconjugate synthesis, the pristine CIZS-NCs were prepared via a two-step, hot-injection, rapid and low-cost domestic-microwave-refluxing (MW-R) method within 6 min. The as-synthesized CIZS-NCs display high photoluminescence quantum yield (approximately 88%) and long-lived lifetime (23.4 mus). In contrast to unlabeled MSCs, the MSCs-CIZS nanobioconjugates show excellent biocompatibility without affecting the stemness, as confirmed by cell viability, immunophenotyping (CD44(+), CD105(+), CD90(+)), multi-lineage-specific gene expressions, and differentiation into adipocytes, osteocytes, and chondrocytes. The in vivo fluorescence tracking analyses revealed that the MSCs-CIZS-NBCs after tail-vein injection were initially trapped in the lungs and gradually engrafted in the injured liver within 2 h. The regeneration potential of MSCs-CIZS-NBCs was confirmed via renewal of the portal tract composed of portal veins, bile ducts, and hepatic arteries around the hepatocytes. Consequently, no apparent inflammations, necrosis, or apoptosis was observed in the acetaminophen (APAP)-induced liver-injured BALB/c mice model over 3 days after transplantation, as corroborated using laser-scanning confocal microscopy and histopathological and hematological analyses. Hence, our innovative NIR-fluorescent MSCs-CIZS-NBCs offer an off-the-self technology for noninvasive tracking of transplanted MSCs in an acute-liver-injured animal model for future image-guided cell-therapies.

Chetty, S. S., et al. (2020). "Human Umbilical Cord Wharton's Jelly-Derived Mesenchymal Stem Cells Labeled with Mn(2+) and Gd(3+) Co-Doped CuInS2-ZnS Nanocrystals for Multimodality Imaging in a Tumor Mice Model." ACS Appl Mater Interfaces **12**(3): 3415-3429.

Mesenchymal stem cell (MSCs) therapy has recently received profound interest as a targeting platform in cancer theranostics because of inherent tumor-homing abilities. However, the terminal tracking of MSCs engraftment by fluorescent in situ hybridization, immuno-histochemistry, and flow-cytometry techniques to translate into clinics is still challenging because of a dearth of inherent MSCs-specific markers and FDA approval for genetic modifications of MSCs. To address this challenge, a cost-effective noninvasive imaging technology based on multifunctional nanocrystals (NCs) with enhanced

detection sensitivity, spatial-temporal resolution, and deep-tissue diagnosis is needed to be developed to track the transplanted stem cells. A hassle-free labeling of human umbilical cord Wharton's Jelly (WJ)-derived MSCs with Mn(2+) and Gd(3+) co-doped CuInS2-ZnS (CIS-ZMGS) NCs has been demonstrated in 2 h without requiring an electroporation process or transfection agents. It has been found that WJ-MSCs labeling did not affect their multilineage differentiation (adipocyte, osteocyte, chondrocyte), immuno-phenotypes (CD44(+), CD105(+), CD90(+)), protein (beta-actin, vimentin, CD73, alpha-SMCA), and gene expressions. Interestingly, CIS-ZMGS-NCs-labeled WJ-MSCs exhibit near-infrared (NIR) fluorescence with a quantum yield of 84%, radiant intensity of approximately 3.999 x 10(11) (p/s/cm(2)/sr)/(muW/cm(2)), magnetic relaxivity (longitudinal r1 = 2.26 mM(-1) s(-1), transverse r2 = 16.47 mM(-1) s(-1)), and X-ray attenuation (78 HU) potential for early noninvasive multimodality imaging of a subcutaneous melanoma in B16F10-tumor-bearing C57BL/6 mice in 6 h. The ex vivo imaging and inductively coupled plasma mass-spectroscopy analyses of excised organs along with confocal microscopy and immunofluorescence of tumor results also significantly confirmed the positive tropism of CIS-ZMGS-NCs-labeled WJ-MSCs in the tumor environment. Hence, we propose the magnetofluorescent CIS-ZMGS-NCs-labeled WJ-MSCs as a next-generation nanobioprobe of three commonly used imaging modalities for stem cell-assisted anticancer therapy and tracking tissue/organ regenerations.

Choi, A. H. and B. Ben-Nissan (2015). "Calcium phosphate nanocoatings and nanocomposites, part I: recent developments and advancements in tissue engineering and bioimaging." Nanomedicine (Lond) **10**(14): 2249-2261.

A number of materials have been applied as implant coatings and as tissue regeneration materials. Calcium phosphate holds a special consideration, due to its chemical similarity to human bone and, most importantly, its dissolution characteristics, which allow for bone growth and regeneration. The applications of molecular and nanoscale-based biological materials have been and will continue to play an ever increasing role in enhancing and improving the osseointegration of dental and orthopedic implants. More recently, extensive research efforts have been focused on the development and applications of fluorescent nanoparticles and nanocoatings for in vivo imaging and diagnostics as well as devising methods of adding luminescent or fluorescent capabilities to enhance the in vivo functionality of calcium phosphate-based biomedical materials.

Chu, P. P., et al. (2012). "Intercellular cytosolic transfer correlates with mesenchymal stromal cell rescue of umbilical cord blood cell viability during ex vivo expansion." Cytotherapy **14**(9): 1064-1079.

BACKGROUND AIMS: Mesenchymal stromal cells (MSC) have been observed to participate in tissue repair and to have growth-promoting effects on ex vivo co-culture with other stem cells. METHODS: In order to evaluate the mechanism of MSC support on ex vivo cultures, we performed co-culture of MSC with umbilical cord blood (UCB) mononuclear cells (MNC) (UCB-MNC). RESULTS: Significant enhancement in cell growth correlating with cell viability was noted with MSC co-culture (defined by double-negative staining for Annexin-V and 7-AAD; $P < 0.01$). This was associated with significant enhancement of mitochondrial membrane potential ($P < 0.01$). We postulated that intercellular transfer of cytosolic substances between MSC and UCB-MNC could be one mechanism mediating the support. Using MSC endogenously expressing green fluorescent protein (GFP) or labeled with quantum dots (QD), we performed co-culture of UCB-MNC with these MSC. Transfer of these GFP and QD was observed from MSC to UCB-MNC as early as 24 h post co-culture. Transwell experiments revealed that direct contact between MSC and UCB-MNC was necessary for both transfer and viability support. UCB-MNC tightly adherent to the MSC layer exhibited the most optimal transfer and rescue of cell viability. DNA analysis of the viable, GFP transfer-positive UCB-MNC ruled out MSC transdifferentiation or MSC-UCB fusion. In addition, there was statistical correlation between higher levels of cytosolic transfer and enhanced UCB-MNC viability ($P < 0.0001$). CONCLUSIONS: Collectively, the data suggest that intercellular transfer of cytosolic materials could be one novel mechanism for preventing UCB cell death in MSC co-culture.

Collet, G., et al. (2013). "Trojan horse at cellular level for tumor gene therapies." Gene **525**(2): 208-216.

Among innovative strategies developed for cancer treatments, gene therapies stand of great interest despite their well-known limitations in targeting, delivery, toxicity or stability. The success of any given gene-therapy is highly dependent on the carrier efficiency. New approaches are often revisiting the mythic trojan horse concept to carry therapeutic nucleic acid, i.e. DNAs, RNAs or small interfering RNAs, to pathologic tumor site. Recent investigations are focusing on engineering carrying modalities to overtake the above limitations bringing new promise to cancer patients. This review describes recent advances and perspectives for gene therapies devoted to tumor treatment, taking advantage of available knowledge in biotechnology and medicine.

Collins, M. C., et al. (2012). "Labeling and imaging mesenchymal stem cells with quantum dots." Methods Mol Biol **906**: 199-210.

Mesenchymal stem cells (MSCs) are multipotent cells with the potential to differentiate into bone, -cartilage, adipose, and muscle cells. Adult derived MSCs are being actively investigated because of their potential to be utilized for therapeutic cell-based transplantation. Methods to track MSCs in vivo are -limited, preventing long-term functional studies of transplanted cells. Quantum Dots (QDs) offer an alternative to organic dyes and fluorescent proteins to label and track cells in vitro and in vivo. Nanoparticles are resistant to chemical and metabolic degradation, demonstrating long-term photostability. Here, we describe the technique to label MSCs with QDs and demonstrate intracellular QD distribution in the labeled MSCs with laser scanning confocal fluorescent microscopy.

Collins, M. C., et al. (2014). "Functional integration of quantum dot labeled mesenchymal stem cells in a cardiac microenvironment." Methods Mol Biol **1199**: 141-154.

Bone marrow derived multipotent mesenchymal stem cells (MSCs) have the potential to differentiate into bone, cartilage, fat, and muscle cells and are being investigated for their utility in cell-based therapies. Stem cell transplantation therapy represents a novel and innovative approach with the promise to restore function to diseased or damaged heart muscle. Transplanted MSCs are expected to engraft, differentiate, and remodel in response to the surrounding cardiac microenvironment significantly changing the therapeutic approach for heart disease. Quantum Dots (QDs) offer an alternative to organic dyes and fluorescent proteins to label and track cells in vitro and in vivo. Here, we describe in vitro QD labeling of MSCs, MSC integration in a cardiomyocyte co-culture microenvironment, and a fluorescent recovery after photobleaching (FRAP) technique to assess functional cell-cell communication. FRAP techniques establish an optical record of dynamic cellular interactions with high spatial and temporal resolution and can be used to successfully evaluate dynamic changes in cellular coupling in multicellular preparations.

Conlon, P., et al. (2008). "Pyrene excimer signaling molecular beacons for probing nucleic acids." J Am Chem Soc **130**(1): 336-342.

Molecular beacon DNA probes, containing 1-4 pyrene monomers on the 5' end and the quencher DABCYL on the 3' end, were engineered and employed for real-time probing of DNA sequences. In the absence of a target sequence, the multiple-pyrene labeled molecular beacons (MBs) assumed a stem-closed conformation resulting in quenching of the pyrene excimer fluorescence. In the presence of target, the beacons switched to a stem-open conformation, which separated the pyrene label from the quencher molecule and generated an excimer emission signal proportional to the target concentration. Steady-state fluorescence assays resulted in a subnanomolar limit of detection in buffer, whereas time-resolved signaling enabled low-nanomolar target detection in cell-growth media. It was found that the excimer emission intensity could be scaled by increasing the number of pyrene monomers conjugated to the 5' terminal. Each additional pyrene monomer resulted in substantial increases in the excimer emission intensities, quantum yields, and excited-state lifetimes of the hybridized MBs. The long fluorescence lifetime (approximately 40 ns), large Stokes shift (130 nm), and tunable intensity of the excimer make this multiple-pyrene moiety a useful alternative to traditional fluorophore labeling in nucleic acid probes.

Cortes, J. E., et al. (2019). "Quizartinib versus salvage chemotherapy in relapsed or refractory FLT3-ITD acute myeloid leukaemia (QuANTUM-R): a multicentre, randomised, controlled, open-label, phase 3 trial." Lancet Oncol **20**(7): 984-997.

BACKGROUND: Patients with relapsed or refractory FLT3 internal tandem duplication (FLT3-ITD)-positive acute myeloid leukaemia have a poor prognosis, including high frequency of relapse, poorer response to salvage therapy, and shorter overall survival than those with FLT3 wild-type disease. We aimed to assess whether single-agent quizartinib, an oral, highly potent and selective type II FLT3 inhibitor, improves overall survival versus salvage chemotherapy. METHODS: QuANTUM-R is a randomised, controlled, phase 3 trial done at 152 hospitals and cancer centres in 19 countries. Eligible patients aged 18 years or older with ECOG performance status 0-2 with relapsed or refractory (duration of first composite complete remission </=6 months) FLT3-ITD acute myeloid leukaemia after standard therapy with or without allogeneic haemopoietic stem-cell transplantation were randomly assigned (2:1; permuted block size of 6; stratified by response to previous therapy and choice of chemotherapy via a phone-based and web-based interactive response system) to quizartinib (60 mg [30 mg lead-in] orally once daily) or investigator's choice of preselected chemotherapy: subcutaneous low-dose cytarabine (subcutaneous injection of cytarabine 20 mg twice daily on days 1-10 of 28-day cycles); intravenous infusions of mitoxantrone (8 mg/m(2) per day), etoposide (100 mg/m(2) per day), and cytarabine (1000 mg/m(2) per day on days 1-5 of up to two 28-day cycles); or intravenous granulocyte colony-stimulating factor (300 mug/m(2) per day or 5 mug/kg per day

subcutaneously on days 1-5), fludarabine (intravenous infusion 30 mg/m(2) per day on days 2-6), cytarabine (intravenous infusion 2000 mg/m(2) per day on days 2-6), and idarubicin (intravenous infusion 10 mg/m(2) per day on days 2-4 in up to two 28-day cycles). Patients proceeding to haemopoietic stem-cell transplantation after quizartinib could resume quizartinib after haemopoietic stem-cell transplantation. The primary endpoint was overall survival in the intention-to-treat population. This trial is registered with ClinicalTrials.gov, number NCT02039726, and follow-up is ongoing. FINDINGS: Between May 7, 2014, and Sept 13, 2017, 367 patients were enrolled, of whom 245 were randomly allocated to quizartinib and 122 to chemotherapy. Four patients in the quizartinib group and 28 in the chemotherapy group were not treated. Median follow-up was 23.5 months (IQR 15.4-32.3). Overall survival was longer for quizartinib than for chemotherapy (hazard ratio 0.76 [95% CI 0.58-0.98; p=0.02]). Median overall survival was 6.2 months (5.3-7.2) in the quizartinib group and 4.7 months (4.0-5.5) in the chemotherapy group. The most common non-haematological grade 3-5 treatment-emergent adverse events (within </=30 days of last dose or >30 days if suspected to be a treatment-related event) for quizartinib (241 patients) and chemotherapy (94 patients) were sepsis or septic shock (46 patients [19%] for quizartinib vs 18 [19%] for chemotherapy), pneumonia (29 [12%] vs eight [9%]), and hypokalaemia (28 [12%] vs eight [9%]). The most frequent treatment-related serious adverse events were febrile neutropenia (18 patients [7%]), sepsis or septic shock (11 [5%]), QT prolongation (five [2%]), and nausea (five [2%]) in the quizartinib group, and febrile neutropenia (five [5%]), sepsis or septic shock (four [4%]), pneumonia (two [2%]), and pyrexia (two [2%]) in the chemotherapy group. Grade 3 QT prolongation in the quizartinib group was uncommon (eight [3%] by central reading, ten [4%] by investigator report); no grade 4 events occurred. There were 80 (33%) treatment-emergent deaths in the quizartinib group (31 [13%] of which were due to adverse events) and 16 (17%) in the chemotherapy group (nine [10%] of which were due to adverse events). INTERPRETATION: Treatment with quizartinib had a survival benefit versus salvage chemotherapy and had a manageable safety profile in patients with rapidly proliferative disease and very poor prognosis. Quizartinib could be considered a new standard of care. Given that there are only a few available treatment options, this study highlights the value of targeting the FLT3-ITD driver mutation with a highly potent and selective FLT3 inhibitor. FUNDING: Daiichi Sankyo.

Costa, C. R. M., et al. (2017). "Labeling of adipose-derived stem cells with quantum dots provides stable and long-term fluorescent signal for ex vivo cell tracking." In Vitro Cell Dev Biol Anim **53**(4): 363-370.

Stem cells derived from adipose tissue (ADSC) have been used in cell therapy as an alternative to treat chronic and degenerative diseases. Using biomedical and image trials to track the cells when infused in the target tissue is essential to control cell migration and adhesion. The objective of the present study was to label and assess the adhesion of goat adipose tissue-derived stem cells (g-ADSC) after cell infusion in animal models by tracking luminescent intracytoplasmatic nanocrystals. The cells were labeled by using Qdots. The g-ADSCs infused with nanocrystal were prepared either fresh or fixed and further visualized under a fluorescence microscope. The labeled cells were infused in the goat mammary glands and mouse testicles and kidneys via tail vein injection. Thirty days after cell infusion, biopsy was carried out for analyses. The g-ADSC cultures were presented with high cellularity and fibroblast morphology, even after infusion of the nanocrystals. It was possible, by processing in paraffin and under fluorescence microscopy, demonstrating the success of the labeling in the long term. Freezing mammary gland biopsies in liquid NO2 did not alter the quality of labeling with Qdots. Therefore, g-ADSCs can be labeled with intracytoplasmatic nanocrystals (Qdots) enabling their in vitro and ex vivo tracking.

Csaszar, E., et al. (2014). "Real-time monitoring and control of soluble signaling factors enables enhanced progenitor cell outputs from human cord blood stem cell cultures." Biotechnol Bioeng **111**(6): 1258-1264.

Monitoring and control of primary cell cultures is challenging as they are heterogenous and dynamically complex systems. Feedback signaling proteins produced from off-target cell populations can accumulate, inhibiting the production of the desired cell populations. Although culture strategies have been developed to reduce feedback inhibition, they are typically optimized for a narrow range of process parameters and do not allow for a dynamically regulated response. Here we describe the development of a microbead-based process control system for the monitoring and control of endogenously produced signaling factors. This system uses quantum dot barcoded microbeads to assay endogenously produced signaling proteins in the culture media, allowing for the dynamic manipulation of protein concentrations. This monitoring system was incorporated into a fed-batch bioreactor to regulate the accumulation of TGF-beta1 in an umbilical cord blood cell expansion system. By maintaining the concentration of TGF-beta1 below an upper threshold throughout the culture, we demonstrate enhanced ex vivo expansion of hematopoietic progenitor cells at higher input cell densities and over longer culture periods. This study demonstrates the potential of a fully automated and

integrated real-time control strategy in stem cell culture systems, and provides a powerful strategy to achieve highly regulated and intensified in vitro cell manufacturing systems.

Dahmke, I. N., et al. (2020). "Correlative Fluorescence- and Electron Microscopy of Whole Breast Cancer Cells Reveals Different Distribution of ErbB2 Dependent on Underlying Actin." Front Cell Dev Biol **8**: 521.

Epidermal growth factor receptor 2 (ErbB2) is found overexpressed in several cancers, such as gastric, and breast cancer, and is, therefore, an important therapeutic target. ErbB2 plays a central role in cancer cell invasiveness, and is associated with cytoskeletal reorganization. In order to study the spatial correlation of single ErbB2 proteins and actin filaments, we applied correlative fluorescence microscopy (FM), and scanning transmission electron microscopy (STEM) to image specifically labeled SKBR3 breast cancer cells. The breast cancer cells were grown on microchips, transformed to express an actin-green fluorescent protein (GFP) fusion protein, and labeled with quantum dot (QD) nanoparticles attached to specific anti-ErbB2 Affibodies. FM was performed to identify cellular regions with spatially correlated actin and ErbB2 expression. For STEM of the intact plasma membrane of whole cells, the cells were fixed and covered with graphene. Spatial distribution patterns of ErbB2 in the actin rich ruffled membrane regions were examined, and compared to adjacent actin-low regions of the same cell, revealing an association of putative signaling active ErbB2 homodimers with actin-rich regions. ErbB2 homodimers were found absent from actin-low membrane regions, as well as after treatment of cells with Cytochalasin D, which breaks up larger actin filaments. In both latter data sets, a significant inter-label distance of 36 nm was identified, possibly indicating an indirect attachment to helical actin filaments via the formation of heterodimers of ErbB2 with epidermal growth factor receptor (EGFR). The possible attachment to actin filaments was further explored by identifying linear QD-chains in actin-rich regions, which also showed an inter-label distance of 36 nm.

Dahmke, I. N., et al. (2017). "Graphene Liquid Enclosure for Single-Molecule Analysis of Membrane Proteins in Whole Cells Using Electron Microscopy." ACS Nano **11**(11): 11108-11117.

Membrane proteins govern many important functions in cells via dynamic oligomerization into active complexes. However, analytical methods to study their distribution and functional state in relation to the cellular structure are currently limited. Here, we introduce a technique for studying single-membrane proteins within their native context of the intact plasma membrane. SKBR3 breast cancer cells were grown on silicon microchips with thin silicon nitride windows. The cells were fixed, and the epidermal growth factor receptor ErbB2 was specifically labeled with quantum dot (QD) nanoparticles. For correlative fluorescence- and liquid-phase electron microscopy, we enclosed the liquid samples by chemical vapor deposited (CVD) graphene films. Depending on the local cell thickness, QD labels were imaged with a spatial resolution of 2 nm at a low electron dose. The distribution and stoichiometric assembly of ErbB2 receptors were determined at several different cellular locations, including tunneling nanotubes, where we found higher levels of homodimerization at the connecting sites. This experimental approach is applicable to a wide range of cell lines and membrane proteins and particularly suitable for studies involving both inter- and intracellular heterogeneity in protein distribution and expression.

Danner, S., et al. (2013). "Quantum Dots Do Not Alter the Differentiation Potential of Pancreatic Stem Cells and Are Distributed Randomly among Daughter Cells." Int J Cell Biol **2013**: 918242.

With the increasing relevance of cell-based therapies, there is a demand for cell-labeling techniques for in vitro and in vivo studies. For the reasonable tracking of transplanted stem cells in animal models, the usage of quantum dots (QDs) for sensitive cellular imaging has major advances. QDs could be delivered to the cytoplasm of the cells providing intense and stable fluorescence. Although QDs are emerging as favourable nanoparticles for bioimaging, substantial investigations are still required to consider their application for adult stem cells. Therefore, rat pancreatic stem cells (PSCs) were labeled with different concentrations of CdSe quantum dots (Qtracker 605 nanocrystals). The QD labeled PSCs showed normal proliferation and their usual spontaneous differentiation potential in vitro. The labeling of the cell population was concentration dependent, with increasing cell load from 5 nM QDs to 20 nM QDs. With time-lapse microscopy, we observed that the transmission of the QD particles during cell divisions was random, appearing as equal or unequal transmission to daughter cells. We report here that QDs offered an efficient and nontoxic way to label pancreatic stem cells without genetic modifications. In summary, QD nanocrystals are a promising tool for stem cell labeling and facilitate tracking of transplanted cells in animal models.

Dapkute, D., et al. (2017). "Skin-derived mesenchymal stem cells as quantum dot vehicles to tumors." Int J Nanomedicine **12**: 8129-8142.

PURPOSE: Cell-mediated delivery of nanoparticles is emerging as a new method of cancer diagnostics and treatment. Due to their inherent

regenerative properties, adult mesenchymal stem cells (MSCs) are naturally attracted to wounds and sites of inflammation, as well as tumors. Such characteristics enable MSCs to be used in cellular hitchhiking of nanoparticles. In this study, MSCs extracted from the skin connective tissue were investigated as transporters of semiconductor nanocrystals quantum dots (QDs). MATERIALS AND METHODS: Cytotoxicity of carboxylated CdSe/ZnS QDs was assessed by lactate dehydrogenase cell viability assay. Quantitative uptake of QDs was determined by flow cytometry; their intracellular localization was evaluated by confocal microscopy. In vitro tumor-tropic migration of skin-derived MSCs was verified by Transwell migration assay. For in vivo migration studies of QD-loaded MSCs, human breast tumor-bearing immunodeficient mice were used. RESULTS: QDs were found to be nontoxic to MSCs in concentrations no more than 16 nM. The uptake studies showed a rapid QD endocytosis followed by saturating effects after 6 h of incubation and intracellular localization in the perinuclear region. In vitro migration of MSCs toward MDA-MB-231 breast cancer cells and their conditioned medium was up to nine times greater than the migration toward noncancerous breast epithelial cells MCF-10A. In vivo, systemically administered QD-labeled MSCs were mainly located in the tumor and metastatic tissues, evading most healthy organs with the exception being blood clearance organs (spleen, kidneys, liver). CONCLUSION: Skin-derived MSCs demonstrate applicability in cell-mediated delivery of nanoparticles. The findings presented in this study promise further development of a cell therapy and nanotechnology-based tool for early cancer diagnostics and therapy.

Darkazalli, A. and C. W. Levenson (2012). "Tracking stem cell migration and survival in brain injury: current approaches and future prospects." Histol Histopathol **27**(10): 1255-1261.

In recent years, stem cell-mediated therapies have gained considerable ground as potential treatments for a wide variety of brain pathologies including traumatic brain injury, stroke and neurodegenerative diseases. Despite extensive preclinical studies, many of these therapies have not been fully translated into viable clinical approaches. This is partly due to our inability to reliably track and monitor transplanted stem cells longitudinally over long periods of time in vivo. In this review, we discuss the predominant histological cell tracing methodologies, such as immunohistochemistry, and fluorescent cellular dyes and proteins, and compare them to emerging cellular imaging technologies. We show that advances in magnetic resonance imaging (MRI) have resulted in opportunities to use this technology to further our understanding of stem cell characteristics and behaviors in vivo. While MRI may not completely replace conventional cell tracking methods in pre-clinical, mechanistic work, it is clear that it has the potential to function as a powerful diagnostic tool for tracking stem cell migration and survival as well as for evaluating the efficacy of stem cell-mediated therapies.

Das, B., et al. (2019). "Doping of carbon nanodots for saving cells from silver nanotoxicity: A study on recovering osteogenic differentiation potential." Toxicol In Vitro **57**: 81-95.

Silver nanoparticles are explored for many advanced biological applications including the development of antimicrobial surfaces on implants, SERS imaging, nanotherapeutics, biosensing and much more. However, recent research findings suggest silver nanoparticles provide blockade of differentiation of mesenchymal stem cells (MSCs), especially into osteogenic developmental pathway via generation of reactive oxygen species. These studies suggest that the application of silver nanoparticles in medical implants should be prohibited. In the current study, carbon nanodots (CND) supported silver clusters (AgC) is explored as a remedy to this problem. The nanostructure was synthesized in microwave irradiation induced rapid method and characterization was conducted via UV-Vis spectroscopy, fluorescence spectroscopy, HRTEM, XRD, FTIR, Raman spectroscopy, DLS, AFM, and XPS. Fluorescence spectrum showed a quantum yield of 0.25 while Raman spectroscopy showed rapid amplification of CND specific peaks implicating significant SERS property. Further in vitro biocompatibility (MTT) and bio-imaging capability was assessed culturing Wharton's Jelly-derived MSCs. In this study, its efficacy as in-situ cellular oxidative stress scavenger is also studied using NBT and DCFH-DA assay. Via ALP assay, alizarin red staining, cell membrane nanoindentation studies, PCR analysis and immunocytochemistry for osteoblast-like gene expression it was confirmed that AgCs can control silver nanoparticle-induced inhibition of osteogenic differentiation in vitro. Thus, AgCs (Carbon nanodots supported silver clusters) are not only considered to be a dual-mode bio-imaging nanoprobe but also a remedy to the silver-induced ROS generation and osteogenic differentiation blockade of MSCs.

Das, B., et al. (2012). "Cyclic RGD peptide conjugated trypsin etched gold quantum clusters: novel biolabeling agents for stem cell imaging." J Stem Cells **7**(3): 189-199.

Quantum clusters are sub-nano sized materials mostly synthesized from noble metals with luminescence property and high quantum yield. They are important to biomedical scientists because of their excellent optical properties. Here we represent a tool for

cell imaging purpose using protein stabilized gold quantum clusters. Intestinal protease Trypsin was used to develop clusters. They were conjugated to cyclic RGD peptides by EDAC coupling. Cell imaging property was checked by transfecting the RGD-conjugated quantum clusters to bone marrow stem cells. For characterization of RGD-conjugated quantum clusters UV-Vis, Fluorescence and FTIR spectroscopy was performed. DLS and Zeta potential measurement also have been done. To check the bio compatibility of the quantum clusters MTT assay, AFM and blood cell adhesion study were performed. The samples are found out to be good for cell imaging as well as bio compatible and hemo-compatible.

Das, B., et al. (2008). "Hypoxia enhances tumor stemness by increasing the invasive and tumorigenic side population fraction." Stem Cells **26**(7): 1818-1830.

Although advances have been made in understanding the role of hypoxia in the stem cell niche, almost nothing is known about a potentially similar role of hypoxia in maintaining the tumor stem cell (TSC) niche. Here we show that a highly tumorigenic fraction of side population (SP) cells is localized in the hypoxic zones of solid tumors in vivo. We first identified a highly migratory, invasive, and tumorigenic fraction of post-hypoxic side population cells (SPm([hox]) fraction) in a diverse group of solid tumor cell lines, including neuroblastoma, rhabdomyosarcoma, and small-cell lung carcinoma. To identify the SPm((hox)) fraction, we used an "injured conditioned medium" derived from bone marrow stromal cells treated with hypoxia and oxidative stress. We found that a highly tumorigenic SP fraction migrates to the injured conditioned medium in a Boyden chamber. We show that as few as 100 SPm((hox)) cells form rapidly growing tumors in vivo. In vitro exposure to hypoxia increases the SPm((hox)) fraction significantly. Quantitative real-time polymerase chain reaction and immunofluorescence studies showed that SPm((hox)) cells expressed Oct-4, a "stemness" gene having a potential role in TSC maintenance. In nude mice xenografts, SPm((hox)) cells were localized to the hypoxic zones, as demonstrated after quantum dot labeling. These results suggest that a highly tumorigenic SP fraction migrates to the area of hypoxia; this migration is similar to the migration of normal bone marrow SP fraction to the area of injury/hypoxia. Furthermore, the hypoxic microenvironment may serve as a niche for the highly tumorigenic fraction of SP cells.

Das, P., et al. (2019). "Converting waste Allium sativum peel to nitrogen and sulphur co-doped photoluminescence carbon dots for solar conversion, cell labeling, and photobleaching diligences: A path from discarded waste to value-added products." J Photochem Photobiol B **197**: 111545.

Proper waste utilization in order to promote value added product is a promising scientific practice in recent era. Inspiring from the recurring trend, we propose a single step oxidative pyrolysis derived fluorescent carbon dots (C-dots) from Allium sativum peel, which is a natural, nontoxic, and waste raw material. Because of its excellent optical properties, and photostability this C-dots have been used in versatile area of applications. Due to its immediate water dispersing character, C-dots reinforced Poly(acrylic acid) (PAA) films revealed improvement in uniaxial stretching behavior and can be used as transparent sunlight conversion film. The nanocomposite film has been tested against rigorous simulated sunlight which proved almost identical sunlight conversion behavior with no photo-bleachable character which is definitely added an extra quality of transparent polymer films. Moreover, the C-dots dispersion has been used as in vitro biomarker for living cells owing to its ease in solubility, biocompatibility, non-cytotoxicity and bright fluorescence even in subcutaneous environment. For this case, adipose derived mesenchymal stem cells (ADMSCs) have been chosen and injected to rabbit ear skin to perform two-photon imaging experiment. The present work opens a new avenue towards the large-scale synthesis of bio-waste based fluorescent C-dots, paving the way for their versatile applications.

Dave, S. D., et al. (2018). "In vitro differentiation of neural cells from human adipose tissue derived stromal cells." Neurol India **66**(3): 716-721.

BACKGROUND: Stem cells, including neural stem cells (NSCs), are endowed with self-renewal capability and hence hold great opportunity for the institution of replacement/protective therapy. We propose a method for in vitro generation of stromal cells from human adipose tissue and their differentiation into neural cells. MATERIALS AND METHODS: Ten grams of donor adipose tissue was surgically resected from the abdominal wall of the human donor after the participants' informed consents. The resected adipose tissue was minced and incubated for 1 hour in the presence of an enzyme (collagenase-type I) at 37(0)C followed by its centrifugation. After centrifugation, the supernatant and pellets were separated and cultured in a medium for proliferation at 37(0)C with 5% CO2 for 9-10 days in separate tissue culture dishes for generation of mesenchymal stromal cells (MSC). At the end of the culture, MSC were harvested and analyzed. The harvested MSC were subjected for further culture for their differentiation into neural cells for 5-7 days using differentiation medium mainly comprising of neurobasal medium. At the end of the procedure, culture cells were isolated and studied for expression of transcriptional factor proteins: orthodenticle homolog-2 (OTX-2), beta-III-tubulin (beta3-Tubulin), glial-

fibrillary acid protein (GFAP) and synaptophysin-beta2. RESULTS: In total, 50 neural cells-lines were generated. In vitro generated MSC differentiated neural cells' mean quantum was 5.4 +/- 6.9 ml with the mean cell count being, 5.27 +/- 2.65 x 10(3/)mul. All of them showed the presence of OTX-2, beta3-Tubulin, GFAP, synaptophysin-beta2. CONCLUSION: Neural cells can be differentiated in vitro from MSC safely and effectively. In vitro generated neural cells represent a potential therapy for recovery from spinal cord injuries and neurodegenerative disease.

Dave, S. D., et al. (2012). "Ex vivo generation of glucose sensitive insulin secreting mesenchymal stem cells derived from human adipose tissue." Indian J Endocrinol Metab **16 Suppl 1**: S65-69.

BACKGROUND: Diabetics are incapable of producing insulin/have autoimmune mechanisms making it ineffective to control glucose secretion. We present a prospective study of glucose-sensitive insulin-secreting mesenchymal stem cells (IS-MSC) generated from human adipose tissue (h-AD) sans xenogenic material. MATERIALS AND METHODS: Ten grams h-AD from donor anterior abdominal wall was collected in proliferation medium composed of alpha-Minimum Essential Media (alpha-MEM), albumin, fibroblast-growth factor and antibiotics, minced, incubated in collagenase-I at 37 degrees C with shaker and centrifuged. Supernatant and pellets were separately cultured in proliferation medium on cell+ plates at 37 degrees C with 5% CO(2) for 10 days. Cells were harvested by trypsinization, checked for viability, sterility, counts, flow-cytometry (CD45(-)/90(+)/73(+)), and differentiated into insulin-expressing cells using medium composed of DMEM, gene expressing up-regulators and antibiotics for 3 days. They were studied for transcriptional factors Pax-6, Isl-1, pdx-1 (immunofluorescence). C-peptide and insulin were measured by chemiluminescence. In vitro glucose sensitivity assay was carried out by measuring levels of insulin and C-peptide secretion in absence of glucose followed by 2 hours incubation after glucose addition. RESULTS: Mean IS-AD-MSC quantum was 3.21 ml, cell count, 1.5 x10(3) cells/mul), CD45(-)/90(+)/73(+) cells were 44.37% /25.52%. All of them showed presence of pax-6, pdx-1, and Isl-1. Mean C-Peptide and insulin levels were 0.36 ng/ml and 234 muU/ml, respectively, pre-glucose and 0.87 ng/ml and 618.3 muU/ml post-glucose additions. The mean rise in secretion levels was 2.42 and 2.65 fold, respectively. CONCLUSION: Insulin-secreting h-AD-MSC can be generated safely and effectively showing in vitro glucose responsive alteration in insulin and C-peptide secretion levels.

Dawson, G. (2016). "Quantum dots and potential therapy for Krabbe's disease." J Neurosci Res **94**(11): 1293-1303.

Enzyme replacement therapy and substrate reduction therapy have proved useful in reversing many pathological consequences of many nonneural lysosomal storage diseases but have not yet reversed pathology or influenced disease outcome in Krabbe's disease (KD). This Review discusses the relative merits of stem cell therapy, molecular chaperone therapy, gene therapy, substrate reduction therapy, enzyme replacement therapy, and combination therapy. Given the limitations of these approaches, this Review introduces the idea of using tiny, 6-nm, intensely fluorescent quantum dots (QDs) to deliver a cell-penetrating peptide and 6 histidine residue-tagged beta-D-galactocerebrosidase across the blood-brain barrier. We can therefore follow the fate of injected material and ensure that all targets are reached and that accumulated material is degraded. Uptake of lysosomal hydrolases is a complex process, and the cell-penetrating peptide JB577 is uniquely able to promote endosomal egress of the QD cargo. This Review further shows that uptake may depend on the charge of the coating of the QD, specifically, that negative charge directs the cargo to neurons. Because KD involves primarily glia, specifically oligodendroglia, we experiment with many coatings and discover a coating (polyethylene glycol 600 amino) that has a positive charge and targets oligodendrocytes. A similar effect is achieved by treating with chondroitinase ABC to degrade the extracellular matrix, indicating that enzyme replacement has several hurdles to overcome before it can become a routine CNS therapy. (c) 2016 Wiley Periodicals, Inc.

de Mel, A., et al. (2012). "Biofunctionalized quantum dots for live monitoring of stem cells: applications in regenerative medicine." Regen Med **7**(3): 335-347.

AIM: This study aimed to live monitor the degree of endothelial progenitor cell (EPC) integration onto tissue-engineering scaffolds by conjugating relevant antibodies to quantum dots (QDs). MATERIALS & METHODS: Biocompatible mercaptosuccinic acid-coated QDs were functionalized with two different antibodies to EPC (CD133 with QDs of 640 nm wavelength [lambda] and later-stage mature EPCs; and von Willebrand factor with QDs of lambda595 and lambda555 nm) using conventional carbomide and N-hydroxysuccinimide chemistry. Biofunctionalization was characterized with Fourier-transform infrared spectroscopy. Cell viability assays and gross morphology observations confirmed cytocompatibility and normal patterns of celluar growth. The antigens corresponding to each state of cell maturation were determined using a single excitation at lambda488 nm. RESULTS: The optimal concentrations

of antibody-QD conjugates were biocompatible, hemocompatible and determined the state of EPC transformation to endothelial cells. CONCLUSION: Antibody-functionalized QDs suggest new applications in tissue engineering of polymer-based implants where cell integration can potentially be monitored without requiring the sacrifice of implants.

Deb, S. and S. Chana (2015). "Biomaterials in Relation to Dentistry." Front Oral Biol **17**: 1-12.

Dental caries remains a challenge in the improvement of oral health. It is the most common and widespread biofilm-dependent oral disease, resulting in the destruction of tooth structure by the acidic attack from cariogenic bacteria. The tooth is a heavily mineralised tissue, and both enamel and dentine can undergo demineralisation due to trauma or dietary conditions. The adult population worldwide affected by dental caries is enormous and despite significant advances in caries prevention and tooth restoration, treatments continue to pose a substantial burden to healthcare. Biomaterials play a vital role in the restoration of the diseased or damaged tooth structure and, despite providing reasonable outcomes, there are some concerns with clinical performance. Amalgam, the silver grey biomaterial that has been widely used as a restorative material in dentistry, is currently in throes of being phased out, especially with the Minimata convention and treaty being signed by a number of countries (January 2013; http://mercuryconvention.org/Convention/) that aims to control the anthropogenic release of mercury in the environment, which naturally impacts the use of amalgam, where mercury is a component. Thus, the development of alternative restoratives and restoration methods that are inexpensive, can be used under different climatic conditions, withstand storage and allow easy handling, the main prerequisites of dental biomaterials, is important. The potential for using biologically engineered tissue and consequent research to replace damaged tissues has also seen a quantum leap in the last decade. Ongoing research in regenerative treatments in dentistry includes alveolar ridge augmentation, bone tissue engineering and periodontal ligament replacement, and a future aim is bioengineering of the whole tooth. Research towards developing bioengineered teeth is well underway and identification of adult stem cell sources to make this a viable treatment is advancing; however, this topic is not in the scope of this chapter. Whilst research focuses on many different aspects, operative dentistry involves the wide use of restorative biomaterials; thus, the development of smart biomaterials to suit the current climes of minimally invasive dentistry is important. The concept of minimally invasive dentistry primarily promotes preservation of the natural tissue, and, thus, the prevention of disease or the advancement of procedures that allow early detection and interception of its progress with minimal tissue loss are of significance. This chapter presents, in brief, the current state of the art of direct restorative biomaterials and their role and future in the field of dentistry. Modern dental practice is highly reliant on the selection of appropriate materials for optimum function and benefit to the patient. Dentistry, perhaps, has the unique distinction of using the widest variety of materials, ranging from polymers, metals, ceramics, inorganic salts to composite materials. So far, aesthetics of restorative materials and their ability to perform in the harsh oral environment without undergoing changes in dimension and stability has been the major focus of materials used in dentistry. Despite advances in tissue engineering and regeneration in the field of regenerative medicine, this concept has found relatively limited application for enamel and dentine due to their limited ability to remodel, but research related to biomimetic approaches for the modification of dentine is a significant step.

Debbage, P. and W. Jaschke (2008). "Molecular imaging with nanoparticles: giant roles for dwarf actors." Histochem Cell Biol **130**(5): 845-875.

Molecular imaging, first developed to localise antigens in light microscopy, now encompasses all imaging modalities including those used in clinical care: optical imaging, nuclear medical imaging, ultrasound imaging, CT, MRI, and photoacoustic imaging. Molecular imaging always requires accumulation of contrast agent in the target site, often achieved most efficiently by steering nanoparticles containing contrast agent into the target. This entails accessing target molecules hidden behind tissue barriers, necessitating the use of targeting groups. For imaging modalities with low sensitivity, nanoparticles bearing multiple contrast groups provide signal amplification. The same nanoparticles can in principle deliver both contrast medium and drug, allowing monitoring of biodistribution and therapeutic activity simultaneously (theranostics). Nanoparticles with multiple bioadhesive sites for target recognition and binding will be larger than 20 nm diameter. They share functionalities with many subcellular organelles (ribosomes, proteasomes, ion channels, and transport vesicles) and are of similar sizes. The materials used to synthesise nanoparticles include natural proteins and polymers, artificial polymers, dendrimers, fullerenes and other carbon-based structures, lipid-water micelles, viral capsids, metals, metal oxides, and ceramics. Signal generators incorporated into nanoparticles include iron oxide, gadolinium, fluorine, iodine, bismuth, radionuclides, quantum dots, and metal nanoclusters. Diagnostic imaging applications, now appearing, include sentinal node localisation and stem cell tracking.

Delsanto, P. P., et al. (2008). "A multilevel approach to cancer growth modeling." J Theor Biol **250**(1): 16-24.

Cancer growth models may be divided into macroscopic models, which describe the tumor as a single entity, and microscopic ones, which consider the tumor as a complex system whose behavior emerges from the local dynamics of its basic components, the neoplastic cells. Mesoscopic models (e.g. as based on the Local Interaction Simulation Approach [Delsanto, P.P., Mignogna, R., Scalerandi, M., Schechter, R., 1998. In: Delsanto, P.P. Saenz, A.W. (Eds.), New Perspectives on Problems in Classical and Quantum Physics, vol. 2. Gordon & Breach, New Delhi, p. 5174]), which explicitly consider the behavior of cell clusters and their interactions, may be used instead of the microscopic ones, in order to study the properties of cancer biology that strongly depend on the interactions of small groups of cells at intermediate spatial and temporal scales. All these approaches have been developed independently, which limits their usefulness, since they all include relevant features and information that should be cross-correlated for a deeper understanding of the mechanisms involved. In this contribution we consider multicellular tumor spheroids as biological reference systems and propose an intermediate model to bridge the gap between a macroscopic formulation of tumor growth and a mesoscopic one. Thus we are able to establish, as an important result of our formalism, a direct correspondence between parameters characterizing processes occurring at different scales. In particular, we analyze their dependence on an important limiting factor to tumor growth, i.e. the extra-cellular matrix pressure. Since the macro and meso-models stem from totally different roots (energy conservation and clinical observations vs. cell groups dynamics), their consistency may be used to validate both approaches. It may also be interesting to note that the proposed formalism fits well into a recently proposed conjecture of growth laws universality.

Dhandayuthapani, B., et al. (2012). "Biomimetic smart nanocomposite: in vitro biological evaluation of zein electrospun fluorescent nanofiber encapsulated CdS quantum dots." Biofabrication **4**(2): 025008.

New hybrid quantum dot (QD)/nanofibers have potential applications in a variety of fields. A novel fluorescent nanocomposite nanofiber material, consisting of CdS and zein has been fabricated through the electrospinning process. A detailed optimization was carried out to fabricate continuous and uniform nanofibers without beads or droplets. The synthesized hybrid nanofibers were characterized by various state-of-the-art techniques such as scanning electron microscopy, transmission electron microscopy (TEM), TEM-energy dispersive spectrometry, atomic force microscopy and confocal fluorescence micrography. The optimization process was carried out to fabricate fibers ranging from 200 to 450 nm in diameter. The electrical conductivity of the zein-CdS hybrid nanofiber substrates was tested. The potential use of the electrospun CdS-encapsulated nanofibrous scaffold as substrates for cell/tissue culture was evaluated with two different cell types, i.e. mesenchymal stem cells and fibroblasts. The results showed that the electrospun fibrous scaffolds could support the attachment and the proliferation of cells. In addition, the cells cultured on the fibrous scaffolds exhibited normal cell shapes and integrated well with surrounding fibers. The obtained results confirmed the potential for the use of the electrospun QD-encapsulated fluorescent nanofiber mats as scaffolds for tissue engineering.

Ding, D., et al. (2014). "Precise and long-term tracking of adipose-derived stem cells and their regenerative capacity via superb bright and stable organic nanodots." ACS Nano **8**(12): 12620-12631.

Monitoring and understanding long-term fate and regenerative therapy of administrated stem cells in vivo is of great importance. Herein we report organic nanodots with aggregation-induced emission characteristics (AIE dots) for long-term tracking of adipose-derived stem cells (ADSCs) and their regenerative capacity in living mice. The AIE dots possess high fluorescence (with a high quantum yield of 25+/-1%), excellent biological and photophysical stabilities, low in vivo toxicity, and superb retention in living ADSCs with negligible interference on their pluripotency and secretome. These AIE dots also exhibit superior in vitro cell tracking capability compared to the most popular commercial cell trackers, PKH26 and Qtracker 655. In vivo quantitative studies with bioluminescence and GFP labeling as the controls reveal that the AIE dots can precisely and quantitatively report the fate of ADSCs and their regenerative capacity for 42 days in an ischemic hind limb bearing mouse model.

Dou, Q. Q., et al. (2015). "Core-shell upconversion nanoparticle - semiconductor heterostructures for photodynamic therapy." Sci Rep **5**: 8252.

Core-shell nanoparticles (CSNPs) with diverse chemical compositions have been attracting greater attention in recent years. However, it has been a challenge to develop CSNPs with different crystal structures due to the lattice mismatch of the nanocrystals. Here we report a rational design of core-shell heterostructure consisting of NaYF4:Yb,Tm upconversion nanoparticle (UCN) as the core and ZnO semiconductor as the shell for potential application in photodynamic therapy (PDT). The core-shell architecture (confirmed by TEM and STEM) enables for improving the loading efficiency of photosensitizer

(ZnO) as the semiconductor is directly coated on the UCN core. Importantly, UCN acts as a transducer to sensitize ZnO and trigger the generation of cytotoxic reactive oxygen species (ROS) to induce cancer cell death. We also present a firefly luciferase (FLuc) reporter gene based molecular biosensor (ARE-FLuc) to measure the antioxidant signaling response activated in cells during the release of ROS in response to the exposure of CSNPs under 980 nm NIR light. The breast cancer cells (MDA-MB-231 and 4T1) exposed to CSNPs showed significant release of ROS as measured by aminophenyl fluorescein (APF) and ARE-FLuc luciferase assays, and ~45% cancer cell death as measured by MTT assay, when illuminated with 980 nm NIR light.

Dudu, V., et al. (2012). "Sendai virus-based liposomes enable targeted cytosolic delivery of nanoparticles in brain tumor-derived cells." J Nanobiotechnology **10**: 9.

BACKGROUND: Nanotechnology-based bioassays that detect the presence and/or absence of a combination of cell markers are increasingly used to identify stem or progenitor cells, assess cell heterogeneity, and evaluate tumor malignancy and/or chemoresistance. Delivery methods that enable nanoparticles to rapidly detect emerging, intracellular markers within cell clusters of biopsies will greatly aid in tumor characterization, analysis of functional state and development of treatment regimens. RESULTS: Experiments utilized the Sendai virus to achieve in vitro, cytosolic delivery of Quantum dots in cells cultured from Human brain tumors. Using fluorescence microscopy and Transmission Electron Microscopy, in vitro experiments illustrated that these virus-based liposomes decreased the amount of non-specifically endocytosed nanoparticles by 50% in the Human glioblastoma and medulloblastoma samples studied. Significantly, virus-based liposome delivery also facilitated targeted binding of Quantum dots to cytosolic Epidermal Growth Factor Receptor within cultured cells, focal to the early detection and characterization of malignant brain tumors. CONCLUSIONS: These findings are the first to utilize the Sendai virus to achieve cytosolic, targeted intracellular binding of Qdots within Human brain tumor cells. The results are significant to the continued applicability of nanoparticles used for the molecular labeling of cancer cells to determine tumor heterogeneity, grade, and chemotherapeutic resistivity.

Dupont, K. M., et al. (2010). "Human stem cell delivery for treatment of large segmental bone defects." Proc Natl Acad Sci U S A **107**(8): 3305-3310.

Local or systemic stem cell delivery has the potential to promote repair of a variety of damaged or degenerated tissues. Although various stem cell sources have been investigated for bone repair, few comparative reports exist, and cellular distribution and viability postimplantation remain key issues. In this study, we quantified the ability of tissue-engineered constructs containing either human fetal or adult stem cells to enhance functional repair of nude rat critically sized femoral defects. After 12 weeks, defects treated with cell-seeded polymer scaffolds had significantly higher bone ingrowth and torsional strength compared to those receiving acellular scaffolds, although there were no significant differences between the cell sources. Next, stem cells were labeled with fluorescent quantum dots (QDs) in an attempt to noninvasively track their distribution after delivery on scaffolds. Clear fluorescence was observed at implantation sites throughout the study; however, beginning 7-10 days after surgery, signals were also observed at contralateral sites treated with acellular QD-free scaffolds. Although immunostaining for human nuclei revealed retention of some cells at the implantation site, no human cells were detected in the control limb defects. Additional histological analysis of implantation and control defect tissues revealed macrophages containing endocytosed QDs. Furthermore, QD-labeling appeared to diminish transplanted cell function resulting in reduced healing responses. In summary, augmentation of polymeric scaffolds with stem cells derived from fetal and adult tissues significantly enhanced healing of large segmental bone defects; however, QD labeling of stem cells eliminated the observed therapeutic effect and failed to conclusively track stem cell location long-term in vivo.

Dzobo, K., et al. (2019). "Targeting the Versatile Wnt/beta-Catenin Pathway in Cancer Biology and Therapeutics: From Concept to Actionable Strategy." OMICS **23**(11): 517-538.

This expert review offers a critical synthesis of the latest insights and approaches at targeting the Wnt/beta-catenin pathway in various cancers such as colorectal cancer, melanoma, leukemia, and breast and lung cancers. Notably, from organogenesis to cancer, the Wnt/beta-catenin signaling displays varied and highly versatile biological functions in animals, with virtually all tissues requiring the Wnt/beta-catenin signaling in one way or the other. Aberrant expression of the members of the Wnt/beta-catenin has been implicated in many pathological conditions, particularly in human cancers. Mutations in the Wnt/beta-catenin pathway genes have been noted in diverse cancers. Biochemical and genetic data support the idea that inhibition of Wnt/beta-catenin signaling is beneficial in cancer therapeutics. The interaction of this important pathway with other signaling systems is also noteworthy, but remains as an area for further research and discovery. In addition, formation of different complexes by components of the Wnt/beta-catenin pathway and the

precise roles of these complexes in the cytoplasmic milieu are yet to be fully elucidated. This article highlights the latest medical technologies in imaging, single-cell omics, use of artificial intelligence (e.g., machine learning techniques), genome sequencing, quantum computing, molecular docking, and computational softwares in modeling interactions between molecules and predicting protein-protein and compound-protein interactions pertinent to the biology and therapeutic value of the Wnt/beta-catenin signaling pathway. We discuss these emerging technologies in relationship to what is currently needed to move from concept to actionable strategies in translating the Wnt/beta-catenin laboratory discoveries to Wnt-targeted cancer therapies and diagnostics in the clinic.

El Baggari, I., et al. (2021). "Charge order textures induced by non-linear couplings in a half-doped manganite." Nat Commun **12**(1): 3747.

The self-organization of strongly interacting electrons into superlattice structures underlies the properties of many quantum materials. How these electrons arrange within the superlattice dictates what symmetries are broken and what ground states are stabilized. Here we show that cryogenic scanning transmission electron microscopy (cryo-STEM) enables direct mapping of local symmetries and order at the intra-unit-cell level in the model charge-ordered system Nd1/2Sr1/2MnO3. In addition to imaging the prototypical site-centered charge order, we discover the nanoscale coexistence of an exotic intermediate state which mixes site and bond order and breaks inversion symmetry. We further show that nonlinear coupling of distinct lattice modes controls the selection between competing ground states. The results demonstrate the importance of lattice coupling for understanding and manipulating the character of electronic self-organization and that cryo-STEM can reveal local order in strongly correlated systems at the atomic scale.

Ellis, D. I., et al. (2013). "Illuminating disease and enlightening biomedicine: Raman spectroscopy as a diagnostic tool." Analyst **138**(14): 3871-3884.

The discovery of the Raman effect in 1928 not only aided fundamental understanding about the quantum nature of light and matter but also opened up a completely novel area of optics and spectroscopic research that is accelerating at a greater rate during the last decade than at any time since its inception. This introductory overview focuses on some of the most recent developments within this exciting field and how this has enabled and enhanced disease diagnosis and biomedical applications. We highlight a small number of stimulating high-impact studies in imaging, endoscopy, stem cell research, and other recent developments such as spatially offset Raman scattering amongst others. We hope this stimulates further interest in this already exciting field, by 'illuminating' some of the current research being undertaken by the latest in a very long line of dedicated experimentalists interested in the properties and potential beneficial applications of light.

El-Sadik, A. O., et al. (2010). "Nanoparticle-labeled stem cells: a novel therapeutic vehicle." Clin Pharmacol **2**: 9-16.

Nanotechnology has been described as a general purpose technology. It has already generated a range of inventions and innovations. Development of nanotechnology will provide clinical medicine with a range of new diagnostic and therapeutic opportunities such as medical imaging, medical diagnosis, drug delivery, and cancer detection and management. Nanoparticles such as manganese, polystyrene, silica, titanium oxide, gold, silver, carbon, quantum dots, and iron oxide have received enormous attention in the creation of new types of analytical tools for biotechnology and life sciences. Labeling of stem cells with nanoparticles overcame the problems in homing and fixing stem cells to their desired site and guiding extension of stem cells to specific directions. Although the biologic effects of some nanoparticles have already been assessed, information on toxicity and possible mechanisms of various particle types remains inadequate. The aim of this review is to give an overview of the mechanisms of internalization and distribution of nanoparticles inside stem cells, as well as the influence of different types of nanoparticles on stem cell viability, proliferation, differentiation, and cytotoxicity, and to assess the role of nanoparticles in tracking the fate of stem cells used in tissue regeneration.

Fakhroueian, Z., et al. (2022). "Anticancer properties of novel zinc oxide quantum dot nanoparticles against breast cancer stem-like cells." Anticancer Drugs **33**(1): e311-e326.

Cancer stem cells (CSCs) play an essential role in cancer development, metastasis, relapse, and resistance to treatment. In this article, the effects of three synthesized ZnO nanofluids on proliferation, apoptosis, and stemness markers of breast cancer stem-like cells are reported. The antiproliferative and apoptotic properties of ZnO nanoparticles were evaluated on breast cancer stem-like cell-enriched mammospheres by MTS assay and flowcytometry, respectively. The expression of stemness markers, including WNT1, NOTCH1, beta-catenin, CXCR4, SOX2, and ALDH3A1 was assessed by real-time PCR. Western blotting was used to analyze the phosphorylation of Janus kinase 2 (JAK2) and Signal Transducer and Activator of Transcription 3 (STAT3). Markers of stemness were significantly decreased by ZnO

nanofluids, especially sample (c) with code ZnO-148 with a different order of addition of polyethylene glycol solution at the end of formulation, which considerably decreased all the markers compared to the controls. All the studied ZnO nanofluids considerably reduced viability and induced apoptosis of spheroidal and parental cells, with ZnO-148 presenting the most effective activity. Using CD95L as a death ligand and ZB4 as an extrinsic apoptotic pathway blocker, it was revealed that none of the nanoparticles induced apoptosis through the extrinsic pathway. Results also showed a marked inhibition of the JAK/STAT pathway by ZnO nanoparticles; confirmed by downregulation of Mcl-1 and Bcl-XL expression. The present data demonstrated that ZnO nanofluids could combat breast CSCs via decreasing stemness markers, stimulating apoptosis, and suppressing JAK/STAT activity.

Falanga, V. (2004). "The chronic wound: impaired healing and solutions in the context of wound bed preparation." Blood Cells Mol Dis **32**(1): 88-94.

In the past few years, a different paradigm for the understanding and treatment of chronic wounds has emerged. The term used to describe this new context in which failure to heal is viewed is "wound bed preparation". This term is revolutionizing the way we approach chronic wounds, and has allowed chronic wounds to gain independence from established models of acute injury. Within the context of wound bed preparation, impaired healing and solutions to it are being addressed in novel ways. In this report, we make use of the diabetic ulcer as an example of a chronic wound, and emphasize the pathophysiological principles, the cellular and molecular abnormalities, and the solutions offered by the new approaches of gene therapy and stem cells. The emerging view is that chronic wounds are characterized by resident cells that have undergone phenotypic changes that need to be corrected for optimal healing to occur. We have established in animal models and in humans that stem cells have the potential to bring about fundamental changes in the repair process and, ultimately, a "quantum" jump in our therapeutic success.

Fasbender, S., et al. (2019). "The Low Toxicity of Graphene Quantum Dots is Reflected by Marginal Gene Expression Changes of Primary Human Hematopoietic Stem Cells." Sci Rep **9**(1): 12028.

Graphene quantum dots (GQDs) are a promising next generation nanomaterial with manifold biomedical applications. For real world applications, comprehensive studies on their influence on the functionality of primary human cells are mandatory. Here, we report the effects of GQDs on the transcriptome of CD34(+) hematopoietic stem cells after an incubation time of 36 hours. Of the 20 800 recorded gene expressions, only one, namely the selenoprotein W, 1, is changed by the GQDs in direct comparison to CD34(+) hematopoietic stem cells cultivated without GQDs. Only a meta analysis reveals that the expression of 1171 genes is weakly affected, taking into account the more prominent changes just by the cell culture. Eight corresponding, weakly affected signaling pathways are identified, which include, but are not limited to, the triggering of apoptosis. These results suggest that GQDs with sizes in the range of a few nanometers hardly influence the CD34(+) cells on the transcriptome level after 36 h of incubation, thereby demonstrating their high usability for in vivo studies, such as fluorescence labeling or delivery protocols, without strong effects on the functional status of the cells.

Feng, G., et al. (2014). "Ultrabright organic dots with aggregation-induced emission characteristics for cell tracking." Biomaterials **35**(30): 8669-8677.

Noninvasive fluorescence cell tracking provides critical information on the physiological displacement and translocation of actively migrating cells, which deepens our understanding of biomedical engineering, oncological research, stem cell transplantation and therapies. Non-viral fluorescent protein transfection based cell tracing has been widely used but with issues related to cell type-dependent expression, lagged readout, immunogenicity and mutagenesis. Alternative cell tracking methods are therefore desired to attain reliable, stable, and efficient labeling over a long time. In this work, we have successfully developed ultra-bright organic dots with aggregation-induced emission (AIE dots) and demonstrated their capabilities for cellular imaging and cell tracking. The AIE dots possess high fluorescence, super photostability, and excellent cellular retention and biocompatibility. As compared to commonly used pMAX-GFP plasmid labeling approach, the organic AIE dots showed excellent cell labeling on all tested human cell lines and superior tracing performance, which opens up new opportunities in the cell-based immunotherapies and other related biological researches.

Ferreira, L. (2009). "Nanoparticles as tools to study and control stem cells." J Cell Biochem **108**(4): 746-752.

The use of nanoparticles in stem cell research is relatively recent, although very significant in the last 5 years with the publication of about 400 papers. The recent advances in the preparation of some nanomaterials, growing awareness of material science and tissue engineering researchers regarding the potential of stem cells for regenerative medicine, and advances in stem cell biology have contributed towards the boost of this research field in the last few years. Most of the research has been focused in the development of new nanoparticles for stem cell imaging; however, these

nanoparticles have several potential applications such as intracellular drug carriers to control stem cell differentiation and biosensors to monitor in real time the intracellular levels of relevant biomolecules/enzymes. This review examines recent advances in the use of nanoparticles for stem cell tracking, differentiation and biosensing. We further discuss their utility and the potential concerns regarding their cytotoxicity.

Ferreira, L., et al. (2008). "New opportunities: the use of nanotechnologies to manipulate and track stem cells." Cell Stem Cell **3**(2): 136-146.

Nanotechnologies are emerging platforms that could be useful in measuring, understanding, and manipulating stem cells. Examples include magnetic nanoparticles and quantum dots for stem cell labeling and in vivo tracking; nanoparticles, carbon nanotubes, and polyplexes for the intracellular delivery of genes/oligonucleotides and protein/peptides; and engineered nanometer-scale scaffolds for stem cell differentiation and transplantation. This review examines the use of nanotechnologies for stem cell tracking, differentiation, and transplantation. We further discuss their utility and the potential concerns regarding their cytotoxicity.

Filali, S., et al. (2020). "Biological Applications and Toxicity Minimization of Semiconductor Quantum Dots." Trends Biotechnol **38**(2): 163-177.

The extraordinary potential of semiconductor quantum dots (QDs) has resulted in their widespread application in various fields, from engineering technology and the development of laboratory techniques to biomedical imaging and therapeutic strategies. However, the toxicity of QDs remains a concern and has limited their applications in human health. Better understanding of the behavior of QDs as it relates to their composition will enable the exploration of their limitations and development of a strategy to control their toxicity for potential therapeutic applications. Here, we describe approaches to minimize their toxicities according to the specific cell type, organ, or animal species, summarizing recent promising research at the cellular, organ, and whole-organism level.

Fishman, J. M., et al. (2012). "Decellularized rabbit cricoarytenoid dorsalis muscle for laryngeal regeneration." Ann Otol Rhinol Laryngol **121**(2): 129-138.

OBJECTIVES: Although considerable progress has been made in regenerative medicine, a quantum step would be the replacement and/or regeneration of functional muscle tissue. For example, although patients' airways can now be successfully replaced with stem cell-based techniques, a much greater patient need would be addressed by regeneration of the muscles required for engineering a functional larynx, in which active movement is critical. The rabbit cricoarytenoid dorsalis muscle was chosen for the present study because it is equivalent to the posterior cricoarytenoid muscle, the only significant abductor muscle in human larynges. METHODS: Rabbit cricoarytenoid dorsalis muscles were harvested, and different decellularization methods were compared by use of a combination of histologic, immunohistochemical, and molecular techniques. Decellularized scaffolds were implanted into Sprague-Dawley rats as part of a 2-week biocompatibility study to assess immunogenicity. RESULTS: Decellularization with a combination of latrunculin B, potassium iodide, potassium chloride, and deoxyribonuclease resulted in total DNA clearance and reduced levels of major histocompatibility complex class II expression, with relative preservation of the scaffold's structural integrity (collagen, elastin, and glycosaminoglycan content). The scaffolds showed minimal signs of rejection at 2 weeks in a cross-species (xenotransplantation) study. CONCLUSIONS: Decellularized laryngeal muscles, which are nonimmunogenic, may provide the optimal scaffold source for the generation of a fully functional tissue-engineered larynx.

Foston, M., et al. (2012). "13C cell wall enrichment and ionic liquid NMR analysis: progress towards a high-throughput detailed chemical analysis of the whole plant cell wall." Analyst **137**(17): 3904-3909.

The ability to accurately and rapidly measure plant cell wall composition, relative monolignol content and lignin-hemicellulose inter-unit linkage distributions has become essential to efforts centered on reducing the recalcitrance of biomass by genetic engineering. Growing (13)C enriched transgenic plants is a viable route to achieve the high-throughput, detailed chemical analysis of whole plant cell wall before and after pretreatment and microbial or enzymatic utilization by (13)C nuclear magnetic resonance (NMR) in a perdeuterated ionic liquid solvent system not requiring component isolation. 1D (13)C whole cell wall ionic liquid NMR of natural abundant and (13)C enriched corn stover stem samples suggest that a high level of uniform labeling (>97%) can significantly reduce the total NMR experiment times up to ~220 times. Similarly, significant reduction in total NMR experiment time (~39 times) of the (13)C enriched corn stover stem samples for 2D (13)C-(1)H heteronuclear single quantum coherence NMR was found.

Frank, N. D., et al. (2019). "Evaluation of reagents used to coat the hollow-fiber bioreactor membrane of the Quantum(R) Cell Expansion System for the culture of human mesenchymal stem cells." Mater Sci Eng C Mater Biol Appl **96**: 77-85.

The addition of a coating reagent to promote cell adherence is necessary to prepare the membrane surface of the Quantum(R) Cell Expansion System hollow-fiber bioreactor for the culture of mesenchymal stem cells. In this study, the efficacy of 8 potential coating reagents has been compared in terms of the doubling times of their cell populations, cell morphology, characterization via flow cytometry, and capacity for trilineage differentiation. Human fibronectin (FN), pooled human cryoprecipitate (CPPT), and recombinant human vitronectin (VN) were successful as coating reagents, and each product has advantages in different cell culture contexts. Mesenchymal stem cells harvested from Quantum cultured with each of these 3 compounds as coating reagents all met International Society for Cellular Therapy standards for plastic adherence, surface marker expression, and successful trilineage differentiation. No significant differences were observed among the doubling times from Quantum harvests using FN, CPPT, or VN as coating reagents (P=0.31). Coating with gelatin, human serum albumin, collagen I, polyllysine, and polydlysine resulted in significantly lower harvest yield; these agents are not recommended for use as coating reagents in the Quantum system.

Fredj, A., et al. (2012). "The single T65S mutation generates brighter cyan fluorescent proteins with increased photostability and pH insensitivity." PLoS One **7**(11): e49149.

Cyan fluorescent proteins (CFP) derived from Aequorea victoria GFP, carrying a tryptophan-based chromophore, are widely used as FRET donors in live cell fluorescence imaging experiments. Recently, several CFP variants with near-ultimate photophysical performances were obtained through a mix of site-directed and large scale random mutagenesis. To understand the structural bases of these improvements, we have studied more specifically the consequences of the single-site T65S mutation. We find that all CFP variants carrying the T65S mutation not only display an increased fluorescence quantum yield and a simpler fluorescence emission decay, but also show an improved pH stability and strongly reduced reversible photoswitching reactions. Most prominently, the Cerulean-T65S variant reaches performances nearly equivalent to those of mTurquoise, with QY = 0.84, an almost pure single exponential fluorescence decay and an outstanding stability in the acid pH range (pK(1/2) = 3.6). From the detailed examination of crystallographic structures of different CFPs and GFPs, we conclude that these improvements stem from a shift in the thermodynamic balance between two well defined configurations of the residue 65 hydroxyl. These two configurations differ in their relative stabilization of a rigid chromophore, as well as in relaying the effects of Glu222 protonation at acid pHs. Our results suggest a simple method to greatly improve numerous FRET reporters used in cell imaging, and bring novel insights into the general structure-photophysics relationships of fluorescent proteins.

Frost, S. H., et al. (2015). "alpha-Imaging Confirmed Efficient Targeting of CD45-Positive Cells After 211At-Radioimmunotherapy for Hematopoietic Cell Transplantation." J Nucl Med **56**(11): 1766-1773.

UNLABELLED: alpha-radioimmunotherapy targeting CD45 may substitute for total-body irradiation in hematopoietic cell transplantation (HCT) preparative regimens for lymphoma. Our goal was to optimize the anti-CD45 monoclonal antibody (mAb; CA12.10C12) protein dose for (211)At-radioimmunotherapy, extending the analysis to include intraorgan (211)At activity distribution and alpha-imaging-based small-scale dosimetry, along with immunohistochemical staining. METHODS: Eight normal dogs were injected with either a 0.75 (n = 5) or 1.00 (n = 3) mg/kg dose of (211)At-B10-CA12.10C12 (11.5-27.6 MBq/kg). Two were euthanized and necropsied 19-22 h after injection, and 6 received autologous HCT 3 d after (211)At-radioimmunotherapy, after lymph node and bone marrow biopsies at 2-4 and/or 19 h after injection. Blood was sampled to study toxicity and clearance; CD45 targeting was evaluated by flow cytometry. (211)At localization and small-scale dosimetry were assessed using two alpha-imaging systems: an alpha-camera and an ionizing-radiation quantum imaging detector (iQID) camera. RESULTS: (211)At uptake was highest in the spleen (0.31-0.61% injected activity [%IA]/g), lymph nodes (0.02-0.16 %IA/g), liver (0.11-0.12 %IA/g), and marrow (0.06-0.08 %IA/g). Lymphocytes in blood and marrow were efficiently targeted using either mAb dose. Lymph nodes remained unsaturated but displayed targeted (211)At localization in T lymphocyte-rich areas. Absorbed doses to blood, marrow, and lymph nodes were estimated at 3.1, 2.4, and 3.4 Gy/166 MBq, respectively. All transplanted dogs experienced transient hepatic toxicity. Liver enzyme levels were temporarily elevated in 5 of 6 dogs; one treated with 1.00 mg mAb/kg developed ascites and was euthanized 136 d after HCT. CONCLUSION: (211)At-anti-CD45 radioimmunotherapy with 0.75 mg mAb/kg efficiently targeted blood and marrow without severe toxicity. Dosimetry calculations and observed radiation-induced effects indicated that sufficient (211)At-B10-CA12.10C12 localization was achieved for efficient conditioning for HCT.

Galeano, C., et al. (2018). "The Route by Which Intranasally Delivered Stem Cells Enter the Central Nervous System." Cell Transplant **27**(3): 501-514.

Intranasal administration is a promising route of delivery of stem cells to the central nervous system (CNS). Reports on this mode of stem cell delivery have not yet focused on the route across the cribriform plate by which cells move from the nasal cavity into the CNS. In the current experiments, human mesenchymal stem cells (MSCs) were isolated from Wharton's jelly of umbilical cords and were labeled with extremely bright quantum dots (QDs) in order to track the cells efficiently. At 2 h after intranasal delivery in immunodeficient mice, the labeled cells were found under the olfactory epithelium, crossing the cribriform plate adjacent to the fila olfactoria, and associated with the meninges of the olfactory bulb. At all times, the cells were separate from actual nerve tracts; this location is consistent with them being in the subarachnoid space (SAS) and its extensions through the cribriform plate into the nasal mucosa. In their location under the olfactory epithelium, they appear to be within an expansion of a potential space adjacent to the turbinate bone periosteum. Therefore, intranasally administered stem cells appear to cross the olfactory epithelium, enter a space adjacent to the periosteum of the turbinate bones, and then enter the SAS via its extensions adjacent to the fila olfactoria as they cross the cribriform plate. These observations should enhance understanding of the mode by which stem cells can reach the CNS from the nasal cavity and may guide future experiments on making intranasal delivery of stem cells efficient and reproducible.

Ganesh, S., et al. (2020). "Quantum scale organic semiconductors for SERS detection of DNA methylation and gene expression." Nat Commun **11**(1): 1135.

Cancer stem cells (CSC) can be identified by modifications in their genomic DNA. Here, we report a concept of precisely shrinking an organic semiconductor surface-enhanced Raman scattering (SERS) probe to quantum size, for investigating the epigenetic profile of CSC. The probe is used for tag-free genomic DNA detection, an approach towards the advancement of single-molecule DNA detection. The sensor detected structural, molecular and gene expression aberrations of genomic DNA in femtomolar concentration simultaneously in a single test. In addition to pointing out the divergences in genomic DNA of cancerous and non-cancerous cells, the quantum scale organic semiconductor was able to trace the expression of two genes which are frequently used as CSC markers. The quantum scale organic semiconductor holds the potential to be a new tool for label-free, ultra-sensitive multiplexed genomic analysis.

Gao, M., et al. (2016). "Long-Term Tracking of the Osteogenic Differentiation of Mouse BMSCs by Aggregation-Induced Emission Nanoparticles." ACS Appl Mater Interfaces **8**(28): 17878-17884.

Bone marrow-derived mesenchymal stem cells (BMSCs) have shown great potential for bone repair due to their strong proliferation ability and osteogenic capacity. To evaluate and improve the stem cell-based therapy, long-term tracking of stem cell differentiation into bone-forming osteoblasts is required. However, conventional fluorescent trackers such as fluorescent proteins, quantum dots, and fluorophores with aggregation-caused quenching (ACQ) characteristics have intrinsic limitations of possible interference with stem cell differentiation, heavy metal cytotoxicity, and self-quenching at a high labeling intensity. Herein, we developed aggregation-induced emission nanoparticles decorated with the Tat peptide (AIE-Tat NPs) for long-term tracking of the osteogenic differentiation of mouse BMSCs without interference of cell viability and differentiation ability. Compared with the ability of the commercial Qtracker 655 for tracking of only 6 passages of mouse BMSCs, AIE-Tat NPs have shown a much superior performance in long-term tracking for over 12 passages. Moreover, long-term tracking of the osteogenic differentiation process of mouse BMSCs was successfully conducted on the biocompatible hydroxyapatite scaffold, which is widely used in bone tissue engineering. Thus, AIE-Tat NPs have promising applications in tracking stem cell fate for bone repair.

Gao, Y., et al. (2016). "A Selective and Purification-Free Strategy for Labeling Adherent Cells with Inorganic Nanoparticles." ACS Appl Mater Interfaces **8**(10): 6336-6343.

Cellular labeling with inorganic nanoparticles such as magnetic iron oxide nanoparticles, quantum dots, and fluorescent silica nanoparticles is an important method for the noninvasive visualization of cells using various imaging modalities. Currently, this is mainly achieved through the incubation of cultured cells with the nanoparticles that eventually reach the intracellular compartment through specific or nonspecific internalization. This classic method is advantageous in terms of simplicity and convenience, but it suffers from issues such as difficulties in fully removing free nanoparticles (suspended in solution) and the lack of selectivity on cell types. This article reports an innovative strategy for the specific labeling of adherent cells without the concern of freely suspended nanoparticles. This method relies on a nanocomposite film that is prepared by homogeneously dispersing nanoparticles within a biodegradable polymeric film. When adherent cells are seeded on the film, they adhere, spread, and filtrate into the film through the micropores formed during the film fabrication. The pre-embedded nanoparticles are thus internalized by the cells during this infiltration process. As an example, fluorescent

silica nanoparticles were homogeneously distributed within a polycaprolactone film by utilizing cryomilling and heat pressing. Upon incubation within physiological buffer, no silica nanoparticles were released from the nanocomposite film even after 20 d of incubation. However, when adherent cells (e.g., human mesenchymal stem cells) were grown on the film, they became fluorescent after 3 d, which suggests internalization of silica nanoparticles by cells. In comparison, the suspension cells (e.g., monocytes) in the medium remained nonfluorescent no matter whether there was the presence of adherent cells or not. This strategy eventually allowed the selective and concomitant labeling of mesenchymal stem cells during their harvest from bone marrow aspiration.

Gao, Y. and T. Zhang (2021). "The Application of Nanomaterials in Cell Autophagy." Curr Stem Cell Res Ther **16**(1): 23-35.

Autophagy is defined as separation and degradation of cytoplasmic components through autophagosomes, which plays an essential part in physiological and pathological events. Hence it is also essential for cellular homeostasis. Autophagy disorder may bring about the failure of stem cells to maintain the fundamental transformation and metabolism of cell components. However, for cancer cells, the disorder of autophagy is a feasible antitumor idea. Nanoparticles, referring to particles of the size range 1-100 nanometers, are appearing as a category of autophagy regulators. These nanoparticles may revolutionize and broaden the therapeutic strategies of many diseases, including neurodegenerative diseases, tumors, muscle disease, and so on. Researches of autophagy-induced nanomaterials mainly focus on silver particles, gold particles, silicon particles, and rare earth oxides. But in recent years, more and more materials have been found to regulate autophagy, such as nano-nucleic acid materials, nanofiber scaffolds, quantum dots, and so on. The review highlights that various kinds of nanoparticles have the power to regulate autophagy intensity in stem cells of interest and further control biological behaviors, which may become a reliable treatment choice for disease therapy.

Genc, D., et al. (2022). "Dental follicle mesenchymal stem cells ameliorated glandular dysfunction in Sjogren's syndrome murine model." PLoS One **17**(5): e0266137.

OBJECTIVE: Dental mesenchymal stem cells (MSCs) are potential for use in tissue regeneration in inflammatory diseases due to their rapid proliferating, multilineage differentiation, and strong anti-inflammatory features. In the present study, immunoregulatory and glandular tissue regeneration effects of the dental follicle (DF)MSCs in Sjogren's Syndrome (SS) were investigated. METHODS: Dental follicle (DF) tissues were obtained from healthy individuals during tooth extraction, tissues were digested enzymatically and DFMSCs were cultured until the third passage. DFMSCs were labeled with Quantum dot 655 for cell tracking analysis. The induction of the SS mouse model was performed by the injection of Ro60-273-289 peptide intraperitoneally. DFMSCs were injected intraperitoneally, or into submandibular, or lacrimal glands. Splenocytes were analyzed for intracellular cytokine (IFN-gamma, IL-17, IL-10) secretion in T helper cells, lymphocyte proliferation, and B lymphocyte subsets. Histologic analysis was done for submandibular and lacrimal glands with hematoxylin-eosin staining for morphologic examination. RESULTS: The systemic injection of DFMSCs significantly reduced intracellular IFN-gamma and IL-17 secreting CD4+ T cells in splenocytes ($p<0.05$), and decreased inflammatory cell deposits and fibrosis in the glandular tissues. DFMSCs differentiated to glandular epithelial cells in submandibular and lacrimal injections with a significant reduction in lymphocytic foci. The results showed that few amounts of DFMSCs were deposited in glandular tissues when applied intraperitoneally, while high amounts of DFMSCs were located in glandular tissues and differentiated to glandular epithelial cells when applied locally in SS murine model. CONCLUSION: DFMSCs have the potential for the regulation of Th1, Th17, and Treg balance in SS, and ameliorate glandular dysfunction. DFMSCs can be a beneficial therapeutic application for SS.

Geng, H., et al. (2018). "Achieving stem cell imaging and osteogenic differentiation by using nitrogen doped graphene quantum dots." J Mater Sci Mater Med **29**(6): 85.

Nitrogen doped graphene quantum dots (N-GQDs) were synthesized to explore and extend their potential applications in biomedical field. The hemocompatibility and cytotoxity of the obtained N-GQDs were primarily assessed at concentrations ranging from 10 to 100 mug/ml. From the results, it was found that the proliferation of rat Bone Mesenchymal Stem Cells (rBMSCs) was depressed to a certain extent after incubating with the high concentration (100 mug/ml) of N-GQDs. The nanoscale size and superior dispersibility endow N-GQDs with good cell permeability. Meanwhile, owing to their intrinsic photoluminescence characteristic, the N-GQDs can be used to label cells with high uniformity and light stability in absence of chemical dyes. More importantly, the up-regulated expression of alkaline phosphate (ALP), extracellular matrix, osteopontin (OPN) and osteocalcin (OCN) in rBMSCs cultured with N-GQDs, indicating N-GQDs have the abilities to promote rBMSCs

osteogenic differentiation. This work would help give a new insight into the advantages of N-GQDs and pave the way for application of N-GQDs in regenerative medicine fields.

Genicio, N., et al. (2015). "Quantum dot labeling and tracking of cultured limbal epithelial cell transplants in vitro." Invest Ophthalmol Vis Sci **56**(5): 3051-3059.

PURPOSE: Cultured human limbal epithelial cells (HLECs) have shown promise in the treatment of limbal stem cell deficiency but little is known about their survival, behavior, and long-term fate after transplantation. The aim of this research was to evaluate, in vitro, quantum dot (Qdot) technology as a tool for tracking transplanted HLECs. METHODS: In vitro cultured HLECs were labeled with Qdot nanocrystals. Toxicity was assessed using live-dead assays. The effect on HLEC function was assessed using colony-forming efficiency assays and expression of CK3, P63alpha, and ABCG2. Sheets of cultured HLECs labeled with Qdot nanocrystals were transplanted onto decellularized human corneoscleral rims in an organ culture model and observed to investigate the behavior of transplanted cells. RESULTS: Quantum dot labeling had no detrimental effect on HLEC viability or function in vitro. Proliferation resulted in a gradual reduction in Qdot signal but sufficient signal was present to allow tracking of cells through multiple generations. Cells labeled with Qdots could be reliably detected and observed using confocal microscopy for at least 2 weeks after transplantation in our organ culture model. In addition, it was possible to label and observe epithelial cells in intact human corneas by using the Rostock corneal module adapted for use with the Heidelberg HRA. CONCLUSIONS: This work demonstrates that Qdots combined with existing clinical equipment could be used to track HLEC for up to 2 weeks after transplantation; however, our model does not permit the assessment of cell labeling beyond 2 weeks. Further characterization in in vivo models are required.

Ghanbari, M., et al. (2021). "Injectable hydrogels based on oxidized alginate-gelatin reinforced by carbon nitride quantum dots for tissue engineering." Int J Pharm **602**: 120660.

Stem cell treatment is promising in the various disorders treatment, but its effect is confined by the adverse conditions in the damaged tissues. The utilization of hydrogels has been suggested as a procedure to defeat this issue by developing the engraftment and survival of injected stem cells. Specifically, injectable hydrogels have drawn much attention due to their shape adaptability, ease of use, and the capability to reach body parts that are hard to access. In this study, the thermosensitive injectable hydrogels based on oxidized alginate, gelatin, and carbon nitride quantum dots (CNQDs) have been fabricated for tissue engineering. The mechanical characteristics of the nanocomposite hydrogels were investigated by rheology analysis. The results show that increasing the amount of CNQDs improve the mechanical strength of the nanocomposite hydrogels. The Cross-section morphology of freeze dried hydrogels comprising 0.25, 1.5, and 3.0% CNQDs indicate porous structure with interrelated pores. Besides, the result of in vitro degradation reveals that the hydrogels comprising CNQDs are more durable than the one without CNQDs. A reduction in the biodegradation and swelling ratio is perceived with the addition of CNQDs. The cell viability and attachment show that the nanocomposite hydrogels are biocompatible (>88%) with great cell adhesion to osteosarcoma cell line MG63 depending on the presence of CNQDs.

Gharbavi, M., et al. (2020). "Mesenchymal Stem Cells: A New Generation of Therapeutic Agents as Vehicles in Gene Therapy." Curr Gene Ther **20**(4): 269-284.

In recent years, mesenchymal stem cells (MSCs) as a new tool for therapeutic gene delivery in clinics have attracted much attention. Their advantages cover longer lifespan, better isolation, and higher transfection efficiency and proliferation rate. MSCs are the preferred approach for cell-based therapies because of their in vitro self-renewal capacity, migrating especially to tumor tissues, as well as anti-inflammatory and immunomodulatory properties. Therefore, they have considerable efficiency in genetic engineering for future clinical applications in cancer gene therapy and other diseases. For improving therapeutic efficiency, targeted therapy of cancers can be achieved through the sustained release of therapeutic agents and functional gene expression induction to the intended tissues. The development of a new vector in gene therapy can improve the durability of a transgene expression. Also, the safety of the vector, if administered systemically, may resolve several problems, such as durability of expression and the host immune response. Currently, MSCs are prominent candidates as cell vehicles for both preclinical and clinical trials due to the secretion of therapeutic agents in several cancers. In the present study, we discuss the status of gene therapy in both viral and non-viral vectors along with their limitations. Throughout this study, the use of several nano-carriers for gene therapy is also investigated. Finally, we critically discuss the promising advantages of MSCs in targeted gene delivery, tumor inhibition and their utilization as the gene carriers in clinical situations.

Ghorbanzade, S., et al. (2020). "Multifunctional Magnetic Nanoparticles-Labeled Mesenchymal Stem Cells for Hyperthermia and Bioimaging Applications." Methods Mol Biol **2125**: 57-72.

Magnetic nanoparticles have demonstrated considerable capacity for theranosis purposes due to their unique characteristics, including magnetic properties, comparable size to biomolecules, favorable conjugations of drugs and biomolecules, ability to labeling, and capability of sensing, separation, detection, and targeted drug delivery. They could be exploited in magnetic resonance imaging as the contrast agents and also warmed as exposed to an external magnetic AC field that could be applied in hyperthermia. Here, progresses and advances in the strategy and assembly of fluorescent magnetic nanoparticles are presented for stem cell tracing and drugs/biomolecules targeting into cells.

Gibney, E. (2016). "2017 sneak peek: What the new year holds for science." Nature **541**(7635): 14-15.

Grady, S. T., et al. (2019). "Persistence of fluorescent nanoparticle-labelled bone marrow mesenchymal stem cells in vitro and after intra-articular injection." J Tissue Eng Regen Med **13**(2): 191-202.

Mesenchymal stem cells (MSCs) improve the osteoarthritis condition, but the fate of MSCs after intra-articular injection is unclear. We used fluorescent nanoparticles (quantum dots [QDs]) to track equine MSCs (QD-labelled MSCs [QD-MSCs]) in vivo after intra-articular injection into normal and osteoarthritic joints. One week after injection of QD-MSCs, unlabelled MSCs, or vehicle, we determined the presence of QD-MSCs in synovium and articular cartilage histologically. In vitro, we evaluated the persistence of QDs in MSCs and whether QDs affected proliferation, immunophenotype, or differentiation. In joints injected with QD-MSCs, labelled cells were identified on the synovial membrane and significantly less often on articular cartilage, without differences between normal and osteoarthritic joints. Joints injected with QD-MSCs and MSCs had increased synovial total nucleated cell count and protein compared with vehicle-injected joints. In vitro, QDs persisted in nonproliferating cells for up to 8 weeks (length of the study), but QD fluorescence was essentially absent from proliferating cells within two passages (approximately 3 to 5 days). QD labelling did not affect MSC differentiation into chondrocytes, adipocytes, and osteocytes. QD-MSCs had slightly different immunophenotype from control cells, but whether this was due to an effect of the QDs or to drift during culture is unknown. QD-MSCs can be visualized in histological sections 1 week after intra-articular injection and are more frequently found in the synovial membrane versus cartilage in both normal and osteoarthritic joints. QDs do not alter MSC viability and differentiation potential in vitro. However, QDs are not optimal markers for long-term tracking of MSCs, especially under proliferative conditions.

Grigsby, C. L., et al. (2013). "Microfluidic preparation of polymer-nucleic acid nanocomplexes improves nonviral gene transfer." Sci Rep **3**: 3155.

As the designs of polymer systems used to deliver nucleic acids continue to evolve, it is becoming increasingly apparent that the basic bulk manufacturing techniques of the past will be insufficient to produce polymer-nucleic acid nanocomplexes that possess the uniformity, stability, and potency required for their successful clinical translation and widespread commercialization. Traditional bulk-prepared products are often physicochemically heterogeneous and may vary significantly from one batch to the next. Here we show that preparation of bioreducible nanocomplexes with an emulsion-based droplet microfluidic system produces significantly improved nanoparticles that are up to fifty percent smaller, more uniform, and are less prone to aggregation. The intracellular integrity of nanocomplexes prepared with this microfluidic method is significantly prolonged, as detected using a high-throughput flow cytometric quantum dot Forster resonance energy transfer nanosensor system. These physical attributes conspire to consistently enhance the delivery of both plasmid DNA and messenger RNA payloads in stem cells, primary cells, and human cell lines. Innovation in processing is necessary to move the field toward the broader clinical implementation of safe and effective nonviral nucleic acid therapeutics, and preparation with droplet microfluidics represents a step forward in addressing the critical barrier of robust and reproducible nanocomplex production.

Gu, H., et al. (2015). "A Stem Cell-Derived Platform for Studying Single Synaptic Vesicles in Dopaminergic Synapses." Stem Cells Transl Med **4**(8): 887-893.

The exocytotic release of dopamine is one of the most characteristic but also one of the least appreciated processes in dopaminergic neurotransmission. Fluorescence imaging has yielded rich information about the properties of synaptic vesicles and the release of neurotransmitters in excitatory and inhibitory neurons. In contrast, imaging-based studies for in-depth understanding of synaptic vesicle behavior in dopamine neurons are lagging largely because of a lack of suitable preparations. Midbrain culture has been one of the most valuable preparations for the subcellular investigation of dopaminergic transmission; however, the paucity and fragility of cultured dopaminergic neurons limits their use for live cell imaging. Recent developments in stem cell technology have led to the successful production of dopamine neurons from embryonic or induced pluripotent stem cells. Although the dopaminergic

identity of these stem cell-derived neurons has been characterized in different ways, vesicle-mediated dopamine release from their axonal terminals has been barely assessed. We report a more efficient procedure to reliably generate dopamine neurons from embryonic stem cells, and it yields more dopamine neurons with more dopaminergic axon projections than midbrain culture does. Using a collection of functional measurements, we show that stem cell-derived dopamine neurons are indistinguishable from those in midbrain culture. Taking advantage of this new preparation, we simultaneously tracked the turnover of hundreds of synaptic vesicles individually using pH-sensitive quantum dots. By doing so, we revealed distinct fusion kinetics of the dopamine-secreting vesicles, which is consistent within both preparations.

Guo, R., et al. (2015). "Rhodamine-Functionalized Graphene Quantum Dots for Detection of Fe(3+) in Cancer Stem Cells." ACS Appl Mater Interfaces **7**(43): 23958-23966.

A turn-on orange-red fluorescent nanosensor based on rhodamine B derivative-functionalized graphene quantum dots (RBD-GQDs) has been successfully synthesized for Fe(3+) detection with high sensitivity and selectivity. By connecting with GQDs, the water solubility, sensitivity, photostability, and biocompatibility of RBD are drastically improved. The most distinctive feature of the RBD-GQDs, which sets them apart from other previously reported fluorophores or GQDs, is that they with the detection limits as low as 0.02 muM are demonstrated as a Fe(3+) turn-on fluorescent nanosensor in cancer stem cells. Fe(3+) binding to such GQDs (RBD-GQDs-Fe(3+)) with orange-red fluorescence of 43% quantum yield were demonstrated to be the biomarkers for cancer stem cell imaging.

Guo, Z., et al. (2018). "Fluorescent Ti3C2 MXene quantum dots for an alkaline phosphatase assay and embryonic stem cell identification based on the inner filter effect." Nanoscale **10**(41): 19579-19585.

As an emerging two-dimensional material, MXenes have attracted much attention due to their unique physicochemical properties, but their application in biosensing has been lagging far behind because of their poor salt tolerance. Herein, a titanium carbide MXene quantum dot (Ti3C2 QD)-based fluorescent probe for the alkaline phosphatase (ALP) activity assay and embryonic stem cell (ESC) identification was developed by taking advantage of the inner filter effect (IFE). Ti3C2 QDs with approximately 4.2 nm in diameter were prepared from Ti3C2 MXenes by hydrothermal treatment, exhibiting excellent salt tolerance, anti-photobleaching and dispersion stability in aqueous solution. Owing to the remarkable overlap between the absorption spectrum of p-nitrophenol (p-NP) and the excitation and emission spectrum of Ti3C2 QDs, p-NP generated from ALP-catalyzed dephosphorylation of the substrate, p-nitrophenyl phosphate (p-NPP), can effectively quench the fluorescence of Ti3C2 QDs through the IFE. As a result, sensitive fluorometric analysis of ALP activity was achieved without complicating the conventional colorimetric ALP detection system. The proposed assay was successfully applied to determine ALP activity with a low limit of detection (0.02 U L(-1)) as well as monitoring the enzyme activity in real time. Finally, accurate analysis of ALP, the biomarker of ESC, in ESC lysates was also achieved using this IFE-based method, affording an alternative method for ESC identification.

Guyette, J. P., et al. (2013). "A novel suture-based method for efficient transplantation of stem cells." J Biomed Mater Res A **101**(3): 809-818.

Advances in regenerative medicine have improved the potential of using cellular therapy for treating several diseases. However, the effectiveness of new cellular therapies is largely limited by low cell engraftment and inadequate localization. To improve on these limitations, we developed a novel delivery mechanism using cell-seeded biological sutures. We demonstrate the ability of cell-seeded biological sutures to efficiently implant human mesenchymal stem cells (hMSCs) to specific regions within the beating heart; a tissue known to have low cell retention and engraftment shortly after delivery. Cell-seeded biological sutures were developed by bundling discrete microthreads extruded from extracellular matrix proteins, attaching a surgical needle to the bundle and seeding the bundle with hMSCs. During cell preparation, hMSCs were loaded with quantum dot nanoparticles for cell tracking within the myocardium. Each biological suture contained an average of 5903 +/- 1966 hMSCs/cm suture length. Delivery efficiency was evaluated by comparing cell-seeded biological suture implantation with intramyocardial (IM) cell injections (10,000 hMSCs in 35 muL) into the left ventricle of normal, noninfarcted rat hearts after 1 h. Delivery efficiency of hMSCs by biological sutures (63.6 +/- 10.6%) was significantly higher than IM injection (11.8 +/- 6.2%; $p < 0.05$). Cell-tracking analysis indicated suture-delivered hMSCs were found throughout the thickness of the ventricular myocardium: along the entire length of the biological suture track, localizing closely with native myocardium. These results suggest cell-seeded biological sutures can deliver cells to the heart more efficiently than conventional methods, demonstrating an effective delivery method for implanting cells in soft tissue.

Haack-Sorensen, M., et al. (2016). "Culture expansion of adipose derived stromal cells. A closed automated Quantum Cell Expansion System compared with manual flask-based culture." J Transl Med **14**(1): 319.

BACKGROUND: Adipose derived stromal cells (ASCs) are a rich and convenient source of cells for clinical regenerative therapeutic approaches. However, applications of ASCs often require cell expansion to reach the needed dose. In this study, cultivation of ASCs from stromal vascular fraction (SVF) over two passages in the automated and functionally closed Quantum Cell Expansion System (Quantum system) is compared with traditional manual cultivation. METHODS: Stromal vascular fraction was isolated from abdominal fat, suspended in alpha-MEM supplemented with 10% Fetal Bovine Serum and seeded into either T75 flasks or a Quantum system that had been coated with cryoprecipitate. The cultivation of ASCs from SVF was performed in 3 ways: flask to flask; flask to Quantum system; and Quantum system to Quantum system. In all cases, quality controls were conducted for sterility, mycoplasmas, and endotoxins, in addition to the assessment of cell counts, viability, immunophenotype, and differentiation potential. RESULTS: The viability of ASCs passage 0 (P0) and P1 was above 96%, regardless of cultivation in flasks or Quantum system. Expression of surface markers and differentiation potential was consistent with ISCT/IFATS standards for the ASC phenotype. Sterility, mycoplasma, and endotoxin tests were consistently negative. An average of 8.0 x 10(7) SVF cells loaded into a Quantum system yielded 8.96 x 10(7) ASCs P0, while 4.5 x 10(6) SVF cells seeded per T75 flask yielded an average of 2.37 x 10(6) ASCs-less than the number of SVF cells seeded. ASCs P1 expanded in the Quantum system demonstrated a population doubling (PD) around 2.2 regardless of whether P0 was previously cultured in flasks or Quantum, while ASCs P1 in flasks only reached a PD of 1.0. CONCLUSION: Manufacturing of ASCs in a Quantum system enhances ASC expansion rate and yield significantly relative to manual processing in T-flasks, while maintaining the purity and quality essential to safe and robust cell production. Notably, the use of the Quantum system entails significantly reduced working hours and thereby costs.

Haack-Sorensen, M., et al. (2018). "Development of large-scale manufacturing of adipose-derived stromal cells for clinical applications using bioreactors and human platelet lysate." Scand J Clin Lab Invest **78**(4): 293-300.

In vitro expanded adipose-derived stromal cells (ASCs) are a useful resource for tissue regeneration. Translation of small-scale autologous cell production into a large-scale, allogeneic production process for clinical applications necessitates well-chosen raw materials and cell culture platform. We compare the use of clinical-grade human platelet lysate (hPL) and fetal bovine serum (FBS) as growth supplements for ASC expansion in the automated, closed hollow fibre quantum cell expansion system (bioreactor). Stromal vascular fractions were isolated from human subcutaneous abdominal fat. In average, 95 x 10(6) cells were suspended in 10% FBS or 5% hPL medium, and loaded into a bioreactor coated with cryoprecipitate. ASCs (P0) were harvested, and 30 x 10(6) ASCs were reloaded for continued expansion (P1). Feeding rate and time of harvest was guided by metabolic monitoring. Viability, sterility, purity, differentiation capacity, and genomic stability of ASCs P1 were determined. Cultivation of SVF in hPL medium for in average nine days, yielded 546 x 10(6) ASCs compared to 111 x 10(6) ASCs, after 17 days in FBS medium. ASCs P1 yields were in average 605 x 10(6) ASCs (PD [population doublings]: 4.65) after six days in hPL medium, compared to 119 x 10(6) ASCs (PD: 2.45) in FBS medium, after 21 days. ASCs fulfilled ISCT criteria and demonstrated genomic stability and sterility. The use of hPL as a growth supplement for ASCs expansion in the quantum cell expansion system provides an efficient expansion process compared to the use of FBS, while maintaining cell quality appropriate for clinical use. The described process is an obvious choice for manufacturing of large-scale allogeneic ASC products.

Haghshenas, M., et al. (2019). "Use of embryonic fibroblasts associated with graphene quantum dots for burn wound healing in Wistar rats." In Vitro Cell Dev Biol Anim **55**(4): 312-322.

Burn is one of the common wounds in the world and using modern methods such as cell therapy can be considered as an effective strategy in the treatment of these wounds. The aim of this study is investigating the effects of using graphene quantum dots (GQDs) associated fibroblasts on treating third-degree burns in Wistar rats. In this experiment, cells were obtained by isolating fibroblasts from 13-day embryos of Wistar rats. MTT assay was performed to determine the dose of nanoparticle and cell tracker. For this study, 40 Wistar rats were burned and randomly divided into two groups of control and treatment. The treatment group was divided into three groups of daily injection of GQD nanoparticle with a concentration of 100 mug/ml, cell therapy, and cell therapy + GQDs. On days 20 and 40, skin tissue sections were prepared and stained with hematoxylin-eosin (H&E) and trichrome Masson for microscopic examination. Macroscopic and microscopic observations showed that in the treatment groups, the recovery was higher than the control. Also, cell therapy and GQD injection and simultaneous injection of cell therapy + GQDs accelerated the wound healing process and the cell therapy + GQDs were significantly more

effective than nanoparticles and cell injection alone after 20 and 40 days. Histological studies indicated a significant increase in angiogenesis, number of cells, collagen synthesis, thickness of skin layers, and ultimately acceleration wound healing in treatment samples compared to controls. Based on these results, it can be concluded that simultaneous cell therapy and GQDs accelerate the repair of skin lesions in the animal models more significantly.

Hajihashemi, S., et al. (2018). "Effect of Cold Stress on Photosynthetic Traits, Carbohydrates, Morphology, and Anatomy in Nine Cultivars of Stevia rebaudiana." Front Plant Sci **9**: 1430.

Stevia rebaudiana Bertoni is a sweet medicinal herb that is cultivated worldwide. This study aimed to identify the genotypic responses and function of nine cultivars of S. rebaudiana (accession numbers 1-9 from the EUSTAS Stevia Gene Bank) to low temperature. Plants were grown in vitro and incubated under controlled conditions at 5 degrees or 25 degrees C for 1 month. Cold stress significantly decreased the maximum quantum yield of photosystem II (Fv/Fm) in all cultivars, which was more pronounced in cultivars 5, 6, 8, and 9. The efficiency of photosystems I and II (PIABS) also declined in cold-stressed plants and was accompanied by reductions in net photosynthesis (PN), intercellular CO2 (Ci), water use efficiency (WUE), and chlorophyll a, chlorophyll b and carotenoid contents, more so in cultivars 5, 6, 8, and 9. Regardless of the downregulation of photosynthetic capacity, the cold stress increased water-soluble carbohydrates in all cultivars, which was accompanied by an increase in fresh leaf mass and area, more so in cultivars 5, 6, 8, and 9. Furthermore, cold stress increased the stomatal index and density, epidermal cell density, stem diameter, xylem vessel width, phloem tissue width, and number of sclerenchyma in all cultivars. Even though the nine cultivars of S. rebaudiana had lower PSII efficiencies at low temperatures, the increase in carbohydrates and leaf mass suggests that damage to PSII is not responsible for the reduction in its efficiency.

Hameroff, S. R. (2004). "A new theory of the origin of cancer: quantum coherent entanglement, centrioles, mitosis, and differentiation." Biosystems **77**(1-3): 119-136.

Malignant cells are characterized by abnormal segregation of chromosomes during mitosis ("aneuploidy"), generally considered a result of malignancy originating in genetic mutations. However, recent evidence supports a century-old concept that maldistribution of chromosomes (and resultant genomic instability) due to abnormalities in mitosis itself is the primary cause of malignancy rather than a mere byproduct. In normal mitosis chromosomes replicate into sister chromatids which are then precisely separated and transported into mirror-like sets by structural protein assemblies called mitotic spindles and centrioles, both composed of microtubules. The elegant yet poorly understood ballet-like movements and geometric organization occurring in mitosis have suggested guidance by some type of organizing field, however neither electromagnetic nor chemical gradient fields have been demonstrated or shown to be sufficient. It is proposed here that normal mirror-like mitosis is organized by quantum coherence and quantum entanglement among microtubule-based centrioles and mitotic spindles which ensure precise, complementary duplication of daughter cell genomes and recognition of daughter cell boundaries. Evidence and theory supporting organized quantum states in cytoplasm/nucleoplasm (and quantum optical properties of centrioles in particular) at physiological temperature are presented. Impairment of quantum coherence and/or entanglement among microtubule-based mitotic spindles and centrioles can result in abnormal distribution of chromosomes, abnormal differentiation and uncontrolled growth, and account for all aspects of malignancy. New approaches to cancer therapy and stem cell production are suggested via non-thermal laser-mediated effects aimed at quantum optical states of centrioles.

Han, H. S., et al. (2015). "Quantum dot/antibody conjugates for in vivo cytometric imaging in mice." Proc Natl Acad Sci U S A **112**(5): 1350-1355.

Multiplexed, phenotypic, intravital cytometric imaging requires novel fluorophore conjugates that have an appropriate size for long circulation and diffusion and show virtually no nonspecific binding to cells/serum while binding to cells of interest with high specificity. In addition, these conjugates must be stable and maintain a high quantum yield in the in vivo environments. Here, we show that this can be achieved using compact (approximately 15 nm in hydrodynamic diameter) and biocompatible quantum dot (QD) -Ab conjugates. We developed these conjugates by coupling whole mAbs to QDs coated with norbornene-displaying polyimidazole ligands using tetrazine-norbornene cycloaddition. Our QD immunoconstructs were used for in vivo single-cell labeling in bone marrow. The intravital imaging studies using a chronic calvarial bone window showed that our QD-Ab conjugates diffuse into the entire bone marrow and efficiently label single cells belonging to rare populations of hematopoietic stem and progenitor cells (Sca1(+)c-Kit(+) cells). This in vivo cytometric technique may be useful in a wide range of structural and functional imaging to study the interactions between cells and between a cell and its environment in intact and diseased tissues.

Hanley, P. J., et al. (2014). "Efficient manufacturing of therapeutic mesenchymal stromal cells with the use of the Quantum Cell Expansion System." Cytotherapy **16**(8): 1048-1058.

BACKGROUND: The use of bone marrow-derived mesenchymal stromal cells (MSCs) as a cellular therapy for various diseases, such as graft-versus-host disease, diabetes, ischemic cardiomyopathy and Crohn's disease, has produced promising results in early-phase clinical trials. However, for widespread application and use in later phase studies, manufacture of these cells must be cost-effective, safe and reproducible. Current methods of manufacturing in flasks or cell factories are labor-intensive, involve a large number of open procedures and require prolonged culture times. METHODS: We evaluated the Quantum Cell Expansion System for the expansion of large numbers of MSCs from unprocessed bone marrow in a functionally closed system and compared the results with a flask-based method currently in clinical trials. RESULTS: After only two passages, we were able to expand a mean of 6.6 x 10(8) MSCs from 25 mL of bone marrow reproducibly. The mean expansion time was 21 days, and cells obtained were able to differentiate into all three lineages: chondrocytes, osteoblasts and adipocytes. The Quantum was able to generate the target cell number of 2.0 x 10(8) cells in an average of 9 fewer days and in half the number of passages required during flask-based expansion. We estimated that the Quantum would involve 133 open procedures versus 54,400 in flasks when manufacturing for a clinical trial. Quantum-expanded MSCs infused into an ischemic stroke rat model were therapeutically active. CONCLUSIONS: The Quantum is a novel method of generating high numbers of MSCs in less time and at lower passages when compared with flasks. In the Quantum, the risk of contamination is substantially reduced because of the substantial decrease in open procedures.

Hansen, K. J., et al. (2016). "Functional Effects of Delivering Human Mesenchymal Stem Cell-Seeded Biological Sutures to an Infarcted Heart." Biores Open Access **5**(1): 249-260.

Stem cell therapy has the potential to improve cardiac function after myocardial infarction (MI); however, existing methods to deliver cells to the myocardium, including intramyocardial injection, suffer from low engraftment rates. In this study, we used a rat model of acute MI to assess the effects of human mesenchymal stem cell (hMSC)-seeded fibrin biological sutures on cardiac function at 1 week after implant. Biological sutures were seeded with quantum dot (Qdot)-loaded hMSCs for 24 h before implantation. At 1 week postinfarct, the heart was imaged to assess mechanical function in the infarct region. Regional parameters assessed were regional stroke work (RSW) and systolic area of contraction (SAC) and global parameters derived from the pressure waveform. MI (n = 6) significantly decreased RSW (0.026 +/- 0.011) and SAC (0.022 +/- 0.015) when compared with sham operation (RSW: 0.141 +/- 0.009; SAC: 0.166 +/- 0.005, n = 6) ($p < 0.05$). The delivery of unseeded biological sutures to the infarcted hearts did not change regional mechanical function compared with the infarcted hearts (RSW: 0.032 +/- 0.004, SAC: 0.037 +/- 0.008, n = 6). The delivery of hMSC-seeded sutures exerted a trend toward increase of regional mechanical function compared with the infarcted heart (RSW: 0.057 +/- 0.011; SAC: 0.051 +/- 0.014, n = 6). Global function showed no significant differences between any group ($p > 0.05$); however, there was a trend toward improved function with the addition of either unseeded or seeded biological suture. Histology demonstrated that Qdot-loaded hMSCs remained present in the infarcted myocardium after 1 week. Analysis of serial sections of Masson's trichrome staining revealed that the greatest infarct size was in the infarct group (7.0% +/- 2.2%), where unseeded (3.8% +/- 0.6%) and hMSC-seeded (3.7% +/- 0.8%) suture groups maintained similar infarct sizes. Furthermore, the remaining suture area was significantly decreased in the unseeded group compared with that in the hMSC-seeded group ($p < 0.05$). This study demonstrated that hMSC-seeded biological sutures are a method to deliver cells to the infarcted myocardium and have treatment potential.

Hao, Z., et al. (2014). "Loss of Arabidopsis GAUT12/IRX8 causes anther indehiscence and leads to reduced G lignin associated with altered matrix polysaccharide deposition." Front Plant Sci **5**: 357.

GAlactUronosylTransferase12 (GAUT12)/IRregular Xylem8 (IRX8) is a putative glycosyltransferase involved in Arabidopsis secondary cell wall biosynthesis. Previous work showed that Arabidopsis irregular xylem8 (irx8) mutants have collapsed xylem due to a reduction in xylan and a lesser reduction in a subfraction of homogalacturonan (HG). We now show that male sterility in the irx8 mutant is due to indehiscent anthers caused by reduced deposition of xylan and lignin in the endothecium cell layer. The reduced lignin content was demonstrated by histochemical lignin staining and pyrolysis Molecular Beam Mass Spectrometry (pyMBMS) and is associated with reduced lignin biosynthesis in irx8 stems. Examination of sequential chemical extracts of stem walls using 2D (13)C-(1)H Heteronuclear Single-Quantum Correlation (HSQC) NMR spectroscopy and antibody-based glycome profiling revealed a reduction in G lignin in the 1 M KOH extract and a concomitant loss of xylan, arabinogalactan and pectin epitopes in the ammonium oxalate, sodium carbonate, and 1 M KOH extracts from the irx8 walls compared with wild-type

walls. Immunolabeling of stem sections using the monoclonal antibody CCRC-M138 reactive against an unsubstituted xylopentaose epitope revealed a bi-lamellate pattern in wild-type fiber cells and a collapsed bi-layer in irx8 cells, suggesting that at least in fiber cells, GAUT12 participates in the synthesis of a specific layer or type of xylan or helps to provide an architecture framework required for the native xylan deposition pattern. The results support the hypothesis that GAUT12 functions in the synthesis of a structure required for xylan and lignin deposition during secondary cell wall formation.

Hashemi, N., et al. (2020). "A novel fluorescent hydroxyapatite based on iron quantum cluster template to enhance osteogenic differentiation." Mater Sci Eng C Mater Biol Appl **111**: 110775.

Template-mediated self-assembly synthesis has produced a diverse range of biomimetic materials with unique physicochemical properties. Here, we fabricated novel fluorescent three-dimensional (3-D) hydroxyapatite (HAP) nanorod-assembled microspheres using iron quantum cluster (FeQC) as a hybrid template, containing three organic components: hemoglobin chains, piperidine, and iron clusters. The material characterization indicated that the synthesized HAP possessed a uniform rod-like morphology, ordered 3-D architecture, high crystallinity, self-activated fluorescence, and remarkable photostability. Our study proposed that this FeQC template is a promising regulating agent to fabricate fluorescent self-assembled HAP microspheres with a controlled morphology. The effect of HAP on stem cell fate and their osteogenic differentiation was investigated by culturing human bone marrow-derived mesenchymal stromal/stem cells (BMSCs) with HAP microspheres. Significant increases in collagen matrix production and gene expression of osteogenic markers, including osteocalcin (OCN), Runt-related transcription factor 2 (Runx2), bone sialoprotein (BSP) and alkaline phosphatase (ALP), were observed compared to the controls after 21 days of culture. Taken together, our data suggest that synthetic HAP nanorod-assembled microspheres represent a promising new biomaterial which exhibits enhanced fluorescent properties and osteoinductive effects on human BMSCs.

Hashemzadeh, H., et al. (2020). "PDMS Nano-Modified Scaffolds for Improvement of Stem Cells Proliferation and Differentiation in Microfluidic Platform." Nanomaterials (Basel) **10**(4).

Microfluidics cell-based assays require strong cell-substrate adhesion for cell viability, proliferation, and differentiation. The intrinsic properties of PDMS, a commonly used polymer in microfluidics systems, regarding cell-substrate interactions have limited its application for microfluidics cell-based assays. Various attempts by previous researchers, such as chemical modification, plasma-treatment, and protein-coating of PDMS revealed some improvements. These strategies are often reversible, time-consuming, short-lived with either cell aggregates formation, not cost-effective as well as not user- and eco-friendly too. To address these challenges, cell-surface interaction has been tuned by the modification of PDMS doped with different biocompatible nanomaterials. Gold nanowires (AuNWs), superparamagnetic iron oxide nanoparticles (SPIONs), graphene oxide sheets (GO), and graphene quantum dot (GQD) have already been coupled to PDMS as an alternative biomaterial enabling easy and straightforward integration during microfluidic fabrication. The synthesized nanoparticles were characterized by corresponding methods. Physical cues of the nanostructured substrates such as Young's modulus, surface roughness, and nanotopology have been carried out using atomic force microscopy (AFM). Initial biocompatibility assessment of the nanocomposites using human amniotic mesenchymal stem cells (hAMSCs) showed comparable cell viabilities among all nanostructured PDMS composites. Finally, osteogenic stem cell differentiation demonstrated an improved differentiation rate inside microfluidic devices. The results revealed that the presence of nanomaterials affected a 5- to 10-fold increase in surface roughness. In addition, the results showed enhancement of cell proliferation from 30% (pristine PDMS) to 85% (nano-modified scaffolds containing AuNWs and SPIONs), calcification from 60% (pristine PDMS) to 95% (PDMS/AuNWs), and cell surface marker expression from 40% in PDMS to 77% in SPION- and AuNWs-PDMS scaffolds at 14 day. Our results suggest that nanostructured composites have a very high potential for stem cell studies and future therapies.

Hayashi, Y., et al. (2021). "Computational screening of cryoprotective agents for regenerative medical products using quantum chemistry and molecular dynamics simulations." Cryobiology **100**: 101-109.

Cryoprotective agents (CPAs) are essential for the cryopreservation of cells. Thus far, dimethyl sulfoxide (DMSO) has been widely used as a CPA; however, DMSO is known to be toxic to cells. The damaged cells by the toxicity can present abnormal conditions, and should not be used for regenerative medical products because the cells/products are implanted directly into human bodies. With the aim of searching for an alternative CPA to DMSO, this work presents a computational screening of CPA candidate compounds using quantum chemistry and molecular dynamics (MD) simulations. Forty compounds were evaluated in regard to the solvation free energy and partition coefficient by quantum chemistry simulation

and the root mean square deviation (RMSD) of a phospholipid bilayer which composes a cell membrane by MD simulation. The solvation free energy, partition coefficient, and RMSD were defined as indicators of osmoregulatory ability, affinity with a cell membrane, and ability to spread a cell membrane, respectively. The quantum chemistry simulation elucidated that the six compounds of trimethylglycine, formamide, urea, thiourea, diethylene glycol, and dulcitol were better than DMSO in either or both of the physical properties considered. This finding is based on the inherent physical property and is thus case-independent. Further characterization with the MD simulation suggested that formamide, thiourea, and urea should be the first candidates to investigate, although the result was valid only in the simulated condition. This work serves as the first step of multi-faceted computational evaluation of multiple compounds in the search for an effective CPA compound after DMSO.

Higuchi, Y. (2012). "[Development of fluorescent labeling methods for stem cells]." Yakugaku Zasshi **132**(4): 433-439.

For successful development of cell-based therapy, both the disposition and differentiation of transplanted cells are directly related to therapeutic effects. In vivo imaging is an attractive tool to obtain real-time information on the disposition of target cells. In various types of imaging methods such as positron emission tomography(PET) and magnetic resonance imaging(MRI), fluorescence imaging is suitable for visualizing the disposition of cells because it can visualize single cells both in vitro and in vivo. For the trafficking of stem cells after transplantation, it is necessary to label living cells for long time periods without disturbing the function or differentiation of the labeled cells. Recently, we have developed quantum dots modified with polyamidoamine(PAMAM) dendrimers. These can more rapidly escape from endosomes and sustain their fluorescence intensity compared with unmodified quantum dots in primary cultured mesenchymal stem cells (MSCs). Fluorescence intensity was also sustained after intravenous injection of MSCs labeled with PAMAM dendrimer-modified quantum dots. To study the dynamics of MSCs in vivo, we constructed a piggyBack transposon vector that can integrate the target gene into the genome in mammalian cells, and established primary MSCs with long-term expression of EmGFP. In addition, we also developed a suction device stabilizing tissue for in vivo real time imaging. In this section, I present our recent findings on long-term fluorescent labeling of MSCs and in vivo visualizing of cell dynamics in a living mouse.

Higuchi, Y., et al. (2011). "Polyamidoamine dendrimer-conjugated quantum dots for efficient labeling of primary cultured mesenchymal stem cells." Biomaterials **32**(28): 6676-6682.

Monitoring of cells in vivo after transplantation could supply important information for determining the efficacy of stem cell therapy. The use of quantum dots (QDs) has several advantages for in vivo imaging, such as remarkable resistance to photo bleaching, high fluorescence efficiency, and size-tunable emission. After they are taken up by cells via endocytosis, QDs lose their fluorescence intensity in endosomes/lysosomes at low pH because the intensity cannot survive under acidic conditions. Moreover, the amount of QD uptake by mesenchymal stem cells (MSCs) is extremely small. Therefore, for effective labeling of MSCs and long observation of MSCs labeled by QDs in vivo, it is essential both to increase cellular uptake of QDs and to promote endosomal escape into the cytosol. The polyamidoamine (PAMAM) dendrimer had plenty of cationic charge, which promoted cellular uptake though electrostatic interactions, and a "buffering capacity," which enhanced endosomal escape into the cytosol. In this study, QDs were modified with PAMAM dendrimer for the efficient labeling of MSCs by QDs. The uptake efficiency and cytosolic distribution of QDs in primary cultured MSCs were increased by the modification of the PAMAM dendrimer. The fluorescence intensity in MSCs labeled by PAMAM dendrimer-conjugated QDs lasted for a longer time in harvested culture plates or in cell-transplanted mice than that in MSCs labeled by non-conjugated QDs.

Hoffmann, A., et al. (2006). "Neotendon formation induced by manipulation of the Smad8 signalling pathway in mesenchymal stem cells." J Clin Invest **116**(4): 940-952.

Tissue regeneration requires the recruitment of adult stem cells and their differentiation into mature committed cells. In this study we describe what we believe to be a novel approach for tendon regeneration based on a specific signalling molecule, Smad8, which mediates the differentiation of mesenchymal stem cells (MSCs) into tendon-like cells. A biologically active Smad8 variant was transfected into an MSC line that coexpressed the osteogenic gene bone morphogenetic protein 2 (BMP2). The engineered cells demonstrated the morphological characteristics and gene expression profile of tendon cells both in vitro and in vivo. In addition, following implantation in an Achilles tendon partial defect, the engineered cells were capable of inducing tendon regeneration demonstrated by double quantum filtered MRI. The results indicate what we believe to be a novel mechanism in which Smad8 inhibits the osteogenic pathway in MSCs known to be induced by BMP2 while promoting tendon differentiation. These findings may have considerable importance for the therapeutic replacement of tendons

or ligaments and for engineering other tissues in which BMP plays a pivotal developmental role.

Hondow, N., et al. (2011). "STEM mode in the SEM: a practical tool for nanotoxicology." Nanotoxicology **5**(2): 215-227.

The addition of a transmitted electron detector to a scanning electron microscope (SEM) allows the recording of bright and dark field scanning transmission electron microscope (STEM) images and the corresponding in-lens secondary electron images from the same region of a thin sample. These combined imaging techniques have been applied here to the analysis of ultrathin sections of cells exposed in vitro to nanomaterials for toxicology investigation. Electron microscopy in general permits the exact nature of the interaction of nanomaterials and cells to be elucidated, and in addition the use of STEM mode in the SEM enables the easy identification and exclusion of artefacts produced by ultramicrotome sectioning. The imaging and analysis obtained by using the STEM mode in the SEM configuration from three different nanomaterial systems of importance (iron oxide nanoparticles, single-walled carbon nanotubes and cadmium selenide quantum dots) indicate that it is a simple, practical and cost-effective tool for nanotoxicological research.

Hori, T., et al. (1999). "Presynaptic mechanism for phorbol ester-induced synaptic potentiation." J Neurosci **19**(17): 7262-7267.

Phorbol ester facilitates transmitter release at a variety of synapses, and the phorbol ester-induced synaptic potentiation (PESP) is a model for presynaptic facilitation. To address the mechanism underlying PESP, we have made paired whole-cell recordings from the giant presynaptic terminal, the calyx of Held, and its postsynaptic target in the medial nucleus of the trapezoid body in rat brainstem slices. Phorbol ester potentiated EPSCs without affecting either presynaptic calcium currents or potassium currents. Protein kinase C inhibitors applied from outside or injected directly into the presynaptic terminal attenuated the PESP. Furthermore, presynaptic loading of a synthetic peptide with the sequence of the N-terminal domain of Doc2alpha interacting with Munc13-1 (Mid peptide) significantly attenuated PESP, whereas mutated Mid peptide had no effect. We conclude that the target of the presynaptic facilitatory effect of phorbol ester resides downstream of calcium influx and may involve both protein kinase C and Doc2alpha - Munc13-1 interaction.

Hossain, M. A., et al. (2015). "Imaging modalities for the in vivo surveillance of mesenchymal stromal cells." J Tissue Eng Regen Med **9**(11): 1217-1224.

Bone marrow stromal cells exist as mesenchymal stromal cells (MSCs) and have the capacity to differentiate into multiple tissue types when subjected to appropriate culture conditions. This property of MSCs creates therapeutic opportunities in regenerative medicine for the treatment of damage to neural, cardiac and musculoskeletal tissues or acute kidney injury. The prerequisite for successful cell therapy is delivery of cells to the target tissue. Assessment of therapeutic outcomes utilize traditional methods to examine cell function of MSC populations involving routine biochemical or histological analysis for cell proliferation, protein synthesis and gene expression. However, these methods do not provide sufficient spatial and temporal information. In vivo surveillance of MSC migration to the site of interest can be performed through a variety of imaging modalities such as the use of radiolabelling, fluc protein expression bioluminescence imaging and paramagnetic nanoparticle magnetic resonance imaging. This review will outline the current methods of in vivo surveillance of exogenously administered MSCs in regenerative medicine while addressing potential technological developments. Furthermore, nanoparticles and microparticles for cellular labelling have shown that migration of MSCs can be spatially and temporally monitored. In vivo surveillance therefore permits time-stratified assessment in animal models without disruption of the target organ. In vivo tracking of MSCs is non-invasive, repeatable and non-toxic. Despite the excitement that nanoparticles for tracking MSCs offer, delivery methods are difficult because of the challenges with imaging three-dimensional systems. The current advances and growth in MSC research, is likely to provide a wealth of evidence overcoming these issues.

Howell, S. L., et al. (2013). "Spatial mapping of efficiency of GaN/InGaN nanowire array solar cells using scanning photocurrent microscopy." Nano Lett **13**(11): 5123-5128.

GaN-InGaN core-shell nanowire array devices are characterized by spectrally resolved scanning photocurrent microscopy (SPCM). The spatially resolved external quantum efficiency is correlated with structure and composition inferred from atomic force microscope (AFM) topography, scanning transmission electron microscope (STEM) imaging, Raman microspectroscopy, and scanning photocurrent microscopy (SPCM) maps of the effective absorption edge. The experimental analyses are coupled with finite difference time domain simulations to provide mechanistic understanding of spatial variations in carrier generation and collection, which is essential to the development of heterogeneous novel architecture solar cell devices.

Hsiao, W. W., et al. (2016). "Fluorescent Nanodiamond: A Versatile Tool for Long-Term Cell Tracking, Super-

Resolution Imaging, and Nanoscale Temperature Sensing." Acc Chem Res **49**(3): 400-407.

Fluorescent nanodiamond (FND) has recently played a central role in fueling new discoveries in interdisciplinary fields spanning biology, chemistry, physics, and materials sciences. The nanoparticle is unique in that it contains a high density ensemble of negatively charged nitrogen-vacancy (NV(-)) centers as built-in fluorophores. The center possesses a number of outstanding optical and magnetic properties. First, NV(-) has an absorption maximum at approximately 550 nm, and when exposed to green-orange light, it emits bright fluorescence at approximately 700 nm with a lifetime of longer than 10 ns. These spectroscopic properties are little affected by surface modification but are distinctly different from those of cell autofluorescence and thus enable background-free imaging of FNDs in tissue sections. Such characteristics together with its excellent biocompatibility render FND ideal for long-term cell tracking applications, particularly in stem cell research. Next, as an artificial atom in the solid state, the NV(-) center is perfectly photostable, without photobleaching and blinking. Therefore, the NV-containing FND is suitable as a contrast agent for super-resolution imaging by stimulated emission depletion (STED). An improvement of the spatial resolution by 20-fold is readily achievable by using a high-power STED laser to deplete the NV(-) fluorescence. Such improvement is crucial in revealing the detailed structures of biological complexes and assemblies, including cellular organelles and subcellular compartments. Further enhancement of the resolution for live cell imaging is possible by manipulating the charge states of the NV centers. As the "brightest" member of the nanocarbon family, FND holds great promise and potential for bioimaging with unprecedented resolution and precision. Lastly, the NV(-) center in diamond is an atom-like quantum system with a total electron spin of 1. The ground states of the spins show a crystal field splitting of 2.87 GHz, separating the ms = 0 and +/-1 sublevels. Interestingly, the transitions between the spin sublevels can be optically detected and manipulated by microwave radiation, a technique known as optically detected magnetic resonance (ODMR). In addition, the electron spins have an exceptionally long coherence time, making FND useful for ultrasensitive detection of temperature at the nanoscale. Pump-probe-type nanothermometry with a temporal resolution of better than 10 mus has been achieved with a three-point sampling method. Gold/diamond nanohybrids have also been developed for highly localized hyperthermia applications. This Account provides a summary of the recent advances in FND-enabled technologies with a special focus on long-term cell tracking, super-resolution imaging, and nanoscale temperature sensing. These emerging and multifaceted technologies are in synchronicity with modern imaging modalities.

Hsieh, S. C., et al. (2006). "The internalized CdSe/ZnS quantum dots impair the chondrogenesis of bone marrow mesenchymal stem cells." J Biomed Mater Res B Appl Biomater **79**(1): 95-101.

Mesenchymal stem cells (MSCs) are capable of differentiating into multiple cell lineages and are useful for therapeutic applications. Labeling the MSCs with fluorescent probes is beneficial in tracing the fate of MSCs after implantation. We have introduced the CdSe/ZnS quantum dots (QDs) into the human bone marrow MSCs and examined the effects of QDs on the proliferation and chondrogenesis of the cells. The internalized QDs were found localized in perinuclear regions and remained there after a number of cell passages. The presence of QDs did not affect the proliferation of cells or the size of chondrospheres formed, when subjected to chondrogenesis induction. However, the expression of mRNA and protein of type II collagen and aggrecan in the chondrospheres was significantly inhibited in cells labeled with QDs, suggesting impaired chondrogenesis. Our results that the presence of QDs interferes with the chondrogenic differentiation of MSCs raise concerns in using the QDs as fluorescence tracers for stem cells.

Hsieh, S. C., et al. (2006). "The inhibition of osteogenesis with human bone marrow mesenchymal stem cells by CdSe/ZnS quantum dot labels." Biomaterials **27**(8): 1656-1664.

CdSe/ZnS quantum dots (QDs) have recently been used as cell tracers for long term imaging of live cells. A number of studies indicate that introduction of quantum dots to cells have no apparent deleterious effects on the morphology or growth of cells. In the present study, the human bone marrow mesenchymal stem cells (hBMSCs) were used as a model to examine the effects of QDs on the growth and osteogenic differentiation of the cells. The CdSe/ZnS QDs were delivered into hBMSCs by liposome-mediated transfection with high efficiency; analysis by transmission electron microscopy revealed that the internalized QDs could be located in the endosome-like vesicles. Uptake of QDs into hBMSCs did not affect the proliferation and cell cycle distribution of the cells. When induced to differentiate along the osteogenic lineage, the QD-containing-hBMSCs were shown to have mineral deposition on the extracellular matrix. However, the cells displayed lower alkaline phosphatase activity as compared to those without QDs. Analysis by reverse transcriptase polymerase chain reaction further demonstrated that the expression of two osteogenic markers, osteopontin and osteocalcin, was significantly inhibited. Together our results show that the presence of

QDs in hBMSCs prevents the full response of the cells to induced osteogenic differentiation.

Hsu, S. H., et al. (2013). "Synthesis of water-dispersible zinc oxide quantum dots with antibacterial activity and low cytotoxicity for cell labeling." Nanotechnology **24**(47): 475102.

Typical photoluminescent semiconductor nanoparticles, called quantum dots (QDs), have potential applications in biological labeling. When used to label stem cells, QDs may impair the differentiation capacity of the stem cells. In this study, we synthesized zinc oxide (ZnO) QDs in methanol with an average size of approximately 2 nm. We then employed two different types of polyethylene glycol (PEG) molecules (SH-PEG-NH2 and NH2-PEG-NH2) to conjugate ZnO QDs and made them water-dispersible. Fourier transform infrared spectroscopy spectra indicated the attachment of PEG molecules on ZnO QDs. No obvious size alteration was observed for ZnO QDs after PEG conjugation. The water-dispersible ZnO QDs still retained the antibacterial activity and fluorescence intensity. The cytotoxicity evaluation revealed that ZnO QDs at higher concentrations decreased cell viability but were generally safe at 30 ppm or below. Cell lines of hepatocytes (HepG2), osteoblasts (MC3T3-E1) and mesenchymal stem cells (MSCs) were successfully labeled by the water-dispersible ZnO QDs at 30 ppm. The ZnO QD-labeled MSCs maintained their stemness and differentiation capacity. Therefore, we conclude that the water-dispersible ZnO QDs developed in this study have antibacterial activity, low cytotoxicity, and proper labeling efficiency, and can be used to label a variety of cells including stem cells.

Huang, S., et al. (2015). "Highly fluorescent and bioresorbable polymeric nanoparticles with enhanced photostability for cell imaging." Nanoscale **7**(3): 889-895.

We report a facile and general strategy for enhancing the photostability of organic fluorophores for bioimaging applications. As a proof of concept, bright and robust fluorescence was observed in solid states of a well-defined synthetic polymer polycaprolactone consisting of di(thiophene-2-yl)-diketopyrrolopyrrole covalently linked in the middle of the polymer chain as a biocompatible and bioresorbable matrix. The nanoparticles prepared through a nanoprecipitation process of these polymers could be internalized by both tumor cells and stem cells with little cytotoxicity. Moreover, these highly fluorescent nanoparticles exhibited significantly enhanced photostability compared to commercial quantum dots or physical blends of dye/polymer complexes in cell imaging and long-term tracing.

Hur, J., et al. (2020). "Microfluidic Cell Stretching for Highly Effective Gene Delivery into Hard-to-Transfect Primary Cells." ACS Nano **14**(11): 15094-15106.

Cell therapy and cellular engineering begin with internalizing synthetic biomolecules and functional nanomaterials into primary cells. Conventionally, electroporation, lipofection, or viral transduction has been used; however, these are limited by their cytotoxicity, low scalability, cost, and/or preparation complexity, especially in primary cells. Thus, a universal intracellular delivery method that outperforms the existing methods must be established. Here, we present a versatile intracellular delivery platform that leverages intrinsic inertial flow developed in a T-junction microchannel with a cavity. The elongational recirculating flows exerted in the channel substantially stretch the cells, creating discontinuities on cell membranes, thereby enabling highly effective internalization of nanomaterials, such as plasmid DNA (7.9 kbp), mRNA, siRNA, quantum dots, and large nanoparticles (300 nm), into different cell types, including hard-to-transfect primary stem and immune cells. We identified that the internalization mechanism of external cargos during the cell elongation-restoration process is achieved by both passive diffusion and convection-based rapid solution exchange across the cell membrane. Using fluidic cell mechanoporation, we demonstrated a transfection yield superior to that of other state-of-the-art microfluidic platforms as well as current benchtop techniques, including lipofectamine and electroporation. In summary, the intracellular delivery platform developed in the present study enables a high delivery efficiency (up to 98%), easy operation (single-step), low material cost (<$1), high scalability (1 x 10(6) cells/min), minimal cell perturbation (up to 90%), and cell type/cargo insensitive delivery, providing a practical and robust approach anticipated to critically impact cell-based research.

Jacob, T., et al. (2016). "Ultrasensitive proteomic quantitation of cellular signaling by digitized nanoparticle-protein counting." Sci Rep **6**: 28163.

Many important signaling and regulatory proteins are expressed at low abundance and are difficult to measure in single cells. We report a molecular imaging approach to quantitate protein levels by digitized, discrete counting of nanoparticle-tagged proteins. Digitized protein counting provides ultrasensitive molecular detection of proteins in single cells that surpasses conventional methods of quantitating total diffuse fluorescence, and offers a substantial improvement in protein quantitation. We implement this digitized proteomic approach in an integrated imaging platform, the single cell-quantum dot platform (SC-QDP), to execute sensitive single cell phosphoquantitation in response to multiple drug

treatment conditions and using limited primary patient material. The SC-QDP: 1) identified pAKT and pERK phospho-heterogeneity and insensitivity in individual leukemia cells treated with a multi-drug panel of FDA-approved kinase inhibitors, and 2) revealed subpopulations of drug-insensitive CD34+ stem cells with high pCRKL and pSTAT5 signaling in chronic myeloid leukemia patient blood samples. This ultrasensitive digitized protein detection approach is valuable for uncovering subtle but important differences in signaling, drug insensitivity, and other key cellular processes amongst single cells.

Jacobi, N. and L. Herich (2016). "Measurement of liver iron concentration by superconducting quantum interference device biomagnetic liver susceptometry validates serum ferritin as prognostic parameter for allogeneic stem cell transplantation." Eur J Haematol **97**(4): 336-341.

INTRODUCTION: There are conflicting data regarding the role of serum ferritin (SF) as surrogate parameter for iron overload as an independent prognostic factor for outcome after allogeneic stem cell transplantation (SCT). Superconducting quantum interference device (SQUID) biomagnetic liver susceptometry, a noninvasive measurement of iron overload, allows measurement of the interference of an exteriorly applied small but highly constant magnetic field by the paramagnetic liver storage iron. By measuring the true iron load of patients through SQUID, we wanted to assess the effect of iron overload on patients undergoing SCT. METHODS: We conducted a single-center retrospective analysis (1994-2010), comparing the effect of SF and liver iron content measured by SQUID shortly before transplantation on overall survival (OS), event-free survival (EFS), and transplant-related mortality (TRM) in 142 patients (median age 54.5 yr, range 5.6-75 yr) undergoing SCT (80% reduced intensity regimen). Patients were subdivided into five groups: myelodysplastic syndrome, de novo acute myeloid leukemia (AML), secondary AML, primary myelofibrosis, and others. RESULTS: Correlation between SF and SQUID was significant (r = 0.6; P < 0.001; log function). The chance of infection was increased 2.4-fold (95% CI 1.22-4.71) when SQUID values ranged >/=1000 mug Fe/g liver (P = 0.012). We found similar results for SF >1000 ng/mL (P = 0.003). A significant association between SQUID and fungal infection was also seen (P = 0.004). For patients with SQUID >/=1000, the risk of proven fungal infection was increased 3.08-fold (95% CI 1.43-6.63). A similar association between SF >1000 and fungal infection was shown (P = 0.01). In univariate analysis, age was a prognostic factor for TRM (P = 0.034, HR 1.04, CI 1.00-1.08). SF >/=1000 was associated with OS (P = 0.033, HR 2.09, CI 1.06-4.11) and EFS (P = 0.016, HR 2.15, 95% CI 1.15-4.10). In multivariate analysis on EFS, only age and SF >1000 remained as independent factors (HR 1.027, P = 0.040, 95% CI 1.001-1.054 and HR 2.058, P = 0.034, 95% CI 1.056-4.008, respectively). The multivariate analysis on TRM left age and SQUID values >/=1000 in the final model (HR 1.045, P = 0.041, 95% CI 1.002-1.090 and HR 2.110, P = 0.103, 95% CI 0.859-5.183, respectively). CONCLUSION: Our data confirmed that SF >/=1000 increases the risk of infection, moreover fungal infection in transplant recipients. As SQUID values correlate well with SF, we could show that SF is indeed a good surrogate parameter for iron overload when measured shortly before SCT. Prospective trials are needed to investigate the effect of iron chelation before or during SCT on transplant outcome.

Jagminas, A., et al. (2017). "Methionine-mediated synthesis of magnetic nanoparticles and functionalization with gold quantum dots for theranostic applications." Beilstein J Nanotechnol **8**: 1734-1741.

Biocompatible superparamagnetic iron oxide nanoparticles (NPs) through smart chemical functionalization of their surface with fluorescent species, therapeutic proteins, antibiotics, and aptamers offer remarkable potential for diagnosis and therapy of disease sites at their initial stage of growth. Such NPs can be obtained by the creation of proper linkers between magnetic NP and fluorescent or drug probes. One of these linkers is gold, because it is chemically stable, nontoxic and capable to link various biomolecules. In this study, we present a way for a simple and reliable decoration the surface of magnetic NPs with gold quantum dots (QDs) containing more than 13.5% of Au(+). Emphasis is put on the synthesis of magnetic NPs by co-precipitation using the amino acid methionine as NP growth-stabilizing agent capable to later reduce and attach gold species. The surface of these NPs can be further conjugated with targeting and chemotherapy agents, such as cancer stem cell-related antibodies and the anticancer drug doxorubicin, for early detection and improved treatment. In order to verify our findings, high-resolution transmission electron microscopy (HRTEM), atomic force microscopy (AFM), FTIR spectroscopy, inductively coupled plasma mass spectroscopy (ICP-MS), and X-ray photoelectron spectroscopy (XPS) of as-formed CoFe2O4 NPs before and after decoration with gold QDs were applied.

Jahed, V., et al. (2020). "Quantum dots-betacyclodextrin-histidine labeled human adipose stem cells-laden chitosan hydrogel for bone tissue engineering." Nanomedicine **27**: 102217.

Mesenchymal stem cells with differentiation ability to diverse cells play a crucial role in tissue engineering. Tracking the fate of these cells during the

regeneration of tissue helps to obtain more information about their function. In this study, histidine conjugated beta-cyclodextrin as a cell-penetrating carrier with drug loading ability was attached to QDs nanoparticle (QD-betaCD-His) for stem cell labeling. Traceability of QD-betaCD-His labeled human adipose stem cells (hASCs) was monitored in 2D cell culture and 3D temperature-sensitive chitosan hydrogel scaffold. Dexamethasone (Dex) as an osteoinductive drug was loaded into QD-betaCD-His nano-carrier (QD-betaCD-His@Dex) to induce bone differentiation of labeled cells. Overall results indicated that QD-betaCD-His@Dex is a promising dual-purpose nano-carrier for stem cell labeling with osteoinductive potential in cell therapy as well as tissue engineering scaffolds.

Jain, K. K. (2007). "Applications of nanobiotechnology in clinical diagnostics." Clin Chem **53**(11): 2002-2009.

BACKGROUND: Nanobiotechnologies are being applied to molecular diagnostics and several technologies are in development. METHODS: This review describes nanobiotechnologies that are already incorporated in molecular diagnostics or have potential applications in clinical diagnosis. Selected promising technologies from published literature as well as some technologies that are in commercial development but have not been reported are included. RESULTS: Nanotechnologies enable diagnosis at the single-cell and molecule levels, and some can be incorporated in current molecular diagnostic methods, such as biochips. Nanoparticles, such as gold nanoparticles and quantum dots, are the most widely used, but various other nanotechnological devices for manipulation at the nanoscale as well as nanobiosensors are also promising for potential clinical applications. CONCLUSIONS: Nanotechnologies will extend the limits of current molecular diagnostics and enable point-of-care diagnostics, integration of diagnostics with therapeutics, and development of personalized medicine. Although the potential diagnostic applications are unlimited, the most important current applications are foreseen in the areas of biomarker discovery, cancer diagnosis, and detection of infectious microorganisms. Safety studies are needed for in vivo use. Because of its close interrelationships with other technologies, nanobiotechnology in clinical diagnosis will play an important role in the development of nanomedicine in the future.

Ji, Y., et al. (2021). "Biological Potential of Polyethylene Glycol (PEG)-Functionalized Graphene Quantum Dots in In Vitro Neural Stem/Progenitor Cells." Nanomaterials (Basel) **11**(6).

Stem cell therapy is one of the novel and prospective fields. The ability of stem cells to differentiate into different lineages makes them attractive candidates for several therapies. It is essential to understand the cell fate, distribution, and function of transplanted cells in the local microenvironment before their applications. Therefore, it is necessary to develop an accurate and reliable labeling method of stem cells for imaging techniques to track their translocation after transplantation. The graphitic quantum dots (GQDs) are selected among various stem cell labeling and tracking strategies which have high photoluminescence ability, photostability, relatively low cytotoxicity, tunable surface functional groups, and delivering capacity. Since GQDs interact easily with the cell and interfere with cell behavior through surface functional groups, an appropriate surface modification needs to be considered to get close to the ideal labeling nanoprobes. In this study, polyethylene glycol (PEG) is used to improve biocompatibility while simultaneously maintaining the photoluminescent potentials of GQDs. The biochemically inert PEG successfully covered the surface of GQDs. The PEG-GQDs composites show adequate bioimaging capabilities when internalized into neural stem/progenitor cells (NSPCs). Furthermore, the bio-inertness of the PEG-GQDs is confirmed. Herein, we introduce the PEG-GQDs as a valuable tool for stem cell labeling and tracking for biomedical therapies in the field of neural regeneration.

Jiang, X., et al. (2010). "[In vitro quantum dot-labeled rat bone marrow mesenchymal stem cells]." Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi **24**(6): 744-748.

UNLABELLED: OBJECTIVE To explore the cytotoxicity, labeled time, marking rate, and effect on adhesion of quantum dot 655 (QD655) labeled rat bone marrow mesenchymal stem cells (BMSCs) in vitro, and to confirm its feasibility for stem cell labeling and tracer means for rat. METHODS: BMSCs were collected from the femur and tibia bone marrow cavity of a 2-week-old SD rat, cultured and identified. The 3rd passage of BMSCs were incubated with QD655 as the experimental group according to the recommended concentration of the markers. The cells were not labeled by QD655 as control group. The cell survival rate after QD655 labeling was detected by trypan-blue exclusion. The effect of QD655 on cell proliferation was observed by MTT. The osteogenic differentiation potential was identified by Alizarin red staining, alkaline phosphatase (ALP) staining, and real-time fluorogenic quantitative PCR. At immediately, 1, 2, 4, and 6 weeks, fluorescent microscopy was used to observe the labeled rate and scanning electron microscope was used to observe the cell adhesion to scaffold (bioglass/collagen composite). RESULTS: The cell survival rates were more than 90% in both experimental group and control group, showing no significant difference ($P > 0.05$). There was no significant difference in the cell proliferation between 2 groups ($P > 0.05$). Alizarin red staining and ALP

staining showed positive results. Real-time fluorogenic quantitative PCR result showed that the mRNA expression levels of osteopontin, osteocalcin, collagen type I, ALP, and BMP-2 in the experimental group was significantly higher than those in the control group. The labeled rates were 96.50% +/- 1.59%, 93.30% +/- 1.51%, 72.40% +/- 2.90%, 40.10% + 3.60%, and 10.00% +/- 1.70% immediately, 1, 2, 4, and 6 weeks after labeling, respectively. The labeled rate in the control group was 0. Scanning electron microscope showed a good distribution of fusiform or polygonal cells in the pores of scaffold. CONCLUSION: QD655 can be used as a labeling marker for BMSCs. Rat BMSCs labeled with QD655 is of high efficiency and safety.

Jindal, S., et al. (2021). "Connexin and gap junctions: perspectives from biology to nanotechnology based therapeutics." Transl Res **235**: 144-167.

The concept of gap junctions and their role in intercellular communication has been known for around 50 years. Considerable progress has been made in understanding the fundamental biology of connexins in mediating gap junction intercellular communication (GJIC) and their role in various cellular processes including pathological conditions. However, this understanding has not led to development of advanced therapeutics utilizing GJIC. Inadequacies in strategies that target specific connexin protein in the affected tissue, with minimal or no collateral damage, are the primary reason for the lack of development of efficient therapeutic models. Herein, nanotechnology has a role to play, giving plenty of scope to circumvent these problems and develop more efficient connexin based therapeutics. AsODN, antisense oligodeoxynucleotides; BMPs, bone morphogenetic proteins; BMSCs, bone marrow stem cells; BG, bioglass; Cx, Connexin; CxRE, connexin-responsive elements; CoCr NPs, cobalt-chromium nanoparticles; cGAMP, cyclic guanosine monophosphate-adenosine monophosphate; cAMP, cyclic adenosine monophosphate; ERK1/2, extracellular signal-regulated kinase 1/2; EMT, epithelial-mesenchymal transition; EPA, eicosapentaenoic acids; FGFR1, fibroblast growth factor receptor 1; FRAP, fluorescence recovery after photobleaching; 5-FU, 5-fluorouracil; GJ, gap junction; GJIC, gap junctional intercellular communication; HGPRTase, hypoxanthine phosphoribosyltransferase; HSV-TK, herpes virus thymidine kinase; HSA, human serum albumin; HA, hyaluronic acid; HDAC, histone deacetylase; IRI, ischemia reperfusion injury; IL-6, interleukin-6; IL-8, interleukin-8; IONPs, iron-oxide nanoparticles; JNK, c-Jun N-terminal kinase; LAMP, local activation of molecular fluorescent probe; MSCs, mesenchymal stem cells; MMP, matrix metalloproteinase; MI, myocardial infarction; MAPK, mitogen-activated protein kinase; NF-kappaB, nuclear factor kappa B; NO, nitric oxide; PKC, protein kinase C; QDs, quantum dots; ROI, region of interest; RGO, reduced graphene oxide; siRNA, small interfering RNA; TGF-beta1, transforming growth factor-beta1; TNF-alpha, tumor necrosis factor-alpha; UCN, upconversion nanoparticles; VEGF, vascular endothelial growth factor. In this review, we discuss briefly the role of connexins and gap junctions in various physiological and pathological processes, with special emphasis on cancer. We further discuss the application of nanotechnology and tissue engineering in developing treatments for various connexin based disorders.

Jones, M., et al. (2013). "Genetic stability of bone marrow-derived human mesenchymal stromal cells in the Quantum System." Cytotherapy **15**(11): 1323-1339.

BACKGROUND AIMS: The Quantum(R) Cell Expansion System (Quantum; Terumo BCT, Inc, Lakewood, CO, USA) is a novel hollow fiber-based device that automates and closes the cell culture process, reducing labor intensive tasks such as manual cell culture feeding and harvesting. The manual cell selection and expansion processes for the production of clinical-scale quantities of bone marrow-derived human mesenchymal stromal cells (BM-hMSCs) have been successfully translated onto the Quantum platform previously. The formerly static, manual, in vitro process performed primarily on tissue culture polystyrene substrates may raise the question of whether BM-hMSCs cultured on a hollow fiber platform yields comparable cell quality. METHODS: A rigorous battery of assays was used to determine the genetic stability of BM-hMSCs selected and produced with the Quantum. In this study, genetic stability was determined by assessing spectral karyotype, micronucleus formation and tumorigenicity to resolve chromosomal aberrations in the stem cell population. Cell phenotype, adherent growth kinetics and tri-lineage differentiation were also evaluated. HMSC bone marrow aspirates, obtained from three approved donors, were expanded in parallel using T225 culture flasks and the Quantum. RESULTS: BM-hMSCs harvested from the Quantum demonstrated immunophenotype, morphology and tri-lineage differentiation capacity characteristics consistent with the International Society of Cell Therapy standard for hMSCs. Cell populations showed no malignant neoplastic formation in athymic mice 60 days post-transplant, no clonal chromosomal aberrations were observed and no DNA damage was found as measured by micronucleus formation. CONCLUSIONS: Quantum-produced BM-hMSCs are of comparable quality and demonstrate analogous genetic stability to BM-hMSCs cultured on tissue culture polystyrene substrates.

Ju, H., et al. (2017). "Impact of Environmental Pollutant Cadmium on the Establishment of a Cancer Stem Cell

Population in Breast and Hepatic Cancer." ACS Omega **2**(2): 563-572.

Cadmium, a heavy metal pollutant, causes cancer. The existence of cancer stem cells (CSCs) in tumors is widely considered to be the reason for the recurrence and treatment failure of cancer. Increasing evidence has confirmed that under certain conditions non-CSCs could be converted into CSCs. The impact of cadmium on the development of CSC lineage in the bulk tumor cell population is not yet studied. The aim of this study was to evaluate the effect of cadmium on the conversion of non-CSCs to CSCs and the identification of CSCs based on the concurrent monitoring of multiple CSC markers. High-content monitoring of molecular markers was performed using quantum dot (QD) nanoprobes and an acousto-optical tunable filter (AOTF)-based imaging device. Cadmium treatment significantly increased the CSC population in MCF-7 and HepG2 cell lines. The cadmium-induced CSCs were identified by a concurrent analysis of stem-cell markers, namely, CD44, CD24, CD133, and ALDH1. Moreover, increased m-RNA expression of CD44, ALDH1, and CD133 and protein expression of p-Ras, p-Raf-1, p-MEK-1, and p-ERK-1 were observed in the cadmium-treated MCF-7 and HepG2 cells. This study demonstrates that cadmium induces the gene expression of CSC markers in the breast and liver cancer cell lineage and promotes the conversion of non-CSCs to CSCs.

Ju, H., et al. (2016). "Crosstalk-eliminated quantitative determination of aflatoxin B1-induced hepatocellular cancer stem cells based on concurrent monitoring of CD133, CD44, and aldehyde dehydrogenase1." Toxicol Lett **243**: 31-39.

Cancer stem cells (CSCs), known as tumor initiating cells, have become a critically important issue for cancer therapy. Although much research has demonstrated the induction of hepato cellular carcinoma by aflatoxin B1, the formation of hepatocellular CSCs and their quantitative determination is hardly reported. In this work, it was found that hepatocellular CSCs were produced from HepG2 cells by aflatoxin B1-induced mutation, and their amount was quantitatively determined using crosstalk-eliminated multicolor cellular imaging based on quantum dot (Qdot) nanoprobes and an acousto-optical tunable filter (AOTF). Hepatocellular CSCs were acquired via magnetic bead-based sorting and observed using concurrent detection of three different markers: CD133, CD44, and aldehyde dehydrogenase1 (ALDH1). The DNA mutation of HepG2 cells caused by aflatoxin B1 was quantitatively observed via absorbance spectra of aflatoxin B1-8, 9-epoxide-DNA adducts. The percentages of hepatocellular CSCs formed in the entire HepG2 cells were determined to be 9.77+/-0.65%, 10.9+/-1.39%, 11.4+/-1.32%, and 12.8+/-0.7%, respectively, at 0 muM, 5 muM, 10 muM, and 20 muM of aflatoxin B1. The results matched well with those obtained utilizing flow cytometry. This study demonstrates that aflatoxin mediated mutation induced the conversion of hepatic cancer cell to hepatic CSCs by using a Qdot based constructed multicolor cellular imaging system.

Kaji, N. and Y. Baba (2014). "Nanobiodevice-based single biomolecule analysis, single-cell analysis, and in vivo imaging for cancer diagnosis, cancer theranostics, and iPS cell-based regenerative medicine." Anal Sci **30**(9): 859-864.

Numerous nanobiodevices have been developed for cancer diagnosis through the analysis of cancer cells and cancer-related biomolecules, cancer gene therapy, and iPS cell-based regenerative medicine. A microchamber array, which is fabricated on a plastic chip, enables to develop a reliable circulating tumor cell (CTC) detection technique for cancer metastasis diagnosis. A nanopillar-array or a nanowire-array on a quartz chip allows ultrafast analysis of DNA and microRNA within 1 s for the molecular diagnosis of cancer. Immunopillar devices, which contain a tremendous amount of antibody-immobilized microparticles inside an immunopillar structure, have realized fast and low invasive "from blood to analysis" type biomarker detection of cancer with a pM detection sensitivity within 5 min. Quantum dots and gene delivery nanodevices are applied to single cancer cell diagnosis, in vivo imaging for iPS cell based regenerative medicine, and theranostic devices for cancer diagnosis and therapy.

Kanda, J., et al. (2011). "Iron overload and allogeneic hematopoietic stem-cell transplantation." Expert Rev Hematol **4**(1): 71-80.

Iron overload is frequently observed in patients with hematologic diseases before and after allogeneic stem-cell transplantation because they usually receive multiple red blood cell transfusions. Elevated pretransplant serum ferritin levels, which are widely used as indicators of body iron status, are significantly associated with a lower overall survival rate and a higher incidence of treatment-related complications; for example, infections and hepatic veno-occlusive disease. As serum ferritin levels are affected, not only by iron loading but also by inflammation, imaging techniques to quantify tissue iron levels have been developed, for example, quantitative MRI using the transverse magnetic relaxation rate, and superconducting quantum interference devices. Iron chelators, such as deferasirox, a new oral iron-chelating agent, reduce iron load in transfusion-dependent patients. Iron-chelating therapy before and/or after transplantation is a promising

strategy to improve the clinical outcomes of transplant patients with iron overload. However, further research is needed to prove the direct relationship between iron overload and adverse outcomes, as well as to determine the effects of treatment for iron overload on outcomes of allogeneic stem-cell transplantation.

Kang, E. S., et al. (2018). "Two-dimensional material-based bionano platforms to control mesenchymal stem cell differentiation." Biomater Res **22**: 10.

BACKGROUND: In the past decade, stem cells, with their ability to differentiate into various types of cells, have been proven to be resourceful in regenerative medicine and tissue engineering. Despite the ability to repair damaged parts of organs and tissues, the use of stem cells still entails several limitations, such as low differentiation efficiency and difficulties in guiding differentiation. To address these limitations, nanotechnology approaches have been recently implemented in stem cell research. It has been discovered that stem cells, in combination with carbon-based functional materials, show enhanced regenerative performances in varying biophysical conditions. In particular, several studies have reported solutions to the conventional quandaries in biomedical engineering, using synergetic effects of nanohybrid materials, as well as further development of technologies to recover from diverse health conditions such as bone fracture and strokes. MAIN TEXT: In this review, we discuss several prior studies regarding the application of various nanomaterials in controlling the behavior of stem cells. We focus on the potential of different types of nanomaterials, such as two-dimensional materials, gold nanoparticles, and three-dimensional nanohybrid composites, to control the differentiation of human mesenchymal stem cells (hMSCs). These materials have been found to affect stem cell functions via the adsorption of growth/differentiation factors on the surfaces of nanomaterials and the activation of signaling pathways that are mostly related to cell adhesion and differentiation (e.g., FAK, Smad, Erk, and Wnt). CONCLUSION: Controlling stem cell differentiation using biophysical factors, especially the use of nanohybrid materials to functionalize underlying substrates wherein the cells attach and grow, is a promising strategy to achieve cells of interest in a highly efficient manner. We hope that this review will facilitate the use of other types of newly discovered and/or synthesized nanomaterials (e.g., metal transition dichalcogenides, non-toxic quantum dots, and metal oxide frameworks) for stem cell-based regenerative therapies.

Kapse-Mistry, S., et al. (2014). "Nanodrug delivery in reversing multidrug resistance in cancer cells." Front Pharmacol **5**: 159.

Different mechanisms in cancer cells become resistant to one or more chemotherapeutics is known as multidrug resistance (MDR) which hinders chemotherapy efficacy. Potential factors for MDR includes enhanced drug detoxification, decreased drug uptake, increased intracellular nucleophiles levels, enhanced repair of drug induced DNA damage, overexpression of drug transporter such as P-glycoprotein(P-gp), multidrug resistance-associated proteins (MRP1, MRP2), and breast cancer resistance protein (BCRP). Currently nanoassemblies such as polymeric/solid lipid/inorganic/metal nanoparticles, quantum dots, dendrimers, liposomes, micelles has emerged as an innovative, effective, and promising platforms for treatment of drug resistant cancer cells. Nanocarriers have potential to improve drug therapeutic index, ability for multifunctionality, divert ABC-transporter mediated drug efflux mechanism and selective targeting to tumor cells, cancer stem cells, tumor initiating cells, or cancer microenvironment. Selective nanocarrier targeting to tumor overcomes dose-limiting side effects, lack of selectivity, tissue toxicity, limited drug access to tumor tissues, high drug doses, and emergence of multiple drug resistance with conventional or combination chemotherapy. Current review highlights various nanodrug delivery systems to overcome mechanism of MDR by neutralizing, evading, or exploiting the drug efflux pumps and those independent of drug efflux pump mechanism by silencing Bcl-2 and HIF1alpha gene expressions by siRNA and miRNA, modulating ceramide levels and targeting NF-kappaB. "Theragnostics" combining a cytotoxic agent, targeting moiety, chemosensitizing agent, and diagnostic imaging aid are highlighted as effective and innovative systems for tumor localization and overcoming MDR. Physical approaches such as combination of drug with thermal/ultrasound/photodynamic therapies to overcome MDR are focused. The review focuses on newer drug delivery systems developed to overcome MDR in cancer cell.

Kawabori, M., et al. (2013). "Timing and cell dose determine therapeutic effects of bone marrow stromal cell transplantation in rat model of cerebral infarct." Neuropathology **33**(2): 140-148.

Stereotactic transplantation of bone marrow stromal cells (BMSCs) enables efficient delivery to the infarct brain. This study was aimed to assess its optimal timing and cell dose for ischemic stroke. The BMSCs were harvested from the green fluorescent protein-transgenic rats and were labeled with quantum dots. The BMSCs (1 x 10(5) or 1 x 10(6)) were stereotactically transplanted into the ipsilateral striatum of the rats subjected to permanent middle cerebral artery occlusion at 1 or 4 weeks post-ischemia. Motor function was

serially assessed. Using in vivo near infrared (NIR) fluorescence imaging, the engrafted BMSCs were visualized at 3 weeks post-transplantation. Immunohistochemistry was performed to evaluate their fate. Functional recovery was significantly enhanced when both low and high doses of BMSCs were transplanted at 1 week post-ischemia, but such therapeutic effects were observed only when the high-dose BMSCs were transplanted at 4 weeks post-ischemia. Both optical imaging and immunohistochemistry revealed their better engraftment in the peri-infarct area when the high-dose BMSCs were transplanted at 1 or 4 weeks post-ischemia. These findings strongly suggest the importance of timing and cell dose to yield therapeutic effects of BMSC transplantation for ischemic stroke. Earlier transplantation requires a smaller number of donor cells for beneficial effects.

Ke, C., et al. (2015). "Migration mechanism of mesenchymal stem cells studied by QD/NSOM." Biochim Biophys Acta **1848**(3): 859-868.

The migration of mesenchymal stem cells (MSCs) plays a key role in tumor-targeted delivery vehicles and tumor-related stroma formation. However, there so far has been no report on the distribution of cell surface molecules during the VEGF-induced migration of MSCs. Here, we have utilized near-field scanning optical microscopy (NSOM) combined with fluorescent quantum dot (QD)-based nano-technology to capture the functional relationship between CD44 and CD29 adhesion molecules on MSCs and the effect of their spatial rearrangements. Before VEGF-induced migration of MSCs, both CD44 and CD29 formed 200-220 nm nano-domains respectively, with little co-localization between the two types of domains. Surprisingly, the size of the CD44 nano-domain rapidly increased in size to 295 nm and apparently larger aggregates were formed following MSC treatment with VEGF for 10 min, while the area of co-localization increased to 0.327 mum2. Compared with CD44, CD29 was activated obviously later, for the fact that CD29 aggregation didn't appear until 30 min after VEGF treatment. Consistently, its co-localization area increased to 0.917 mum2. The CD44 and CD29 nano-domains further aggregated into larger nano-domains or even formed micro-domains on the membrane of activated MSCs. The aggregation and co-localization of these molecules promoted FAK formation and cytoskeleton rearrangement. All of the above changes induced by VEGF contributed to MSC migration. Taken together, our data of NSOM-based dual color fluorescent imaging demonstrated for the first time that CD44, together with CD29, involved in VEGF-induced migration of MSCs through the interaction between CD44 and its co-receptor of VEGFR-2.

Khodair, A. I., et al. (2019). "Synthesis, molecular modeling and anti-cancer evaluation of a series of quinazoline derivatives." Carbohydr Res **486**: 107832.

Quinazolines were surveyed as biologically relevant moieties against different cancer cell lines, so in the present study, we analyzed novel derivatives as target-oriented chemotherapeutic anti-cancer drugs. A series of 3-substituted 2-thioxo-2,3-dihydro-1H-quinazolin-4-ones 4a-e were synthesized via the reaction of 2-aminobenzoic acid (1) with isothiocyanate derivatives 2a-e. S-alkylation and S-glycosylation were carried via the reaction of 4a-e with alkyl halides and alpha-glycopyranosyl bromides 7a,b under anhydrous alkaline and glycoside conditions, respectively. The S-alkylated and S-glycosylated structures, and not that of the N-alkylated and N-glycosylated isomers, have been selected for the products. Conformational analysis has been studied by homo- and heteronuclear two-dimensional NMR methods (DQF-COSY, HMQC, and HMBC). The S site of alkylation and glycosylation were determined from the (1)H, (13)C heteronuclear multiple-quantum coherence (HMQC) experiments. All derivatives were subjected to molecular docking calculations, which selected some derivatives (5n, 8c, 8g, 9c, and 9a) as promising ones based on their excellent binding affinities towards the EGFR tyrosine kinase molecular target. The in vitro cytotoxic activity against MCF-7 and HepG2 cell lines showed effective anti-proliferative activity of the analyzed derivatives with lower IC50 values especially 9a with IC50=2.09 and 2.08muM against MCF-7 and HepG2, respectively, and their treatments were safe against the normal cell line Gingival mesenchymal stem cells (GMSC). Moreover, RT-PCR reaction investigated the apoptotic pathway for the compound 9a, which activated the P53 genes and its related genes. So, further work is recommended for developing it as a chemotherapeutic drug.

Ki, J., et al. (2017). "High-content cell death imaging using quantum dot-based TIRF microscopy for the determination of anticancer activity against breast cancer stem cell." J Biophotonics **10**(1): 118-127.

We report a two color monitoring of drug-induced cell deaths using total internal reflection fluorescence (TIRF) as a novel method to determine anticancer activity. Instead of cancer cells, breast cancer stem cells (CSCs) were directly tested in the present assay to determine the effective concentration (EC50) values of camptothecin and cisplatin. Phosphatidylserine and HMGB1 protein were concurrently detected to observe apoptotic and necrotic cell death induced by anticancer drugs using quantum dot (Qdot)-antibody conjugates. Only 50-to-100 breast CSCs were consumed at each cell chamber due to the high sensitivity of Qdot-based TIRF. The high

sensitivity of Qdot-based TIRF, that enables the consumption of a small number of cells, is advantageous for cost-effective large-scale drug screening. In addition, unlike MTT assay, this approach can provide a more uniform range of EC50 values because the average values of single breast CSCs fluorescence intensities are observed to acquire EC50 values as a function of dose. This research successfully demonstrated the possibility that Qdot-based TIRF can be widely used as an improved alternative to MTT assay for the determination of anticancer drug efficacies.

Kim, J., et al. (2016). "Multiphoton luminescent graphene quantum dots for in vivo tracking of human adipose-derived stem cells." Nanoscale **8**(16): 8512-8519.

The applicability of graphene quantum dots (GQDs) for the in vitro and in vivo live imaging and tracking of different types of human stem cells is investigated. GQDs synthesized by the modified graphite intercalated compound method show efficient cellular uptake with improved biocompatibility and highly sensitive optical properties, indicating their feasibility as a bio-imaging probe for stem cell therapy.

Kim, T. H., et al. (2014). "Electrically controlled delivery of cargo into single human neural stem cell." ACS Appl Mater Interfaces **6**(23): 20709-20716.

Nanoprobe-based techniques have emerged as an efficient tool for the manipulation and analysis of single cells. Here, we report a powerful whole-electrical single-cell manipulation tool that enables rapid and controllable delivery of cargo into single neural stem cells with precision monitoring of the cell penetration process using a conductive nanoprobe. The highly electrically sensitive nanoprobes that were fabricated and the indium tin oxide electrode-integrated cell chip were found to be very effective for monitoring the cell penetration process via current changes that appear as spike-like negative currents. Moreover, the assembly of cargoes onto the nanoprobes was controllable and could reach its maximum load in a very short period of time (<10 min) based on the same electrical system that was used for monitoring cell penetration and without the need for any complex chemical linkers or mediators. Even more remarkably, the cargo assembled on the surface of the nanoprobe was successfully released in a very short period of time (<10 s), regardless of the surrounding intracellular or extracellular environments. The monitoring of cell penetration, assembly of quantum dots (QDs), and release of QDs into the intracellular environment were all accomplished using our whole-electrical system that combined a conductive nanoprobe with cell chip technology. This is a novel technology, which can eliminate complex and time-consuming steps owing to chemical modifications, as well as reduce the time needed for the delivery of cargo into the cell cytosol/nucleus during cell penetration, which is very important for reducing cell damage.

Kim, T. H., et al. (2017). "Live cell biosensing platforms using graphene-based hybrid nanomaterials." Biosens Bioelectron **94**: 485-499.

A novel strategy to precisely detect or monitor various biomaterials in living cells poses paramount importance in understanding cellular processes. Graphene, a newly emerged two-dimensional carbon material, has been widely utilized for biosensors owing to its multifarious characteristics including mechanical, electrical, and optical properties (e.g. stability, conductivity, fluorescence quenching and photoluminescence). In addition, graphene derivatives and their innate characteristics, such as biocompatibility low cytotoxicity and water solubility have facilitated the use of graphene-based materials for live cell biosensing, wherein graphene is utilized as a core material by itself or in combination with other functional nanomaterials to load target-specific probes, fluorescent dyes, and other signaling molecules. Such graphene-based hybrid nanomaterials have been employed to detect various cellular entities in living cells, including ions, biomolecules, genetic molecules, proteins, enzymes, and even whole cells. The following review will discuss a number of previous studies in which graphene-based hybrid constructs were used for live cell biosensing, and their potential applications in cancer research and stem cell therapy.

Kim, Y. S., et al. (2014). "Tracking intravenous adipose-derived mesenchymal stem cells in a model of elastase-induced emphysema." Tuberc Respir Dis (Seoul) **77**(3): 116-123.

BACKGROUND: Mesenchymal stem cells (MSCs) obtained from bone marrow or adipose tissue can successfully repair emphysematous animal lungs, which is a characteristic of chronic obstructive pulmonary disease. Here, we describe the cellular distribution of MSCs that were intravenously injected into mice with elastase-induced emphysema. The distributions were also compared to the distributions in control mice without emphysema. METHODS: We used fluorescence optical imaging with quantum dots (QDs) to track intravenously injected MSCs. In addition, we used a human Alu sequence-based real-time polymerase chain reaction method to assess the lungs, liver, kidney, and spleen in mice with elastase-induced emphysema and control mice at 1, 4, 24, 72, and 168 hours after MSCs injection. RESULTS: The injected MSCs were detected with QD fluorescence at 1- and 4-hour postinjection, and the human Alu sequence was detected at 1-, 4- and 24-hour postinjection in control mice (lungs only). Injected MSCs remained more in mice with

elastase-induced emphysema at 1, 4, and 24 hours after MSCs injection than the control lungs without emphysema. CONCLUSION: In conclusion, our results show that injected MSCs were observed at 1 and 4 hours post injection and more MSCs remain in lungs with emphysema.

Kitase, Y., et al. (2020). "A Novel Treatment with Stem Cells from Human Exfoliated Deciduous Teeth for Hypoxic-Ischemic Encephalopathy in Neonatal Rats." Stem Cells Dev **29**(2): 63-74.

Recently, cell therapy has been developed as a novel treatment for perinatal hypoxic-ischemic encephalopathy (HIE), which is an important cause of neurological disorder and death, and stem cells from human exfoliated deciduous teeth (SHED) express early markers for mesenchymal and neuroectodermal stem cells. We investigated the treatment effect of SHED for HIE in neonatal rats. Seven-day-old rats underwent ligation of the left carotid artery and were exposed to 8% hypoxic treatment. SHED (1 x 10(5) cells) were injected via the right external jugular vein 24 h after the insult. The effect of intravenous administration of SHED cells was evaluated neurologically and pathophysiologically. In the evaluation of engraftment using quantum dots 655, only a few SHED were detected in the injured cortex. In the immunohistological evaluation 24 h after injection, the numbers of positive cells of active caspase-3 and anti-4 hydroxynonenal antiserum were lower in the SHED group than in the vehicle group. The number of Iba-1(+) cells in the cortex was higher in the SHED group. However, the proportion of M1 microglia (Iba-1(+)/ED-1(+)) was significantly decreased, whereas M2 microglia (Iba-1(+)/CD206(+)) tended to increase in the SHED group. In the behavioral tests performed 5 months after hypoxic treatment, compared to the vehicle group, the SHED group showed significant elongation of the endurance time in the rotarod treadmill test, significantly ameliorated proportion of using the impaired hand in the cylinder test, significantly lower ratio of right/left front paw area in gait analysis, and significantly higher avoidance rate in the active avoidance test. In the in vitro experiment with cultured neurons exposed to oxygen-glucose deprivation, we confirmed the neuroprotective effect of the condition medium of SHED. These results suggested that intravenous administration of SHED exerted a treatment effect both histologically and functionally, possibly via a paracrine effect.

Kozanoglu, I., et al. (2017). "Quantum cell expansion system: Safe and rapid expansion." Cytotherapy **19**(10): 1246-1247.

Kravets, V., et al. (2016). "Imaging of Biological Cells Using Luminescent Silver Nanoparticles." Nanoscale Res Lett **11**(1): 30.

The application of luminescent silver nanoparticles as imaging agents for neural stem and rat basophilic leukemia cells was demonstrated. The experimental size dependence of the extinction and emission spectra for silver nanoparticles were also studied. The nanoparticles were functionalized with fluorescent glycine dimers. Spectral position of the resonance extinction and photoluminescence emission for particles with average diameters ranging from 9 to 32 nm were examined. As the particle size increased, the spectral peaks for both extinction and the intrinsic emission of silver nanoparticles shifted to the red end of the spectrum. The intrinsic photoluminescence of the particles was orders of magnitude weaker and was spectrally separated from the photoluminescence of the glycine dimer ligands. The spectral position of the ligand emission was independent of the particle size; however, the quantum yield of the nanoparticle-ligand system was size-dependent. This was attributed to the enhancement of the ligand's emission caused by the local electric field strength's dependence on the particle size. The maximum quantum yield determined for the nanoparticle-ligand complex was (5.2 +/- 0.1) %. The nanoparticles were able to penetrate cell membranes of rat basophilic leukemia and neural stem cells fixed with paraformaldehyde. Additionally, toxicity studies were performed. It was found that towards rat basophilic leukemia cells, luminescent silver nanoparticles had a toxic effect in the silver atom concentration range of 10-100 muM.

Kumar, S., et al. (2018). "Induced Pluripotent Stem Cells in Disease Modeling and Gene Identification." Methods Mol Biol **1706**: 17-38.

Experimental modeling of human inherited disorders provides insight into the cellular and molecular mechanisms involved, and the underlying genetic component influencing, the disease phenotype. The breakthrough development of induced pluripotent stem cell (iPSC) technology represents a quantum leap in experimental modeling of human diseases, providing investigators with a self-renewing and, thus, unlimited source of pluripotent cells for targeted differentiation. In principle, the entire range of cell types found in the human body can be interrogated using an iPSC approach. Therefore, iPSC technology, and the increasingly refined abilities to differentiate iPSCs into disease-relevant target cells, has far-reaching implications for understanding disease pathophysiology, identifying disease-causing genes, and developing more precise therapeutics, including advances in regenerative medicine. In this chapter, we discuss the technological perspectives and recent developments in the application

of patient-derived iPSC lines for human disease modeling and disease gene identification.

Kundrotas, G., et al. (2019). "Uptake and distribution of carboxylated quantum dots in human mesenchymal stem cells: cell growing density matters." J Nanobiotechnology **17**(1): 39.

BACKGROUND: Human mesenchymal stem cells (MSCs) have drawn much attention in the field of regenerative medicine for their immunomodulatory and anti-inflammatory effects. MSCs possess specific tumor-oriented migration and incorporation highlighting the potential for MSCs to be used as an ideal carrier for anticancer agents. Bone marrow is the main source of MSCs for clinical applications. MSCs tracking in vivo is a critical component of the safety and efficacy evaluation of therapeutic cell products; therefore, cells must be labeled with contrast agents to enable visualization of the MSCs migration in vivo. Due to their unique properties, quantum dots (QDs) are emerging as optimal tools in long-term MSC optical imaging applications. The aim of this study was to investigate the uptake dynamics, cytotoxity, subcellular and extracellular distribution of non-targeted carboxylated quantum dots in human bone marrow MSCs at different cell growing densities. RESULTS: QDs had no negative impact on MSC viability throughout the experiment and accumulated in all observed cells efficiently; however, in some MSCs QDs induced formation of lipid droplets. At low cell growing densities QDs distribute within MSCs cytoplasm already after 1 h of incubation reaching saturation after 6 h. After 24 h QDs localize mainly in the perinuclear region of the cells in endosomes. Interestingly, in more confluent culture QDs localize mostly outside MSCs. QDs abundantly mark MSC long filopodia-like structures attaching neighboring cells. At high cell density cultivation, we for the first time demonstrated that carboxylated QDs localize in human bone marrow MSC extracellular matrix. Moreover, we observed that average photoluminescence lifetime of QDs distributed in extracellular matrix are longer than lifetimes of QDs entrapped in endocytic vesicles; thus, for the first time showing the possibility to identify and distinguish localization of QDs in various extracellular and intracellular structures using fluorescence-lifetime imaging microscopy without additional staining assays. CONCLUSION: Carboxylated QDs can be used as nonspecific and effective dye for staining of human bone marrow MSCs and their specific extracellular structures. These results are promising in fundamental stem cell biology as well as in cellular therapy, anticancer drug delivery and tissue engineering.

Kuo, C. W., et al. (2019). "Real-time in vivo imaging of subpopulations of circulating tumor cells using antibody conjugated quantum dots." J Nanobiotechnology **17**(1): 26.

INTRODUCTION: The detection of circulating tumor cells (CTCs) is very important for cancer diagnosis. CTCs can travel from primary tumors through the circulation to form secondary tumor colonies via bloodstream extravasation. The number of CTCs has been used as an indicator of cancer progress. However, the population of CTCs is very heterogeneous. It is very challenging to identify CTC subpopulations such as cancer stem cells (CSCs) with high metastatic potential, which are very important for cancer diagnostic management. RESULTS: We report a study of real-time CTC and CSC imaging in the bloodstreams of living animals using multi-photon microscopy and antibody conjugated quantum dots. We have developed a cancer model for noninvasive imaging wherein pancreatic cancer cells expressing fluorescent proteins were subcutaneously injected into the earlobes of mice and then formed solid tumors. When the cancer cells broke away from the solid tumor, CTCs with fluorescent proteins in the bloodstream at different stages of development could be monitored noninvasively in real time. The number of CTCs observed in the blood vessels could be correlated to the tumor size in the first month and reached a maximum value of approximately 100 CTCs/min after 5 weeks of tumor inoculation. To observe CTC subpopulations, conjugated quantum dots were used. It was found that cluster of differentiation (CD)24+ CTCs can move along the blood vessel walls and migrate to peripheral tissues. CD24+ cell accumulation on the solid tumors' sides was observed, which may provide valuable insight for designing new drugs to target cancer subpopulations with high metastatic potential. We also demonstrated that our system is capable of imaging a minor population of cancer stem cells, CD133+ CTCs, which are found in 0.7% of pancreatic cancer cells and 1%-3% of solid tumors in patients. CONCLUSIONS: With the help of quantum dots, CTCs with higher metastatic potential, such as CD24+ and CD133+ CTCs, have been identified in living animals. Using our approach, it may be possible to investigate detailed metastatic mechanism such as tumor cell extravasation to the blood vessels. In addition, the number of observed CTCs in the blood stream could be correlated with tumor stage in the early stage of cancer.

Kyle, S. and S. Saha (2014). "Nanotechnology for the detection and therapy of stroke." Adv Healthc Mater **3**(11): 1703-1720.

Over the years, nanotechnology has greatly developed, moving from careful design strategies and synthesis of novel nanostructures to producing them for specific medical and biological applications. The use of nanotechnology in diagnostics, drug delivery, and tissue

engineering holds great promise for the treatment of stroke in the future. Nanoparticles are employed to monitor grafted cells upon implantation, or to enhance the imagery of the tissue, which is coupled with a noninvasive imaging modality such as magnetic resonance imaging, computed axial tomography or positron emission tomography scan. Contrast imaging agents used can range from iron oxide, perfluorocarbon, cerium oxide or platinum nanoparticles to quantum dots. The use of nanomaterial scaffolds for neuroregeneration is another area of nanomedicine, which involves the creation of an extracellular matrix mimic that not only serves as a structural support but promotes neuronal growth, inhibits glial differentiation, and controls hemostasis. Promisingly, carbon nanotubes can act as scaffolds for stem cell therapy and functionalizing these scaffolds may enhance their therapeutic potential for treatment of stroke. This Progress Report highlights the recent developments in nanotechnology for the detection and therapy of stroke. Recent advances in the use of nanomaterials as tissue engineering scaffolds for neuroregeneration will also be discussed.

Lambrechts, T., et al. (2016). "Large-scale progenitor cell expansion for multiple donors in a monitored hollow fibre bioreactor." Cytotherapy **18**(9): 1219-1233.

BACKGROUND AIMS: With the increasing scale in stem cell production, a robust and controlled cell expansion process becomes essential for the clinical application of cell-based therapies. The objective of this work was the assessment of a hollow fiber bioreactor (Quantum Cell Expansion System from Terumo BCT) as a cell production unit for the clinical-scale production of human periosteum derived stem cells (hPDCs). METHODS: We aimed to demonstrate comparability of bioreactor production to standard culture flask production based on a product characterization in line with the International Society of Cell Therapy in vitro benchmarks and supplemented with a compelling quantitative in vivo bone-forming potency assay. Multiple process read-outs were implemented to track process performance and deal with donor-to-donor-related variation in nutrient needs and harvest timing. RESULTS: The data show that the hollow fiber bioreactor is capable of robustly expanding autologous hPDCs on a clinical scale (yield between 316 million and 444 million cells starting from 20 million after +/- 8 days of culture) while maintaining their in vitro quality attributes compared with the standard flask-based culture. The in vivo bone-forming assay on average resulted in 10.3 +/- 3.7% and 11.0 +/- 3.8% newly formed bone for the bioreactor and standard culture flask respectively. The analysis showed that the Quantum system provides a reproducible cell expansion process in terms of yields and culture conditions for multiple donors.

Laver, C. R., et al. (2015). "Bimodal in vivo imaging provides early assessment of stem-cell-based photoreceptor engraftment." Eye (Lond) **29**(5): 681-690.

PURPOSE: Subretinal transplantation of stem-cell-derived photoreceptor precursor cells (PPCs) is a promising and innovative approach to treating a range of blinding diseases. However, common barriers to efficient preclinical transplantation comes in the form of suboptimal graft architecture, limited graft survival, and immune-rejection, each of which cannot be assessed using conventional in vivo imaging (i.e., rodent ophthalmoscopy). With the majority of PPCs reported to die within the first few weeks after transplantation, understanding the mechanisms of graft failure, and ultimately devising preventative methods, currently relies on lengthy end point histology. To address these limitations, we hypothesized that combining two imaging modalities, optical coherence tomography (OCT) and fluorescence confocal scanning laser ophthalmoscopy (fcSLO), could provide a more rapid and comprehensive view of PPC engraftment. METHODS: Human ESC-derived PPCs were transplanted into 15 retinal dystrophic rats that underwent bimodal imaging at 0, 8, and 15 days posttransplant. RESULTS: Bimodal imaging provided serial detection of graft: placement, architecture, and survival; each undetectable under ophthalmoscopy. Bimodal imaging determined graft placement to be either: subretinal (n=7), choroidal (n=4), or vitreal (n=4) indicating neural retinal perforation. Graft architecture was highly variable at the time of transplantation, with notable redistribution over time, while complete, or near complete, graft loss was observed in the majority of recipients after day 8. Of particular importance was detection of vitreal aggregates overlying the graft-possibly an indicator of host-site inflammation and rejection. CONCLUSION: Early real-time feedback of engraftment has the potential to greatly increase efficiency of preclinical trials in cell-based retinal therapeutics.

Leapman, R. D. (2017). "Application of EELS and EFTEM to the life sciences enabled by the contributions of Ondrej Krivanek." Ultramicroscopy **180**: 180-187.

The pioneering contributions of Ondrej Krivanek to the development of electron energy loss spectrometers, energy filters, and detectors for transmission and scanning transmission electron microscopes have provided researchers with indispensible tools across a wide range of disciplines in the physical sciences, ranging from condensed matter physics, to chemistry, mineralogy, materials science, and nanotechnology. In addition, the same instrumentation has extended its reach into the life sciences, and it is this aspect of Ondrej Krivanek's

influential contributions that will be surveyed here, together with some personal recollections. Traditionally, electron microscopy has given a purely morphological view of the biological structures that compose cells and tissues. However, the availability of high-performance electron energy loss spectrometers and energy filters offers complementary information about the elemental and chemical composition at the subcellular scale. Such information has proven to be valuable for applications in cell and structural biology, microbiology, histology, pathology, and more generally in the biomedical sciences.

Lee, K. H., et al. (2021). "Ginkwanghols A and B, osteogenic coumaric acid-aliphatic alcohol hybrids from the leaves of Ginkgo biloba." Arch Pharm Res **44**(5): 514-524.

Ginkgo biloba (Ginkgoaceae), commonly known as "ginkgo", is called a living fossil, and it has been cultivated early in human history for various uses in traditional medicine and as a source of food. As part of ongoing research to explore the chemical diversity and biologically active compounds from natural resources, two new coumaric acid-aliphatic alcohol hybrids, ginkwanghols A (1) and B (2) were isolated from the leaves of G. biloba. The coumaric acid-aliphatic alcohol hybrids of natural products have rarely been reported. The structures of the new compounds were determined by extensive NMR spectroscopic analysis, HRESI-MS, and quantum chemical ECD calculations, and by comparing the experimental HRESI-MS/MS spectrum of chemically transformed compound 1a with the predicted HRESI-MS/MS spectra proposed from CFM-ID 3.0, a software tool for MS/MS spectral prediction and MS-based compound identification. Ginkwanghols A (1) and B (2) increased alkaline phosphatase (ALP) production in C3H10T1/2, a mouse mesenchymal stem cell line, in a dose-dependent manner. In addition, ginkwanghols A and B mediated the promotion of osteogenic differentiation as indicated by the induction of the mRNA expression of the osteogenic markers ALP and osteopontin (OPN).

Lei, Y., et al. (2009). "Applications of fluorescent quantum dots to stem cell tracing in vivo." J Nanosci Nanotechnol **9**(10): 5726-5730.

Fluorescent quantum dots have great potential to act as labels in biological research, especially cellular tracking in vivo. Here, small thiol molecules-capped CdSe/ZnS quantum dots attached to Tat peptide were used for label agents, and introduced into living stem cells. The stem cells labeled with quantum dots were intravenously injected into the tail veins of NOD/SCID beta2M null mice, and whose tissue sections of major organs of null mice were examined with fluorescence microscope to assess the distribution of transplanted stem cells. Stem cells with internalized quantum dots offer a promising approach in stem cell transplantation, which will hold a significant impact on stem cell based therapy for several disorders.

Lei, Y., et al. (2008). "Applications of mesenchymal stem cells labeled with Tat peptide conjugated quantum dots to cell tracking in mouse body." Bioconjug Chem **19**(2): 421-427.

Fluorescent quantum dots have great potential in cellular labeling and tracking. Here, PEG encapsulated CdSe/ZnS quantum dots have been conjugated with Tat peptide, and introduced into living mesenchymal stem cells. The Tat peptide conjugated quantum dots in mesenchymal stem cells were assessed by fluorescent microscopy, laser confocal microscope and. flow cytometry. The result shows that Tat peptide conjugated quantum dots could enter mesenchymal stem cells efficiently. The Tat-quantum dots labeled stem cells were further injected into the tail veins of NOD/SCID beta2 M null mice, and the tissue distribution of these labeled cells in nude mice were examined with fluorescence microscope. The result shows that characteristic fluorescence of quantum dots was observed primarily in the liver, the lung and the spleen, with little or no quantum dots accumulation in the brain, the heart, or the kidney.

Lei, Y., et al. (2012). "Labeling of hematopoietic stem cells by Tat peptide conjugated quantum dots for cell tracking in mouse body." J Nanosci Nanotechnol **12**(9): 6880-6886.

Fluorescent quantum dots have great potential to act as labeling tools in biological research, especially cellular tracking and imaging. Tat peptide conjugated quantum dots were introduced into living human hematopoietic stem cells (HSCs). The internalized quantum dots were assessed by laser confocal microscope and flow cytometer. The quantum dots labeled HSCs were injected intravenously into the tail veins of NOD/SCID beta2M null mice. The tissue collections in the major organs were examined with fluorescence microscope to assess the distribution of transplanted stem cells. HSCs with internalized quantum dots offer a promising approach for stem cell transplantation, which will hold a significant impact on stem cell based therapy for several disorders, especially to cure leukemia in current China.

Leung, K. (2004). Alexa Fluor 680-Bevacizumab. Molecular Imaging and Contrast Agent Database (MICAD). Bethesda (MD).

Optical fluorescence imaging is increasingly used to obtain biological functions of specific targets (1, 2). However, the intrinsic fluorescence of biomolecules poses a problem when visible light (350-700 nm)

absorbing fluorophores are used. Near-infrared (NIR) fluorescence (700-1,000 nm) detection avoids the background fluorescence interference of natural biomolecules, providing a high contrast between target and background tissues. NIR fluorophores have wider dynamic range and minimal background as a result of reduced scattering compared with visible fluorescence detection. They also have high sensitivity, resulting from low infrared background, and high extinction coefficients, which provide high quantum yields. The NIR region is also compatible with solid-state optical components, such as diode lasers and silicon detectors. NIR fluorescence imaging is becoming a non-invasive alternative to radionuclide imaging. Vascular endothelial growth factor (VEGF) consists of at least six isoforms of various numbers of amino acids (121, 145, 165, 183, 189, and 206 amino acids) produced through alternative splicing (3). VEGF121, VEGF165, and VEGF189 are the forms secreted by most cell types and are active as homodimers linked by disulfide bonds. VEGF121 does not bind to heparin like the other VEGF species do (4). VEGF is a potent angiogenic factor that induces proliferation, sprouting, migration, and tube formation of endothelial cells. There are three high-affinity tyrosine kinase VEGF receptors (VEGFR-1, Flt-1; VEGFR-2, KDR/Flt-1; and VEGFR-3, Flt-4) on endothelial cells. Several types of non-endothelial cells such as hematopoietic stem cells, melanoma cells, monocytes, osteoblasts, and pancreatic beta cells also express VEGF receptors (3). VEGF is overexpressed in various tumor cells and tumor-associated endothelial cells (5). Inhibition of VEGF receptor function has been shown to inhibit pathological angiogenesis as well as tumor growth and metastasis (6, 7). Radiolabeled VEGF has been developed as a single-photon emission computed tomography tracer for imaging solid tumors and angiogenesis in humans (8-10). However, several studies have shown that cancer treatments (photodynamic therapy, radiotherapy, and chemotherapy) can lead to increased tumor VEGF expression and subsequently to more aggressive disease (11, 12). Therefore, it is important to measure VEGF levels in the tumors to design better anti-cancer treatment protocols. Bevacizumab is a humanized antibody against VEGF-A. It binds to all VEGF isoforms. Bevacizumab is approved for clinical use in metastatic colon carcinoma and non-small cell lung cancer. Chang et al. (13) prepared Alexa Fluor 680-bevacizumab (Alexa680-bevacizumab) for imaging VEGF expression in tumors. Alexa680 is a NIR fluorescent dye with absorbance maximum at 684 nm and emission maximum at 707 nm with a high extinction coefficient of 183,000 (mol/L)(-1)cm(-1).

Leung, K. (2004). Biotinylated vascular endothelial growth factor121-Avi-streptavidin-IRDye800. Molecular Imaging and Contrast Agent Database (MICAD). Bethesda (MD).

Optical fluorescence imaging is increasingly being used to monitor biological functions of specific targets in small animals (1-3). However, the intrinsic fluorescence of biomolecules poses a problem when fluorophores that absorb visible light (350-700 nm) are used. Near-infrared (NIR) fluorescence (700-1,000 nm) detection avoids the natural background fluorescence interference of biomolecules, providing a high contrast between target and background tissues in small animals. NIR fluorophores have a wider dynamic range and minimal background fluorescence as a result of reduced scattering compared with visible fluorescence detection. NIR fluorophores also have high sensitivity, attributable to low background fluorescence, and high extinction coefficients, which provide high quantum yields. The NIR region is compatible with solid-state optical components, such as diode lasers and silicon detectors. NIR fluorescence imaging is a non-invasive complement to radionuclide imaging in small animals. Vascular endothelial growth factor (VEGF) consists of at least six isoforms of various numbers of amino acids (121, 145, 165, 183, 189, and 206 amino acids) produced through alternative splicing (4). VEGF121, VEGF165, and VEGF189 are the forms secreted by most cell types and are active as homodimers linked by disulfide bonds. VEGF121 does not bind to heparin like the other VEGF species (5). VEGF is a potent angiogenic factor that induces proliferation, sprouting, migration, and tube formation of endothelial cells. There are three high-affinity tyrosine kinase VEGF receptors (VEGFR-1, Flt-1; VEGFR-2, KDR/Flt-1; and VEGFR-3, Flt-4) on endothelial cells. Several types of non-endothelial cells such as hematopoietic stem cells, melanoma cells, monocytes, osteoblasts, and pancreatic beta cells also express VEGF receptors (4). VEGF receptors were found to be overexpressed in various tumor cells and tumor-associated endothelial cells (6). Inhibition of VEGF receptor function has been shown to inhibit pathological angiogenesis as well as tumor growth and metastasis (7, 8). Radiolabeled VEGF tracers have been developed for imaging solid tumors and angiogenesis in humans (9-11). Wang et al. (12) fused the Avi peptide (14 amino acids) to the C-terminus of VEGF121 to allow site-specific biotinylation of the epsilon amine group of a central lysine residue of Avi. The binotinylated VEGF121-Avi (VEGF121-Avib) was able to form a tight complex with streptavidin-IRDye800 (SA800) as VEGF121-Avib-SA800 for NIR imaging of VEGFR expression in vivo to study tumor angiogenesis.

Leung, K. (2004). Cy5.5-Single-chain Cys-tagged vascular endothelial growth factor-121. Molecular Imaging and Contrast Agent Database (MICAD). Bethesda (MD).

Optical fluorescence imaging is increasingly used to observe biological functions of specific targets (1, 2) in small animals. However, the intrinsic fluorescence of biomolecules poses a problem when fluorophores that absorb visible light (350-700 nm) are used. Near-infrared (NIR) fluorescence (700-1,000 nm) detection avoids the background fluorescence interference of natural biomolecules, providing a high contrast between target and background tissues. NIR fluorophores have wider dynamic range and minimal background as a result of reduced scattering compared with visible fluorescence detection. They also have high sensitivity, resulting from low infrared background, and high extinction coefficients, which provide high quantum yields. The NIR region is also compatible with solid-state optical components, such as diode lasers and silicon detectors. NIR fluorescence imaging is becoming a non-invasive alternative to radionuclide imaging in small animals. Vascular endothelial growth factor (VEGF) consists of at least six isoforms with various numbers of amino acids (121, 145, 165, 183, 189, and 206 amino acids) produced through alternative splicing (3). VEGF121, VEGF165, and VEGF189 are the forms secreted by most cell types and are active as homodimers linked by disulfide bonds. VEGF121 does not bind to heparin like the other VEGF species (4). VEGF is a potent angiogenic factor that induces proliferation, sprouting, migration, and tube formation of endothelial cells. There are three high-affinity tyrosine kinase VEGF receptors on endothelial cells (VEGFR-1, Flt-1; VEGFR-2, KDR/Flt-1; and VEGFR-3, Flt-4). Several types of non-endothelial cells such as hematopoietic stem cells, melanoma cells, monocytes, osteoblasts, and pancreatic beta cells also express VEGF receptors (3). VEGF receptors were found to be overexpressed in various tumor cells and tumor-associated endothelial cells (5). Inhibition of VEGF receptor function has been shown to inhibit pathological angiogenesis as well as tumor growth and metastasis (6, 7). Radiolabeled VEGF has been developed as a tracer for imaging solid tumors and angiogenesis in humans (8-10). Cys-tag, a fusion tag comprising 15 amino acids, was developed for site-specific conjugation via the free sulfhydryl group of Cys. Backer et al. (11) prepared a Cys-tagged vector of VEGF121 by cloning two single-chain fragments (amino acid sequence 3-112) of VEGF121 joining head-to-tail to express as scVEGF, which can be labeled as (64)Cu-1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA)-scVEGF ((64)Cu-DOTA-scVEGF), (99m)Tc-hydrazinonicotinic acid (HYNIC)-scVEGF ((99m)Tc-HYNIC-scVEGF), and Cy5.5-scVEGF for imaging VEGFR expression to study tumor angiogenesis (12). Cy5.5 is a NIR fluorescent dye with an absorbance maximum at 675 nm and an emission maximum at 694 nm with a high extinction coefficient of 250,000 M(-1)cm(-1) according to in vitro measurement. Cy5.5-scVEGF is being developed for NIR fluorescence imaging of VEGFR-2 in tumor vasculature.

Leung, K. (2004). IRDye800-Anti-vascular endothelial growth factor receptor-2 monoclonal antibody Avas12a1. Molecular Imaging and Contrast Agent Database (MICAD). Bethesda (MD).

Optical fluorescence imaging is increasingly being used to monitor biological functions of specific targets in small animals (1-3). However, the intrinsic fluorescence of biomolecules poses a problem when fluorophores that absorb visible light (350-700 nm) are used. Near-infrared (NIR) fluorescence (700-1,000 nm) detection avoids the natural background fluorescence interference of biomolecules, providing a high contrast between target and background tissues in small animals. NIR fluorophores have a wider dynamic range and minimal background fluorescence as a result of reduced scattering compared with visible fluorescence detection. NIR fluorophores also have high sensitivity, attributable to low background fluorescence, and high extinction coefficients, which provide high quantum yields. The NIR region is also compatible with solid-state optical components, such as diode lasers and silicon detectors. NIR fluorescence imaging is a non-invasive complement to radionuclide imaging in small animals. Vascular endothelial growth factor (VEGF) consists of at least six isoforms of various numbers of amino acids (121, 145, 165, 183, 189, and 206 amino acids) produced through alternative splicing (4). VEGF121, VEGF165, and VEGF189 are the forms secreted by most cell types and are active as homodimers linked by disulfide bonds. VEGF121 does not bind to heparin like the other VEGF species (5). VEGF is a potent angiogenic factor that induces proliferation, sprouting, migration, and tube formation of endothelial cells. There are three high-affinity tyrosine kinase VEGF receptors (VEGFR-1, Flt-1; VEGFR-2, KDR/Flt-1; and VEGFR-3, Flt-4) on endothelial cells. Several types of non-endothelial cells such as hematopoietic stem cells, melanoma cells, monocytes, osteoblasts, and pancreatic beta cells also express VEGF receptors (4). VEGF receptors were found to be overexpressed in various tumor cells and tumor-associated endothelial cells (6). Inhibition of VEGF receptor function has been shown to inhibit pathological angiogenesis as well as tumor growth and metastasis (7, 8). Radiolabeled VEGF tracers have been developed for imaging solid tumors and angiogenesis in humans (9-11). Virostko et al. (12) conjugated the monoclonal antibody Avas12a1 (rat anti-mouse VEGFR-2) with NIR dye IRDye 800CW as IRDye800-Avas12a1 for NIR imaging of VEGFR expression in vivo to study tumor angiogenesis.

Levy, M., et al. (2012). "How cellular processing of superparamagnetic nanoparticles affects their magnetic behavior and NMR relaxivity." Contrast Media Mol Imaging **7**(4): 373-383.

Cellular processing of nanomaterials may affect their physical properties at the root of various biomedical applications. When nanoparticles interact with living cells, their spatial distribution is progressively modified by cellular activity, which tends to concentrate them into intracellular compartments, changing in turn their responsivity to physical stimuli. In this paper, we investigate the consequences of cellular uptake on the related magnetic properties and NMR relaxivity of iron oxide nanoparticles. The superparamagnetic behavior (field-dependent and temperature-dependent magnetization curves investigated by SQUID (Superconducting Quantum Interference Device) measurements) and nuclear magnetic relaxation dispersion (NMRD) R(1) profiles of citrate-coated maghemite nanoparticles (mean diameter 8 nm) were characterized in colloidal suspension and after being uptaken by several types of cells (tumor cells, stem cells and macrophages). The temperature-dependent magnetization as well as the NMRD profile were changed following cellular uptake depending on the stage of endocytosis process while the field-dependent magnetization at room temperature remained unchanged. Magnetic coupling between nanoparticles confined in cell lysosomes accounts for the modification in magnetic behavior, thereby reflecting the local organization of nanoparticles. NMR longitudinal relaxivity was directly sensitive to the intracellular distribution of nanoparticles, in line with Transmission Electron Microscopy TEM observations. This study is the first attempt to link up magnetic properties and NMR characterization of iron oxide nanoparticles before and after their cell processing.

Li, J., et al. (2016). "Multifunctional Quantum Dot Nanoparticles for Effective Differentiation and Long-Term Tracking of Human Mesenchymal Stem Cells In Vitro and In Vivo." Adv Healthc Mater **5**(9): 1049-1057.

Human mesenchymal stem cells (hMSCs) hold great potential for regenerative medicine. Efficient induction of hMSC differentiation and better understanding of hMSCs behaviors in vitro and in vivo are essential to the clinical translation of stem cell therapy. Here a quantum dots (QDs)-based multifunctional nanoparticle (RGD-beta-CD-QDs) is developed for effective enhancing differentiation and long-term tracking of hMSCs in vitro and in vivo. The RGD-beta-CD-QDs are modified with beta-cyclodextrin (beta-CD) and Cys-Lys-Lys-Arg-Gly-Asp (CKKRGD) peptide on the surface. The beta-CD can harbor hydrophobic osteogenic small molecule dexamethasone (Dex) and the RGD peptide not only facilitates the complexation of siRNA and delivers siRNA into hMSCs but also leads to cellular uptake of nanoparticles by RGD receptor. Co-delivery of Dex and siRNA by RGD-beta-CD-QDs nanocarrier significantly expedites and enhances the osteogenesis differentiation of hMSCs in vitro and in vivo by combined effect of small molecule and RNAi. Furthermore, the RGD-beta-CD-QDs can be labeled with hMSCs for a long-term tracking (3 weeks) in vivo to observe the behaviors of implanted hMSCs in animal level. These findings demonstrate that the RGD-beta-CD-QDs nanocarrier provides a powerful tool to simultaneously enhance differentiation and long-term tracking of hMSCs in vitro and in vivo for regenerative medicine.

Li, K., et al. (2013). "Photostable fluorescent organic dots with aggregation-induced emission (AIE dots) for noninvasive long-term cell tracing." Sci Rep **3**: 1150.

Long-term noninvasive cell tracing by fluorescent probes is of great importance to life science and biomedical engineering. For example, understanding genesis, development, invasion and metastasis of cancerous cells and monitoring tissue regeneration after stem cell transplantation require continual tracing of the biological processes by cytocompatible fluorescent probes over a long period of time. In this work, we successfully developed organic far-red/near-infrared dots with aggregation-induced emission (AIE dots) and demonstrated their utilities as long-term cell trackers. The high emission efficiency, large absorptivity, excellent biocompatibility, and strong photobleaching resistance of the AIE dots functionalized by cell penetrating peptides derived from transactivator of transcription proteins ensured outstanding long-term noninvasive in vitro and in vivo cell tracing. The organic AIE dots outperform their counterparts of inorganic quantum dots, opening a new avenue in the development of fluorescent probes for following biological processes such as carcinogenesis.

Li, K., et al. (2017). "Effects of quantum dots on the ROS amount of liver cancer stem cells." Colloids Surf B Biointerfaces **155**: 193-199.

Liver cancer (LC) is a serious disease that threatens human lives. LC has a high recurrence rate and poor prognosis. LC stem cells (LCSCs) play critical roles in these processes. However, the mechanism remains unclear. Reactive oxygen species (ROS) can be used to determine cell apoptosis and proliferation. However, studies of the effects of exogenous nanomaterials on LCSC ROS changes are rarely reported. In this work, quantum dots (QDs) were prepared using a hydrothermal method, and QDs were further modified with polyethylene glycol (PEG) and bovine serum albumin (BSA) using a chemical approach. The effects of QDs, PEG-modified QDs (PEG@QDs)

and BSA-modified QDs (BSA@QDs) on the amounts of ROS in liver cancer PLC/PRF/5 (PLC) cells and liver cancer stem cells (LCSCs) were principally investigated. The results showed that when the concentration of QDs, PEG@QDs, and BSA@QDs were 10nM and 90nM, the ROS amount in PLC cells increased by approximately 2- to 5-fold. However, when the concentrations of these nanomaterials were 10nM and 90nM, ROS levels in LCSCs were reduced by approximately 50%. This critical path potentially leads to drug resistance and recurrence of LC. This work provides an important indication for further study of LC drug resistance and recurrence.

Li, S. C., et al. (2010). "A biological global positioning system: considerations for tracking stem cell behaviors in the whole body." Stem Cell Rev Rep **6**(2): 317-333.

Many recent research studies have proposed stem cell therapy as a treatment for cancer, spinal cord injuries, brain damage, cardiovascular disease, and other conditions. Some of these experimental therapies have been tested in small animals and, in rare cases, in humans. Medical researchers anticipate extensive clinical applications of stem cell therapy in the future. The lack of basic knowledge concerning basic stem cell biology-survival, migration, differentiation, integration in a real time manner when transplanted into damaged CNS remains an absolute bottleneck for attempt to design stem cell therapies for CNS diseases. A major challenge to the development of clinical applied stem cell therapy in medical practice remains the lack of efficient stem cell tracking methods. As a result, the fate of the vast majority of stem cells transplanted in the human central nervous system (CNS), particularly in the detrimental effects, remains unknown. The paucity of knowledge concerning basic stem cell biology--survival, migration, differentiation, integration in real-time when transplanted into damaged CNS remains a bottleneck in the attempt to design stem cell therapies for CNS diseases. Even though excellent histological techniques remain as the gold standard, no good in vivo techniques are currently available to assess the transplanted graft for migration, differentiation, or survival. To address these issues, herein we propose strategies to investigate the lineage fate determination of derived human embryonic stem cells (hESC) transplanted in vivo into the CNS. Here, we describe a comprehensive biological Global Positioning System (bGPS) to track transplanted stem cells. But, first, we review, four currently used standard methods for tracking stem cells in vivo: magnetic resonance imaging (MRI), bioluminescence imaging (BLI), positron emission tomography (PET) imaging and fluorescence imaging (FLI) with quantum dots. We summarize these modalities and propose criteria that can be employed to rank the practical usefulness for specific applications. Based on the results of this review, we argue that additional qualities are still needed to advance these modalities toward clinical applications. We then discuss an ideal procedure for labeling and tracking stem cells in vivo, finally, we present a novel imaging system based on our experiments.

Li, X., et al. (2019). "Fluorescent labelling in living dental pulp stem cells by graphene oxide quantum dots." Artif Cells Nanomed Biotechnol **47**(1): 115-122.

Cellular labelling is possible to offer significant information after transplantation for the purpose of determining stem cell therapy's efficacy. According to the research, it has been reported that graphene oxide quantum dots (GOQDs) are a kind of healthy biological labelling agent for stem cells which show little cytotoxicity. GOQDs' interactions have been examined on the dental pulp stem cells (hDPSCs) of human beings for the purpose of investigating GOQD's biocompatibility and uptake and explored GOQDs' effects on hDPSCs' metabolic activity and the proliferation. According to the outcomes, GDQDs have been accepted by hDPSCs in a time-dependent and concentration-dependent behaviour. Moreover, no important changes have been discovered within hDOPSCs' proliferation, viability as well as metabolic activity after treatment with GOQDs. Therefore, such resources have shown that GOQDs can be multifunctional agents for cell therapy, drug delivery as well as cell imaging and also as outstanding candidates for labelling stem cells.

Li, X., et al. (2020). "Graphene Oxide Quantum Dots-Induced Mineralization via the Reactive Oxygen Species-Dependent Autophagy Pathway in Dental Pulp Stem Cells." J Biomed Nanotechnol **16**(6): 965-974.

As an important recycling and degradation system, autophagy is considered to be critical in regulating stem cell differentiation. It has been shown that graphene oxide quantum dots (GOQDs) are a robust biological labelling tool for stem cells with little cytotoxicity. In this study, we explored the role of autophagy in regulating the impact of GOQDs on the odontoblastic differentiation of DPSCs during autophagy. Western blotting and immunofluorescence staining were used to evaluate the autophagic activity of DPSCs. Quantitative PCR, alizarin red S staining, and alkaline phosphatase staining were used to examine DPSC odontoblastic differentiation. The impacts of ROS scavengers on autophagy induction and reactive oxygen species (ROS) levels were also measured. Lentiviral vectors carrying Beclin1 siRNA sequences, as well as autophagy inhibitors (3-MA and bafilomycin A1), were used to inhibit autophagy. Initial exposure to GOQDs increased autophagic activity and enhanced DPSC mineralization. Autophagy inhibition suppressed GOQD-induced odontoblastic differentiation. Moreover,

GOQD treatment induced autophagy in a ROS-dependent manner. GOQDs promoted differentiation, which could be modulated via ROS-induced autophagy.

Li, Y. Y., et al. (2012). "Adipose-derived mesenchymal stem cells ameliorate STZ-induced pancreas damage in type 1 diabetes." Biomed Mater Eng **22**(1-3): 97-103.

OBJECTIVE: To investigate the possibility of adipose-derived mesenchymal stem cells (ADSC) in the treatment of type 1 diabetes (T1D). METHODS: ADSC were isolated from the adipotic tissue of abdomen in Sprague-Dawley rats (4-6 week-old,female) and expanded in vitro. Cells were then identified by testing their phenotypes through flow cytometry. Balb/c mice (8 week-old, male) were divided into 3 groups: T1D group, ADSC group and control group. Streptozocin (50 mg/kg.d) were injected intraperitoneally into mice of T1D group and ADSC group for 5 consecutive days to establish the T1D model. In ADSC group, ADSC were injected intravenously on day 3 of STZ injection. In control group, only PBS was injected. Fasting blood glucose (FGB) level was examined once a week. At the end of the 4th week, animals were killed. The pathological changes of islet were showed by histochemistry through hematoxylin-eosin staining (HE staining). beta cell insulin expression was detected by quantum dots immunofluorescence histochemistry. RESULTS: After ADSC administration, FGB levels decreased significantly from the second week. Whereas FGB levels in T1D group increased significantly and continuously during the experimental period. Moreover, ADSC effectively suppressed pancreatic islet damage induced by STZ and increased the expression of insulin protein in pancreatic beta cells. CONCLUSIONS: Intravenuously injected ADSC can prevent STZ induced beta-cell destruction and decrease blood glucose level.

Lin, S., et al. (2007). "Quantum dot imaging for embryonic stem cells." BMC Biotechnol **7**: 67.

BACKGROUND: Semiconductor quantum dots (QDs) hold increasing potential for cellular imaging both in vitro and in vivo. In this report, we aimed to evaluate in vivo multiplex imaging of mouse embryonic stem (ES) cells labeled with Qtracker delivered quantum dots (QDs). RESULTS: Murine embryonic stem (ES) cells were labeled with six different QDs using Qtracker. ES cell viability, proliferation, and differentiation were not adversely affected by QDs compared with non-labeled control cells (P = NS). Afterward, labeled ES cells were injected subcutaneously onto the backs of athymic nude mice. These labeled ES cells could be imaged with good contrast with one single excitation wavelength. With the same excitation wavelength, the signal intensity, defined as (total signal-background)/exposure time in millisecond was 11 +/- 2 for cells labeled with QD 525, 12 +/- 9 for QD 565, 176 +/- 81 for QD 605, 176 +/- 136 for QD 655, 167 +/- 104 for QD 705, and 1,713 +/- 482 for QD 800. Finally, we have shown that QD 800 offers greater fluorescent intensity than the other QDs tested. CONCLUSION: In summary, this is the first demonstration of in vivo multiplex imaging of mouse ES cells labeled QDs. Upon further improvements, QDs will have a greater potential for tracking stem cells within deep tissues. These results provide a promising tool for imaging stem cell therapy non-invasively in vivo.

Lin, Z., et al. (2014). "A novel aptamer functionalized CuInS2 quantum dots probe for daunorubicin sensing and near infrared imaging of prostate cancer cells." Anal Chim Acta **818**: 54-60.

In this paper, a novel daunorubicin (DNR)-loaded MUC1 aptamer-near infrared (NIR) CuInS2 quantum dot (DNR-MUC1-QDs) conjugates were developed, which can be used as a targeted cancer imaging and sensing system. After the NIR CuInS2 QDs conjugated with the MUC1 aptamer-(CGA)7, DNR can intercalate into the double-stranded CG sequence of the MUC1-QDs. The incorporation of multiple CG sequences within the stem of the aptamers may further increase the loading efficiency of DNR on these conjugates. DNR-MUC1-QDs can be used to target prostate cancer cells. We evaluated the capacity of MUC1-CuInS2 QDs for delivering DNR to cancer cells in vitro, and its binding affinity to MUC1-positive and MUC1-negative cells. This novel aptamer functionalized QDs bio-nano-system can not only deliver DNR to the targeted prostate cancer cells, but also can sense DNR by the change of photoluminescence intensity of CuInS2 QDs, which concurrently images the cancer cells. The quenched fluorescence intensity of MUC1-QDs was proportional to the concentration of DNR in the concentration ranges of 33-88 nmol L(-1). The detection limit (LOD) for DNR was 19 nmol L(-1). We demonstrate the specificity and sensitivity of this DNR-MUC1-QDs probe as a cancer cell imaging, therapy and sensing system in vitro.

Lira, R. B., et al. (2013). "Studies on intracellular delivery of carboxyl-coated CdTe quantum dots mediated by fusogenic liposomes." J Mater Chem B **1**(34): 4297-4305.

The use of Quantum Dots (QDs) as fluorescent probes for understanding biological functions has emerged as an advantageous alternative over application of conventional fluorescent dyes. Intracellular delivery of QDs is currently a specific field of research. When QDs are tracking a specific target in live cells, they are mostly applied for extracellular membrane labeling. In order to study intracellular molecules and structures it is necessary to deliver free QDs into the cell cytosol. In this work, we adapted the freeze and thaw method to

encapsulate water dispersed carboxyl-coated CdTe QDs into liposomes of different compositions, including cationic liposomes with fusogenic properties. We showed that labeled liposomes were able to fuse with live human stem cells and red blood cells in an endocytic-independent way. We followed the interactions of liposomes containing QDs with the cells. The results were minutely discussed and showed that QDs were delivered, but they were not freely diffused in the cytosol of those cells. We believe that this approach has the potential to be applied as a general route for encapsulation and delivery of any membrane-impermeant material into living cells.

Liu, G. and J. L. Feldman (1992). "Quantal synaptic transmission in phrenic motor nucleus." J Neurophysiol **68**(4): 1468-1471.

1. The quantal nature of excitatory synaptic transmission was studied in respiratory interneurons and phrenic motoneurons of intact neonatal rat brain stem-spinal cord preparations in vitro. Synaptic currents were recorded with whole-cell patch-clamp recording techniques. 2. Because the most important factor for quantal detection is the ratio of quantal size to quantal standard deviation, factors that influence this ratio were evaluated so that experimental techniques that enhance this ratio could be defined. 3. Under favorable conditions, we directly observed quantal amplitude fluctuations in spontaneous excitatory postsynaptic currents (EPSCs) in spinal cord respiratory neurons. The quantal conductance size was 55-100 pS. With fast decay of these EPSCs, the charge reaching the soma for a single quantum is only approximately 15 fC (Vh = -80 mV). 4. We also studied miniature EPSC amplitude distributions. These were skewed, as previously reported; however, distinct quantal intervals were observed. Furthermore, in three cells tested, the quantal size in the miniature EPSC amplitude distribution was similar to the quantal size in the spontaneous EPSC amplitude distribution. 5. We conclude that excitatory synaptic transmission in the mammalian spinal cord is quantal and that the apparent skewness of miniature EPSC distributions results from summation of events with multiple quantal peak amplitudes.

Liu, J. H., et al. (2015). "Carbon "Quantum" Dots for Fluorescence Labeling of Cells." ACS Appl Mater Interfaces **7**(34): 19439-19445.

The specifically synthesized and selected carbon dots of relatively high fluorescence quantum yields were evaluated in their fluorescence labeling of cells. For the cancer cell lines, the cellular uptake of the carbon dots was generally efficient, resulting in the labeling of the cells with bright fluorescence emissions for both one- and two-photon excitations from predominantly the cell membrane and cytoplasm. In the exploration on labeling the live stem cells, the cellular uptake of the carbon dots was relatively less efficient, though fluorescence emissions could still be adequately detected in the labeled cells, with the emissions again predominantly from the cell membrane and cytoplasm. This combined with the observed more efficient internalization of the same carbon dots by the fixed stem cells might suggest some significant selectivity of the stem cells toward surface functionalities of the carbon dots. The needs and possible strategies for more systematic and comparative studies on the fluorescence labeling of different cells, including especially live stem cells, by carbon dots as a new class of brightly fluorescent probes are discussed.

Liu, K. X., et al. (2020). "Populeuphrines A and B, two new cembrane diterpenoids from the resins of Populus euphratica." Nat Prod Res **34**(21): 3108-3116.

Two new cembrane diterpenoids, named populeuphrines A and B (1 and 2), together with three known analogues (3-5) were isolated from the resins of Populus euphratica. The planar structures and relative configurations of 1 and 2 were elucidated by detailed 1 D and 2 D NMR spectroscopic analyses. The absolute configurations of 1 and 2 were determined by X-ray diffraction analysis and quantum chemical computation. Biological activities of all the isolates against proliferation of human cancer cells and umbilical cord mesenchymal stem cells were evaluated.

Liu, T., et al. (2014). "Hematopoiesis toxicity induced by CdTe quantum dots determined in an invertebrate model organism." Biomaterials **35**(9): 2942-2951.

Quantum dots (QDs) have gained significant attention due to their superior optical properties and wide usage in biological and biomedical studies. In recent years, there has been intense concern regarding the in vivo toxicity of QDs. This study was undertaken to examine the toxicity of CdTe QDs on hematopoiesis in an invertebrate model organism, Bombyx mori. Vascular injection of sub-lethal doses of QDs in B. mori larvae caused time- and dose-dependent damage in the hematopoietic organ and hematocytes. QDs with the maximum emission wavelength of 530 nm (QDs530) were quickly observed in cystocytes and plasmacytes, and gradually bleached their green fluorescence, followed by a decrease in peripheral hematocytes. Additionally, the proportion of abnormal hematocytes increased. In marked contrast, QDs with the maximum emission wavelength of 720 nm (QDs720) were quickly surrounded by hematocytes and subsequently enriched in cystocytes like the human's leukocytes, but with weaker cytotoxicity. QDs exposure promoted the mitotic nucleus in prohemocytes and hematocytes similar to peripheral blood stem cells in humans, but aggravated apoptosis. A decrease in hematopoiesis was

accompanied by shrinkage and death of hematopoietic organs via an increase in reactive oxygen species. QDs with smaller size resulted in more severe hematopoiesis toxicity.

Liu, X., et al. (2018). "A cyclo-trimer of acetonitrile combining fluorescent property with ability to induce osteogenesis and its potential as multifunctional biomaterial." Acta Biomater **65**: 163-173.

A biomaterial combining fluorescent property with ability to induce osteogenesis can serve as an ideal multifunctional scaffold in bone tissue engineering. However, the frequently used fluorescent agents can only serve as imaging probes. The polymer or oligomer with a conjugated system containing nitrogen atoms will fulfill these criteria. In this study, a cyclo-trimer of acetonitrile is synthesized using a facile method, which is proved to be 4-amino-2,6-dimethylpyrimidine. The cyclo-trimer of acetonitrile demonstrates strong intrinsic photoluminescence and has the potential for in vivo imaging. The cyclo-trimer of acetonitrile shows no toxicity both in vitro and in vivo. Moreover, the cyclo-trimer of acetonitrile significantly promotes the osteogenesis of SaOS-2 cells by improving alkaline phosphatase activity, collagen type I and osteocalcin expression, as well as expressions of osteoblastic genes, and enhances the matrix mineralization of rBMSCs. Thus, the cyclo-trimer of acetonitrile synthesized in present study illustrates the employment of this kind multifunctional biomaterial in bone tissue engineering and may offer great potential in biomedical applications where bioimaging and osteogenesis are both required. STATEMENT OF SIGNIFICANCE: A conjugated cyclo-trimer of acetonitrile combining intrinsic fluorescent property with ability to induce osteogenesis was reported. Different from the traditional fluorescent dye or quantum dots, which are just "imaging agents", the cyclo-trimer of acetonitrile can serve as a multifunctional biomaterial and offer great potential in biomedical applications where bioimaging and osteogenesis are both required. To our best knowledge, the fluorescent property, especially fluorescent property in vivo and the ability of this molecule to induce osteogenesis have not been reported before. Our work illustrates the employment of this kind multifunctional biomaterial in bone tissue engineering and will highlight the importance of multifunctional biomaterial in biomedical applications.

Liu, Z., et al. (2019). "AIEgen Nanoparticles of Arylamino Fumaronitrile Derivative with High Near-Infrared Emission for Two-Photon Imaging and in Vivo Cell Tracking." ACS Appl Bio Mater **2**(1): 430-436.

Developing of two-photon materials for live-cell imaging and in vivo analysis in-depth have received great attention, and it is urgent so that such microscopy techniques could be promoted and advanced using the powerful probes. Herein, a new arylamino fumaronitrile derivative NPAPF was synthesized and transferred as aggregation-induced emission luminogen (AIEgen) fluorescent nanoparticles (AF-NPs) via assembly technique. This AF-NP exhibited a two-photon absorption cross-section at 2.6 x 10(6) GM with 19.5% of fluorescence quantum yield. Moreover, utilizing the great potential of AF-NPs, two-photon imaging of live cells with good cytocompatibility is realized upon two-photon microscopy. By in vivo long-term tracing studies of mesenchymal stem cells, we demonstrated the tremendous advantage of AF-NPs tracer in monitoring the stem cells transplant. Therefore, our unique AF-NPs provided an efficient two-photon-absorbing probe for investigating biological mechanism and behavior, and opened a new avenue for spatiotemporal visualization of transplanted stem cells.

Long, X., et al. (2013). "Effect of human mesenchymal stem cells on the growth of HepG2 and Hela cells." Cell Struct Funct **38**(1): 109-121.

Human mesenchymal stem cells (hMSCs) accumulate at carcinomas and have a great impact on cancer cell's behavior. Here we demonstrated that hMSCs could display both the promotional and inhibitive effects on growth of HepG2 and Hela cells by using the conditioned media, indirect co-culture, and cell-to-cell co-culture. Cell growth was increased following the addition of lower proportion of hMSCs while decreased by treatment of higher proportion of hMSCs. We also established a novel noninvasive label way by using internalizing quantum dots (i-QDs) for study of cell-cell contact in the co-culture, which was effective and sensitive for both tracking and distinguishing different cells population without the disturbance of cells. Furthermore, we investigated the role of hMSCs in regulation of cell growth and showed that mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) signaling pathways were involved in hMSC-mediated cell inhibition and proliferation. Our findings suggested that hMSCs regulated cancer cell function by providing a suitable environment, and the discovery from the study would provide some clues for development of effective strategy for hMSC-based cancer therapies.

Loukanov, A., et al. (2010). "Immunolocalization of multiple membrane proteins on a carbon replica with STEM and EDX." Ultramicroscopy **110**(4): 366-374.

We present a method for immunolabeling of multiple species of membrane proteins with high spatial resolution. It allows differentiation of equally sized very small markers with different chemical compositions, which leads to high labeling efficiency and reduces steric hindrance of closely spaced immunolabeled

biomolecules. Markers such as CdSe/ZnS semiconductor quantum dots and colloidal gold particles are distinguished by differential contrast in high-angle annular detector dark-field STEM mode or by EDX microanalysis of their elemental contents. This method was tested by observation of labeled AMPA- and NMDA-type glutamate receptors on sodium-dodecyl-sulfate-digested replica prepared from rat hippocampus. To improve particle visibility and detectability, the replica films were made exclusively with carbon to avoid the high background of conventional platinum/carbon replica. Extension of the method is suggested by detection of 1.4 nm nanogold particles and its potential application in the biological imaging research.

Lu, S., et al. (2010). "Targeting of embryonic stem cells by peptide-conjugated quantum dots." PLoS One **5**(8): e12075.

BACKGROUND: Targeting stem cells holds great potential for studying the embryonic stem cell and development of stem cell-based regenerative medicine. Previous studies demonstrated that nanoparticles can serve as a robust platform for gene delivery, non-invasive cell imaging, and manipulation of stem cell differentiation. However specific targeting of embryonic stem cells by peptide-linked nanoparticles has not been reported. METHODOLOGY/PRINCIPAL FINDINGS: Here, we developed a method for screening peptides that specifically recognize rhesus macaque embryonic stem cells by phage display and used the peptides to facilitate quantum dot targeting of embryonic stem cells. Through a phage display screen, we found phages that displayed an APWHLSSQYSRT peptide showed high affinity and specificity to undifferentiated primate embryonic stem cells in an enzyme-linked immunoabsorbent assay. These results were subsequently confirmed by immunofluorescence microscopy. Additionally, this binding could be completed by the chemically synthesized APWHLSSQYSRT peptide, indicating that the binding capability was specific and conferred by the peptide sequence. Through the ligation of the peptide to CdSe-ZnS core-shell nanocrystals, we were able to, for the first time, target embryonic stem cells through peptide-conjugated quantum dots. CONCLUSIONS/SIGNIFICANCE: These data demonstrate that our established method of screening for embryonic stem cell specific binding peptides by phage display is feasible. Moreover, the peptide-conjugated quantum dots may be applicable for embryonic stem cell study and utilization.

Luo, M., et al. (2022). "Micro-/nano-fluidic devices and in vivo fluorescence imaging based on quantum dots for cytologic diagnosis." Lab Chip **22**(12): 2223-2236.

Semiconductor quantum dots (QDs) possess attractive merits over traditional organic dyes, such as tunable emission, narrow emission spectra and good resistance against optical bleaching, and play a vital role in biosensing and bioimaging for cytologic diagnoses. Microfluidic technology is a potentially useful strategy, as it provides a rapid platform for tracing of disease markers. In vivo fluorescence imaging (FI) based on QDs has become popular for the analysis of complex biological processes. We herein report the applications of multifunctional fluorescent QDs as sensitive probes for diagnoses on cancer medicine and stem cell therapy via microfluidic chips and in vivo imaging.

Lyberopoulou, A., et al. (2015). "Mutational analysis of circulating tumor cells from colorectal cancer patients and correlation with primary tumor tissue." PLoS One **10**(4): e0123902.

Circulating tumor cells (CTCs) provide a non-invasive accessible source of tumor material from patients with cancer. The cellular heterogeneity within CTC populations is of great clinical importance regarding the increasing number of adjuvant treatment options for patients with metastatic carcinomas, in order to eliminate residual disease. Moreover, the molecular profiling of these rare cells might lead to insight on disease progression and therapeutic strategies than simple CTCs counting. In the present study we investigated the feasibility to detect KRAS, BRAF, CD133 and Plastin3 (PLS3) mutations in an enriched CTCs cell suspension from patients with colorectal cancer, with the hypothesis that these genes` mutations are of great importance regarding the generation of CTCs subpopulations. Subsequently, we compared CTCs mutational status with that of the corresponding primary tumor, in order to access the possibility of tumor cells characterization without biopsy. CTCs were detected and isolated from blood drawn from 52 colorectal cancer (CRC) patients using a quantum-dot-labelled magnetic immunoassay method. Mutations were detected by PCR-RFLP or allele-specific PCR and confirmed by direct sequencing. In 52 patients, discordance between primary tumor and CTCs was 5.77% for KRAS, 3.85% for BRAF, 11.54% for CD133 rs3130, 7.69% for CD133 rs2286455 and 11.54% for PLS3 rs6643869 mutations. Our results support that DNA mutational analysis of CTCs may enable non-invasive, specific biomarker diagnostics and expand the scope of personalized medicine for cancer patients.

Ma, K., et al. (2011). "Effects of nanofiber/stem cell composite on wound healing in acute full-thickness skin wounds." Tissue Eng Part A **17**(9-10): 1413-1424.

Acute full-thickness skin wounds (FTSW) caused by extensive burns or high-energy trauma are not adequately addressed by current clinical treatments.

This study hypothesized that biomimetic nanofiber scaffolds (NFSs) functionalized with rich attachment of bone-marrow-derived mesenchymal stem cells (BM-MSCs) can promote wound healing in acute FTSW. Results in a rat model showed that both NFS and BM-MSCs contributed to the wound healing. Wounds in NFS group with a higher density of BM-MSCs achieved complete closure 8 days earlier than the control group. Implanted BM-MSCs were found to promote epithelial edge ingrowth and collagen synthesis. The colocation of BM-MSCs (tagged with quantum-dots) with the expression of keratin 10 and filaggrin indicated the participation of BM-MSCs in epidermal differentiation at early and intermediate stages under the local wounding environment. Overall, this study suggests a great potential of using NFS/BM-MSC composites for the treatment of acute FTSW.

Madhankumar, A. B., et al. (2017). "Interleukin-13 conjugated quantum dots for identification of glioma initiating cells and their extracellular vesicles." Acta Biomater **58**: 205-213.

Cadmium selenide (CdSe) based quantum dots modified with polyethylene glycol and chemically linked to interleukin-13 (IL13) were prepared with the aim of identifying the high affinity receptor (IL13Ralpha2) which is expressed in glioma stem cells and exosomes secreted by these cancer stem cells. IL13 conjugated quantum dots (IL13QD) were thoroughly characterized for their physicochemical properties including particle size and surface morphology. Furthermore, the specific binding of the IL13QD to glioma cells and to glioma stem cells (GSC) was verified using a competitive binding study. The exosomes were isolated from the GSC conditioned medium and the expression of IL13Ralpha2 in the GSC and exosomes was verified. The binding property of IL13QD to the tumor associated exosomes was initially confirmed by transmission electron microscopy. The force of attraction between the quantum dots and U251 glioma cells and the exosomes was investigated by atomic force microscopy, which indicated a higher force of binding interaction between the IL13QD and IL13Ralpha2 expressing glioma cells and exosomes secreted by glioma stem cells. Flow cytometry of the IL13QD and exosomes from the culture media and cerebrospinal fluid (CSF) of patients with glioma tumors indicated a distinctly populated complex pattern different from that of non-targeted quantum dots and bovine serum albumin (BSA) conjugated quantum dots confirming specific binding potential of the IL13QD to the tumor associated exosomes. The results of this study demonstrate that IL13QD can serve as an ex vivo marker for glioma stem cells and exosomes that can inform diagnosis and prognosis of patients harboring malignant disease. STATEMENT OF SIGNIFICANCE: Functionalized quantum dots are flexible semiconductor nanomaterials which have an immense application in biomedical research. In particular, when they are functionalized with biomolecules like proteins or antibodies, they have the specialized ability to detect the expression of receptors and antigens in cells and tissues. In this study we designed a cytokine (interleukin-13) functionalized quantum dot to detect a cancer associated receptor expressed in cancer stem cells and the extracellular vesicles (exosomes) secreted by the cancer cells themselves. The binding pattern of these cytokine modified quantum dots to the cancer stem cells and exosomes alters the physical properties of the complex in the fixed and suspended form. This altered binding pattern can be monitored by a variety of techniques, including transmission electron microscopy, atomic force microscopy and flow cytometry, and subsequent characterization of this quantum dot binding profile provides useful data that can be utilized as a fingerprint to detect cancer disease progression. This type of functionalized quantum dot fingerprint is especially useful for invasive cancers including brain and other metastatic cancers and may allow for earlier detection of disease progression or recurrence, thus saving the lives of patients suffering from this devastating disease.

Mangoni, M., et al. (2012). "Stem cell tracking: toward clinical application in oncology?" Tumori **98**(5): 535-542.

Noninvasive cellular imaging allows the tracking of grafted cells as well as the monitoring of their migration, suggesting potential applications to track both cancer and therapeutic stem cells. Cell tracking can be performed by two approaches: direct labeling (cells are labeled with tags) and indirect labeling (cells are transfected with a reporter gene and visualized after administration of a reporter probe). Techniques for in vivo detection of grafted cells include optic imaging, nuclear medicine imaging, magnetic resonance imaging, microCT imaging and ultrasound imaging. The ideal imaging modality would bring together high sensitivity, high resolution and low toxicity. All of the available imaging methods are based on different principles, have different properties and different limitations, so several of them can be considered complementary. Transfer of these preclinical cellular imaging modalities to stem cells has already been reported, and transfer to clinical practice within the next years can be reasonably considered.

Mannucci, S., et al. (2017). "Quantum dots labelling allows detection of the homing of mesenchymal stem cells administered as immunomodulatory therapy in an experimental model of pancreatic islets transplantation." J Anat **230**(3): 381-388.

Cell transplantation is considered a promising therapeutic approach in several pathologies but still needs innovative and non-invasive imaging technologies to be validated. The use of mesenchymal stem cells (MSCs) attracts major interest in clinical transplantation thanks to their regenerative properties, low immunogenicity and ability to regulate immune responses. In several animal models, MSCs are used in co-transplantation with pancreatic islets (PIs) for the treatment of type I diabetes, supporting graft survival and prolonging normal glycaemia levels. In this study we investigated the homing of systemically administered MSCs in a rat model of pancreatic portal vein transplantation. MSCs labelled with quantum dots (Qdots) were systemically injected by tail vein and monitored by optical fluorescence imaging. The fluorescence signal of the liver in animals co-transplanted with MSCs and PIs was significantly higher than in control animals in which MSCs alone were transplanted. By using magnetic labelling of PIs, the homing of PIs into liver was independently confirmed. These results demonstrate that MSCs injected in peripheral blood vessels preferentially accumulate into liver when PIs are transplanted in the same organ. Moreover, we prove that bimodal MRI-fluorescence imaging allows specific monitoring of the fate of two types of cells.

Manoukian, O. S., et al. (2017). "Electrospun Nanofiber Scaffolds and Their Hydrogel Composites for the Engineering and Regeneration of Soft Tissues." Methods Mol Biol **1570**: 261-278.

Electrospinning has emerged as a simple, elegant, and scalable technique that can be used to fabricate polymeric nanofibers. Pure polymers as well as blends and composites of both natural and synthetic ones have been successfully electrospun into nanofiber matrices for many biomedical applications. Tissue-engineered medical implants, such as polymeric nanofiber scaffolds, are potential alternatives to autografts and allografts, which are short in supply and carry risks of disease transmission. These scaffolds have been used to engineer various soft tissues, including connective tissues, such as skin, ligament, and tendon, as well as nonconnective ones, such as vascular, muscle, and neural tissue. Electrospun nanofiber matrices show morphological similarities to the natural extracellular matrix (ECM), characterized by ultrafine continuous fibers, high surface-to-volume ratios, high porosities, and variable pore-size distributions. The physiochemical properties of nanofiber matrices can be controlled by manipulating electrospinning parameters so that they meet the requirements of a specific application.Nanostructured implants show improved biological performance over bulk materials in aspects of cellular infiltration and in vivo integration, taking advantage of unique quantum, physical, and atomic properties. Furthermore, the topographies of such scaffolds has been shown to dictate cellular attachment, migration, proliferation, and differentiation, which are critical in engineering complex functional tissues with improved biocompatibility and functional performance. This chapter discusses the use of the electrospinning technique in the fabrication of polymer nanofiber scaffolds utilized for the regeneration of soft tissues. Selected scaffolds will be seeded with human mesenchymal stem cells (hMSCs), imaged using scanning electron and confocal microscopy, and then evaluated for their mechanical properties as well as their abilities to promote cell adhesion, proliferation , migration, and differentiation.

Manshian, B. B., et al. (2015). "High content analysis at single cell level identifies different cellular responses dependent on nanomaterial concentrations." Sci Rep **5**: 13890.

A mechanistic understanding of nanomaterial (NM) interaction with biological environments is pivotal for the safe transition from basic science to applied nanomedicine. NM exposure results in varying levels of internalized NM in different neighboring cells, due to variances in cell size, cell cycle phase and NM agglomeration. Using high-content analysis, we investigated the cytotoxic effects of fluorescent quantum dots on cultured cells, where all effects were correlated with the concentration of NMs at the single cell level. Upon binning the single cell data into different categories related to NM concentration, this study demonstrates, for the first time, that quantum dots activate both cytoprotective and cytotoxic mechanisms, resulting in a zero net result on the overall cell population, yet with significant effects in cells with higher cellular NM levels. Our results suggest that future NM cytotoxicity studies should correlate NM toxicity with cellular NM numbers on the single cell level, as conflicting mechanisms in particular cell subpopulations are commonly overlooked using classical toxicological methods.

Marrache, S. and S. Dhar (2013). "Biodegradable synthetic high-density lipoprotein nanoparticles for atherosclerosis." Proc Natl Acad Sci U S A **110**(23): 9445-9450.

Atherosclerosis remains one of the most common causes of death in the United States and throughout the world because of the lack of early detection. Macrophage apoptosis is a major contributor to the instability of atherosclerotic lesions. Development of an apoptosis targeted high-density lipoprotein (HDL)-mimicking nanoparticle (NP) to carry contrast agents for early detection of vulnerable plaques and the initiation of preventative therapies that exploit the

vascular protective effects of HDL can be attractive for atherosclerosis. Here, we report the construction of a synthetic, biodegradable HDL-NP platform for detection of vulnerable plaques by targeting the collapse of mitochondrial membrane potential that occurs during apoptosis. This HDL mimic contains a core of biodegradable poly(lactic-co-glycolic acid), cholesteryl oleate, and a phospholipid bilayer coat that is decorated with triphenylphosphonium (TPP) cations for detection of mitochondrial membrane potential collapse. The lipid layer provides the surface for adsorption of apolipoprotein (apo) A-I mimetic 4F peptide, and the core contains diagnostically active quantum dots (QDs) for optical imaging. In vitro uptake, detection of apoptosis, and cholesterol binding studies indicated promising detection ability and therapeutic potential of TPP-HDL-apoA-I-QD NPs. In vitro studies indicated the potential of these NPs in reverse cholesterol transport. In vivo biodistribution and pharmacokinetics indicated favorable tissue distribution, controlled pharmacokinetic parameters, and significant triglyceride reduction for i.v.-injected TPP-HDL-apoA-I-QD NPs in rats. These HDL NPs demonstrate excellent biocompatibility, stability, nontoxic, and nonimmunogenic properties, which prove to be promising for future translation in early plaque diagnosis and might find applications to prevent vulnerable plaque progression.

Martinez-Cuadron, D., et al. (2020). "Practical Considerations for Treatment of Relapsed/Refractory FLT3-ITD Acute Myeloid Leukaemia with Quizartinib: Illustrative Case Reports." Clin Drug Investig **40**(3): 227-235.

Quizartinib is a tyrosine kinase inhibitor selectively targeting the FMS-like tyrosine kinase 3 (FLT3) receptor that has been developed for the treatment of acute myeloid leukaemia (AML). The Phase 3 QuANTUM-R study investigated the efficacy of quizartinib monotherapy in patients with relapsed/refractory FLT3-ITD mutation-positive AML. The clinical course of four QuANTUM-R participants exemplifies issues specific to quizartinib treatment and is described here. Patient 1 was FLT3-ITD mutation-negative at AML diagnosis, but became FLT3-ITD mutation-positive during treatment that included several lines of chemotherapy and was therefore a suitable candidate for quizartinib. Because of the clonal shifts of AML during treatment, retesting genetic alterations at each relapse or resistance may help to identify candidates for targeted treatment options. Patient 2 developed QTc prolongation during quizartinib treatment, but the QTc interval normalised after dose reduction, allowing the patient to continue treatment and eventually resume the recommended dose. Patient 3 responded to quizartinib and was scheduled for haematopoietic stem cell transplant (HSCT), but developed febrile neutropenia and invasive aspergillosis during conditioning and subsequently died (to avoid drug-drug interactions, no azole antifungal was administered concomitantly). Care is required when selecting concomitant medications, and if there is potential for interactions (e.g. if prophylactic azole antifungals are required) the quizartinib dose should be reduced to minimise the risk of QTc prolongation. Patient 4 was able to undergo HSCT after responding to quizartinib and experienced a durable response after HSCT while on quizartinib maintenance therapy. Together, these cases illustrate the main issues to be addressed when managing patients under quizartinib, allowing for adequate scheduling and tolerability, bridging to HSCT, and durable remission on maintenance therapy in some patients.

Martin-Manso, G. and P. J. Hanley (2015). "Using the quantum cell expansion system for the automated expansion of clinical-grade bone marrow-derived human mesenchymal stromal cells." Methods Mol Biol **1283**: 53-63.

Bone marrow-derived human mesenchymal stromal cells (hMSCs) constitute a promising therapeutic approach. However, the extremely low frequency of hMSCs in bone marrow makes the translation of these regulatory cells to clinical therapies difficult for large patient populations. Here, we describe a good manufacturing practices-compliant procedure for the expansion of hMSCs using the Quantum Cell Expansion System. This closed and automated system allows the large-scale expansion of hMSCs while maintaining their multipotency, immunophenotype, morphology, and karyotype.

McCabe-Lankford, E., et al. (2020). "Binding of Targeted Semiconducting Photothermal Polymer Nanoparticles for Intraperitoneal Detection and Treatment of Colorectal Cancer." Nanotheranostics **4**(3): 107-118.

Nanoparticles offer many promising advantages for improving current surgical regimens through their ability to detect and treat disseminated colorectal cancer (CRC). Hybrid Donor-Acceptor Polymer Particles (HDAPPs) have recently been shown to fluorescently detect and thermally ablate tumors in a murine model. Here, HDAPPS were functionalized with hyaluronic acid (HA) to improve their binding specificity to CT26 mouse CRC cells using HA to target the cancer stem cell marker CD44. In this work, we compared the binding of HA functionalized HDAPPs (HA-HDAPPs) in in vitro, ex vivo, and in vivo environments. The HA-HDAPPs bound to CT26 cells 2-fold more in vitro and 2.3-fold higher than un-functionalized HDAPPs ex vivo. Compared to

intraoperative abdominal perfusion, intraperitoneal injection prior to laser stimulation for nanoparticle heat generation provides a superior modality of HA-HDAPPs delivery for CRC tumor selectivity. Photothermal treatment of disseminated CRC showed that only HA-HDAPPs delivered via intraperitoneal injection had a reduction in the tumor burden, and these nanoparticles also remained in the abdomen following resolution of the tumor. The results of this work confirm that HA-HDAPPs selectively bind to disseminated CRC, with ex vivo tumors having bound HA-HDAPPs capable of photothermal ablation. HA-HDAPPs demonstrated superior binding to tumor regions compared to HDAPPs. Overall, this study displays the theranostic potential of HDAPPs, emphasizing their capacity to detect and photothermally treat disseminated CRC tumors.

McCloskey, K. E., et al. (2003). "Magnetophoretic cell sorting is a function of antibody binding capacity." Biotechnol Prog **19**(3): 899-907.

Antibody binding capacity (ABC) is a term representing a cell's ability to bind antibodies, correlating with the number of specific cellular antigens expressed on that cell. ABC allows magnetically conjugated antibodies to bind to the targeted cells, imparting a magnetophoretic mobility on each targeted cell. This enables sorting based on differences in the cell magnetophoretic mobility and, potentially, a magnetic separation based on the differences in the cell ABC values. A cell's ABC value is a particularly important factor in continuous magnetic cell separation. This work investigates the relationship between ABC and magnetic cell separation efficiency by injection of a suspension of immunomagnetically labeled quantum simply cellular calibration microbeads of known ABC values into fluid flowing through a quadrupole magnetic sorter. The elution profiles of the outlet streams were evaluated using UV detectors. Optimal separation flow rate was shown to correlate with the ABC of these microbeads. Comparing experimental and theoretical results, the theory correctly predicted maximum separation flow rates but overestimated the separation fractional recoveries.

Meng, Z., et al. (2017). "Peptide-Coated Semiconductor Polymer Dots for Stem Cells Labeling and Tracking." Chemistry **23**(28): 6836-6844.

Stem cell therapy is rapidly moving toward translation to clinical application. To elucidate the therapeutic effect, a robust method that allows tracking of the stem cells over an extended period of time is required. Herein, semiconducting polymer dots (Pdots) are demonstrated for their use in bright labeling and tracking of human mesenchymal stem cells (MSCs) in vitro and in vivo. The Pdots coated with a cell-penetrating peptide (R8) showed remarkable endocytic uptake efficiency that was 15 times higher than that of carboxyl Pdots and more than 200 times than that of bare Pdots. The Pdot-labeled MSCs can be traced for 15 generations in vitro and tracked over 2 weeks in vivo after subcutaneous transplantation. The labeled MSCs administered through the tail vein were preferentially accumulated in the lung; this was distinctive from the distribution of free Pdots, which were primarily distributed in the liver. Based on the properties of bright labeling, excellent tracking capability, and great biocompatibility, the Pdots will be valuable in the applications of stem cell biology and regenerative medicine.

Mennan, C., et al. (2019). "A comprehensive characterisation of large-scale expanded human bone marrow and umbilical cord mesenchymal stem cells." Stem Cell Res Ther **10**(1): 99.

BACKGROUND: The manufacture of mesenchymal stem/stromal cells (MSCs) for clinical use needs to be cost effective, safe and scaled up. Current methods of expansion on tissue culture plastic are labour-intensive and involve several 'open' procedures. We have used the closed Quantum(R) hollow fibre bioreactor to expand four cultures each of MSCs derived from bone marrow (BM) and, for the first time, umbilical cords (UCs) and assessed extensive characterisation profiles for each, compared to parallel cultures grown on tissue culture plastic. METHODS: Bone marrow aspirate was directly loaded into the Quantum(R), and cells were harvested and characterised at passage (P) 0. Bone marrow cells were re-seeded into the Quantum(R), harvested and further characterised at P1. UC-MSCs were isolated enzymatically and cultured once on tissue culture plastic, before loading cells into the Quantum(R), harvesting and characterising at P1. Quantum(R)-derived cultures were phenotyped in terms of immunoprofile, tri-lineage differentiation, response to inflammatory stimulus and telomere length, as were parallel cultures expanded on tissue culture plastic. RESULTS: Bone marrow cell harvests from the Quantum(R) were 23.1 +/- 16.2 x 10(6) in 14 +/- 2 days (P0) and 131 +/- 84 x 10(6) BM-MSCs in 13 +/- 1 days (P1), whereas UC-MSC harvests from the Quantum(R) were 168 +/- 52 x 10(6) UC-MSCs after 7 +/- 2 days (P1). Quantum(R)- and tissue culture plastic-expanded cultures at P1 adhered to criteria for MSCs in terms of cell surface markers, multipotency and plastic adherence, whereas the integrins, CD29, CD49c and CD51/61, were found to be elevated on Quantum(R)-expanded BM-MSCs. Rapid culture expansion in the Quantum(R) did not cause shortened telomeres when compared to cultures on tissue culture plastic. Immunomodulatory gene expression was variable between donors but showed that all MSCs upregulated indoleamine 2, 3-dioxygenase (IDO). CONCLUSIONS: The results

presented here demonstrate that the Quantum(R) can be used to expand large numbers of MSCs from bone marrow and umbilical cord tissues for next-generation large-scale manufacturing, without impacting on many of the properties that are characteristic of MSCs or potentially therapeutic. Using the Quantum(R), we can obtain multiple MSC doses from a single manufacturing run to treat many patients. Together, our findings support the development of cheaper cell-based treatments.

Mias, C., et al. (2009). "Mesenchymal stem cells promote matrix metalloproteinase secretion by cardiac fibroblasts and reduce cardiac ventricular fibrosis after myocardial infarction." Stem Cells **27**(11): 2734-2743.

Recent studies showed that mesenchymal stem cells (MSCs) transplantation significantly decreased cardiac fibrosis; however, the mechanisms involved in these effects are still poorly understood. In this work, we investigated whether the antifibrotic properties of MSCs involve the regulation of matrix metalloproteinases (MMPs) and matrix metalloproteinase endogenous inhibitor (TIMP) production by cardiac fibroblasts. In vitro experiments showed that conditioned medium from MSCs decreased viability, alpha-smooth muscle actin expression, and collagen secretion of cardiac fibroblasts. These effects were concomitant with the stimulation of MMP-2/MMP-9 activities and membrane type 1 MMP expression. Experiments performed with fibroblasts from MMP2-knockout mice demonstrated that MMP-2 plays a preponderant role in preventing collagen accumulation upon incubation with conditioned medium from MSCs. We found that MSC-conditioned medium also decreased the expression of TIMP2 in cardiac fibroblasts. In vivo studies showed that intracardiac injection of MSCs in a rat model of postischemic heart failure induced a significant decrease in ventricular fibrosis. This effect was associated with the improvement of morphological and functional cardiac parameters. In conclusion, we showed that MSCs modulate the phenotype of cardiac fibroblasts and their ability to degrade extracellular matrix. These properties of MSCs open new perspectives for understanding the mechanisms of action of MSCs and anticipate their potential therapeutic or side effects.

Mittal, R. and M. P. Bruchez (2009). "Calibration of Flow Cytometry for Quantitative Quantum Dot Measurements." Curr Protoc Cytom **Chapter 6**: Unit6 26.

Observations of quantum dot (QD) labeled cells in biomedical research are mainly qualitative in nature, which limits the ability of researchers to compare results experiment-to-experiment and lab-to-lab to improve the state-of-the-art. Labeled cells are useful in a range of in vitro and in vivo assays where tracking behavior of administered cells is integral for answering research questions in areas such as tissue engineering and stem cell therapy. Before the full potential of QD based toolsets can be realized in the clinic, uptake of QDs by cells must be quantified and standardized. This unit describes a novel, simple method to assess the number of QDs per cell using flow cytometry and commercially available standards. This quick and easy method can be used by all researchers to calibrate their flow cytometry instruments and settings, and quantify QD uptake by cells for in vitro and in vivo experimentation for comparable results across QD conjugate types, cell types, research groups, lots of commercial QDs, and homemade QDs.

Miyazaki, Y., et al. (2013). "Adipose Tissue-Derived Stem Cell Imaging Using Cadmium-Free Quantum Dots." Cell Med **6**(1-2): 91-97.

Quantum dots (QDs) have received much attention for biomolecule and cell imaging applications because of their superior optical properties such as high quantum efficiency, size-tunable emission, and resistance to photobleaching process. However, QDs that are commercially available contain cadmium (Cd), a highly toxic element. Thus, the development of Cd-free and less toxic QDs is strongly desired. In this study, we developed Cd-free QDs (ZnS-coated ZnS-AgInS2 solid solution nanoparticles with a sulfo group: ZnS-ZAIS-SO3H) and investigated the ability of this material to label stem cells. ZnS-ZAIS-SO3H could be transduced into mouse adipose tissue-derived stem cells (mASCs) using octaarginine peptides (R8), known as cell-penetrating peptides. The optimal ratio of ZnS-ZAIS-SO3H:R8 was found to be 1:100 for labeling mASCs. More than 80% of mASCs labeled with 500 nM ZnS-ZAIS-SO3H were found to be alive, and the proliferation rates of labeled mASCs were maintained at the same rate as that of nonlabeled mASCs. In addition, no abnormalities in the morphology of mASCs labeled with ZnS-ZAIS-SO3H could be observed. These data suggest that ZnS-ZAIS-SO3H may be effective for the labeling of mASCs.

Mohapatra, D., et al. (2021). "Carbon dots from an immunomodulatory plant for cancer cell imaging, free radical scavenging and metal sensing applications." Nanomedicine (Lond) **16**(23): 2039-2059.

Aim: This work aimed to develop Tinospora cordifolia stem-derived carbon dots (TCSCD) for cancer cell imaging, free radical scavenging and metal sensing applications. Method: The TCSCDs were synthesized by a simple, one-step, and ecofriendly hydrothermal carbonization method and characterized for their optical properties, morphology, hydrodynamic size, surface functionality, crystallinity, stability, bacterial biocompatibility, in vitro cellular imaging, free radical

scavenging and metal sensing ability. Results: The TCSCDs exhibited excellent biocompatibility with dose-dependent bioimaging results in melanoma (B16F10) and cervical cancer (SiHa) cell lines. They exerted good free radical scavenging, Fe(3+) sensing, bacterial biocompatibility, photostability, colloidal dispersion stability and thermal stability. Conclusion: The results reflect the potential of TCSCDs for biomedical and pharmaceutical applications.

Molnar, M., et al. (2010). "Effects of Quantum Dot Labeling on Endothelial Progenitor Cell Function and Viability." Cell Med **1**(2): 105-112.

Endothelial progenitor cells (EPC) play an important role in repairing damaged endothelium. An effective imaging method for in vivo tracking of EPCs is essential for understanding EPC-based cell therapy. Fluorescent quantum dots (QDs) have attractive optical characteristics such as extreme brightness and photostability. QDs are currently being investigated as probes for stem cell labeling; however, there is concern about whether QDs can be used safely. We investigated whether quantum dot (QD) labeling would influence EPC viability and function. Rat bone marrow-derived EPCs were cultured and characterized. The cells were labeled with near-infrared-emitting, carboxyl-coated QDs (8 nM) for 24 h. QD labeling efficiency was higher than 97%. Using WST-1 assay, we showed that the viability of the QD-labeled EPCs was not different from that of the control EPCs. Moreover, QD labeling did not influence the ability of EPCs to form capillary tubes on Matrigel and to migrate. The percentage of QD-positive cells decreased with time, probably due to the rapid division of EPCs. These data suggest that the carboxyl-coated QD705 can be useful for labeling EPCs without interrupting their viability and functions.

Moradi-Kalbolandi, S., et al. (2020). "Development of an anti-CD45RA-quantum dots conjugated scFv to detect leukemic cancer stem cells." Mol Biol Rep **47**(1): 225-234.

Leukemic cancer stem cells (LSCs), aberrantly overexpressing CD45RA are among the major causes of relapse following chemotherapy in patients with acute myeloid leukemia and serve as a highly sensitive marker for predicting relapse occurrence following chemotherapy. The main purpose of current study was to develop a sensitive approach for detecting LSCs based on a conjugate of an anti-CD45 scFv and quantum dot. The variable light and heavy chain sequences of a recently developed anti-CD45RA monoclonal antibody were derived from hybridoma cells and PCR amplified to construct scFv. Following insertion of scFv gene into a pET32a-lic vector and expression in Escherichia coli and purification, the purified scFv, was conjugated with carbon dots (C dots) and used for the detection of CD45RA (+)cells while CD45RA-cells served as negative control. Subsequently, Functional activity of the conjugate was analyzed by flow cytometry and ICC to detect the cell surface antigen binding and detection ability. Based on results, purified CD45RA scFv conjugated C dots could specifically recognize CD45RA positive cells, but not any CD45RA negative ones. In conclusion, here we developed a low-cost but very efficient approach for detection of CD45RA positive cells including LSCs.

Morais, P. C. and D. C. Silva (2022). "Mathematical Modeling for an MTT Assay in Fluorine-Containing Graphene Quantum Dots." Nanomaterials (Basel) **12**(3).

The paper reports on a new mathematical model, starting with the original Hill equation which is derived to describe cell viability (V) while testing nanomaterials (NMs). Key information on the sample's morphology, such as mean size (s) and size dispersity (sigma) is included in the new model via the lognormal distribution function. The new Hill-inspired equation is successfully used to fit MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) data from assays performed with the HepG2 cell line challenged by fluorine-containing graphene quantum dots (F:GQDs) under light (400-700 nm wavelength) and dark conditions. The extracted "biological polydispersity" (light: sMTT=1.77+/-0.02 nm and sigmaMTT=0.21+/-0.02); dark: sMTT=1.87+/-0.02 nm and sigmaMTT=0.22+/-0.01) is compared with the "morphological polydispersity" (sTEM=1.98+/-0.06 nm and sigmaTEM=0.19+/-0.03), the latter obtained from TEM (transmission electron microscopy). The fitted data are then used to simulate a series of V responses. Two aspects are emphasized in the simulations: (i) fixing sigma, one simulates V versus s and (ii) fixing s, one simulates V versus sigma. Trends observed in the simulations are supported by a phenomenological model picture describing the monotonic reduction in V as s increases (V~pa/(s)p-a; p and a are fitting parameters) and accounting for two opposite trends of V versus sigma: under light (V~sigma) and under dark (V~1/sigma).

Mulay, S. V., et al. (2016). "Substituent Effects in BODIPY in Live Cell Imaging." Chem Asian J **11**(24): 3598-3605.

Small-molecule organoselenium-based fluorescent probes possess great capacity in understanding biological processes through the detection of various analytes such as reactive oxygen/nitrogen species (ROS/RNS), biothiols (cysteine, homocysteine and glutathione), lipid droplets, etc. Herein, we present how substituents on the BODIPY system play a significant part in the detection of biologically important analytes for in vitro conditions

and live cell imaging studies. The fluorescence of the probe was quenched by 2-chloro and 6-phenyl selenium groups; the probe shows high selectivity with NaOCl among other ROS/RNS, and gives a turn-on response. The maximum fluorescence intensity is attained within approximately 1-2 min with a low detection limit (19.6 nm), and shows a approximately 110-fold fluorescence enhancement compared to signals generated for other ROS/RNS. Surprisingly, in live cell experiments, the probe specifically located and accumulated in lipid droplets, and showed a fluorescence turn-on response. We believe this turn-on response occurred because of aggregation-induced emission (AIE), which surprisingly occurred only by introducing one lipophilic mesityl group at the meso position of the BODIPY.

Muller-Borer, B. J., et al. (2007). "Quantum dot labeling of mesenchymal stem cells." J Nanobiotechnology **5**: 9.

BACKGROUND: Mesenchymal stem cells (MSCs) are multipotent cells with the potential to differentiate into bone, cartilage, fat and muscle cells and are being investigated for their utility in cell-based transplantation therapy. Yet, adequate methods to track transplanted MSCs in vivo are limited, precluding functional studies. Quantum Dots (QDs) offer an alternative to organic dyes and fluorescent proteins to label and track cells in vitro and in vivo. These nanoparticles are resistant to chemical and metabolic degradation, demonstrating long term photostability. Here, we investigate the cytotoxic effects of in vitro QD labeling on MSC proliferation and differentiation and use as a cell label in a cardiomyocyte co-culture. RESULTS: A dose-response to QDs in rat bone marrow MSCs was assessed in Control (no-QDs), Low concentration (LC, 5 nmol/L) and High concentration (HC, 20 nmol/L) groups. QD yield and retention, MSC survival, proinflammatory cytokines, proliferation and DNA damage were evaluated in MSCs, 24 -120 hrs post QD labeling. In addition, functional integration of QD labeled MSCs in an in vitro cardiomyocyte co-culture was assessed. A dose-dependent effect was measured with increased yield in HC vs. LC labeled MSCs (93 +/- 3% vs. 50% +/- 15%, $p < 0.05$), with a larger number of QD aggregates per cell in HC vs. LC MSCs at each time point ($p < 0.05$). At 24 hrs >90% of QD labeled cells were viable in all groups, however, at 120 hrs increased apoptosis was measured in HC vs. Control MSCs (7.2% +/- 2.7% vs. 0.5% +/- 0.4%, $p < 0.05$). MCP-1 and IL-6 levels doubled in HC MSCs when measured 24 hrs after QD labeling. No change in MSC proliferation or DNA damage was observed in QD labeled MSCs at 24, 72 and 120 hrs post labeling. Finally, in a cardiomyocyte co-culture QD labeled MSCs were easy to locate and formed functional cell-to-cell couplings, assessed by dye diffusion. CONCLUSION: Fluorescent QDs label MSC effectively in an in vitro co-culture model. QDs are easy to use, show a high yield and survival rate with minimal cytotoxic effects. Dose-dependent effects suggest limiting MSC QD exposure.

Murasawa, S., et al. (2005). "Niche-dependent translineage commitment of endothelial progenitor cells, not cell fusion in general, into myocardial lineage cells." Arterioscler Thromb Vasc Biol **25**(7): 1388-1394.

OBJECTIVE: Previous studies from our laboratory have shown therapeutic potential of ex vivo expanded endothelial progenitor cells (EPCs) for myocardial ischemia. Our purpose was to investigate the mechanisms regulating EPC contribution to myocardial regeneration. METHODS AND RESULTS: To evaluate niche-dependent expression profiles of EPCs in vitro, we performed coculture using cultured EPCs derived from human peripheral blood and rat cardiac myoblast cell line (H9C2). Reverse-transcription polymerase chain reaction (PCR) disclosed the expression of human-specific cardiac markers as well as human-specific smooth muscle markers. Cytoimmunochemistry presented several cocultured cells stained with human specific cardiac antibody. To prove this translineage differentiation in vivo, human cultured EPCs were injected into nude rat myocardial infarction model. Reverse-transcription PCR as well as immunohistochemistry of rat myocardial samples demonstrated the expression of human specific cardiac, vascular smooth muscle, and endothelial markers. We observed the distribution of colors (Qtracker; Quantum Dot Corp) in coculture to detect the fused cells, and the frequency of cell fusion was <1%. CONCLUSIONS: EPCs can contribute to not only vasculogenesis but also myogenesis in the ischemic myocardium in vivo. Transdifferentiation, not cell fusion, is dominant for EPCs commitment to myocardial lineage cells. Ex vivo expanded EPCs transplantation might have enhanced therapeutic potential for myocardial regeneration.

Murata, Y., et al. (2020). "Visualization of Human Induced Pluripotent Stem Cells-Derived Three-Dimensional Cartilage Tissue by Gelatin Nanospheres." Tissue Eng Part C Methods **26**(5): 244-252.

Recently, many studies on the three-dimensional (3D) fabrication of cells have been performed. Under these circumstances, it is indispensable to develop the imaging technologies and methodologies for noninvasive visualization of 3D cells fabricated. The objective of this study is to develop the labeling method of human induced pluripotent stem (iPS) cells-derived 3D cartilage tissue with gelatin nanospheres coincorporating three kinds of quantum dots (QD) and iron oxide nanoparticles (IONP) (GNSQD+IONP). In this study, two labeling methods were performed. One is that a cartilage tissue was labeled directly by incubating with octaarginine (R8)-

treated GNSQD+IONP (direct labeling method). The other one is a "dissociation and labeling method." First, the cartilage tissue was dissociated to cells in a single dispersed state. Then, the cells were incubated with R8-GNSQD+IONP in a monolayer culture. Finally, the cells labeled were fabricated to 3D pellets or cell sheets. By the direct labeling method, only cells residing in the surrounding site of cartilage tissue were labeled. On the contrary, the 3D cartilage pellets and the cell sheets were homogenously labeled and maintained fluorescently visualized over 4 weeks. In addition, the cartilage properties were histologically detected even after the process of dissociation and labeling. Homogenous labeling and visualization of human iPS cells-derived 3D cartilage tissue was achieved by the dissociation and labeling method with GNSQD+IONP. Impact statement The homogenous labeling and visualization of human iPS cells-derived three-dimensional (3D) cartilage tissue was achieved over 4 weeks by the dissociation and labeling method with gelatin nanospheres coincorporating quantum dots (QD) and iron oxide nanoparticles (IONP) (GNSQD+IONP). The cartilage properties of cells treated were maintained. It is concluded that the dissociation and labeling method with GNSQD+IONP is a promising to visualize the human iPS cells-derived 3D cartilage tissue.

Muroski, M. E., et al. (2014). "A gold nanoparticle pentapeptide: gene fusion to induce therapeutic gene expression in mesenchymal stem cells." J Am Chem Soc **136**(42): 14763-14771.

Mesenchymal stem cells (MSC) have been identified as having great potential as autologous cell therapeutics to treat traumatic brain injury and spinal injury as well as neuronal and cardiac ischemic events. All future clinical applications of MSC cell therapies must allow the MSC to be harvested, transfected, and induced to express a desired protein or selection of proteins to have medical benefit. For the full potential of MSC cell therapy to be realized, it is desirable to systematically alter the protein expression of therapeutically beneficial biomolecules in harvested MSC cells with high fidelity in a single transfection event. We have developed a delivery platform on the basis of the use of a solid gold nanoparticle that has been surface modified to produce a fusion containing a zwitterionic, pentapeptide designed from Bax inhibiting peptide (Ku70) to enhance cellular uptake and a linearized expression vector to induce enhanced expression of brain-derived neurotrophic factor (BDNF) in rat-derived MSCs. Ku70 is observed to effect >80% transfection following a single treatment of femur bone marrow isolated rat MSCs with efficiencies for the delivery of a 6.6 kbp gene on either a Au nanoparticle (NP) or CdSe/ZnS quantum dot (QD). Gene expression is observed within 4 d by optical measurements, and secretion is observed within 10 d by Western Blot analysis. The combination of being able to selectively engineer the NP, to colocalize biological agents, and to enhance the stability of those agents has provided the strong impetus to utilize this novel class of materials to engineer primary MSCs.

Nagesha, D., et al. (2007). "In vitro imaging of embryonic stem cells using multiphoton luminescence of gold nanoparticles." Int J Nanomedicine **2**(4): 813-819.

Recent advances in nonlinear optical techniques and materials such as quantum wells, nanowires and noble-metal nanoparticles have led to advances in cellular imaging wherein various nanoparticles have been shown to improve both in vitro and in vivo visualization. In this paper, we demonstrate in vitro imaging using multi-photon photoluminescence of gold nanoparticles from two different cell types Dictyostelium discoideum and mouse embryonic stem cells. By observing nanoparticles we show that embryonic stem cells maintained their ability to proliferate for several passages while grown in the presence of gold nanoparticles. The advantages of multi-photon luminescence using gold nanoparticles have important implications for use in stem cell proliferation experiments and in vitro experiments to monitor differentiation.

Narayanan, K., et al. (2013). "Mimicking cellular transport mechanism in stem cells through endosomal escape of new peptide-coated quantum dots." Sci Rep **3**: 2184.

Protein transport is an important phenomenon in biological systems. Proteins are transported via several mechanisms to reach their destined compartment of cell for its complete function. One such mechanism is the microtubule mediated protein transport. Up to now, there are no reports on synthetic systems mimicking the biological protein transport mechanism. Here we report a highly efficient method of mimicking the microtubule mediated protein transport using newly designed biotinylated peptides encompassing a microtubule-associated sequence (MTAS) and a nuclear localization signaling (NLS) sequence, and their final conjugation with streptavidin-coated CdSe/ZnS quantum dots (QDs). Our results demonstrate that these novel bio-conjugated QDs enhance the endosomal escape and promote targeted delivery into the nucleus of human mesenchymal stem cells via microtubules. Mimicking the cellular transport mechanism in stem cells is highly desirable for diagnostics, targeting and therapeutic applications, opening up new avenues in the area of drug delivery.

Nasrin, A., et al. (2022). "3D-printed bioresorbable poly(lactic-co-glycolic acid) and quantum-dot nanocomposites: Scaffolds for enhanced bone mineralization and inbuilt co-monitoring." J Biomed Mater Res A **110**(4): 916-927.

Multifunctional 3D-printed nanocomposites based on poly(lactic-co-glycolic acid), that is, PLGA (RESOMER(R) LG857S) were developed for simultaneous monitoring of cells and scaffold as a function of time and spectral responses. These were achieved by impregnating carbon quantum dots (CQDs) on PLGA using melt-blending, plasticating extrusion, and 3D-printing. The nanocomposites enabled enhanced bio-affinity and cellular interactions for bone tissue engineering (TE). PLGA (control) and PLGA-CQD scaffolds were used for growing human adipose-derived-stem-cells (ADSCs) and tested for cell biocompatibility, cellular adhesion, growth, and osteogenesis. CQDs were found to enhance the hydrophilicity of nanocomposites and promote cellular nesting. MTS assays confirmed that CQDs on PLGA act as cell anchoring sites, thereby enhancing seeding efficiency and cell proliferation. Alkaline phosphate tests showed increased osteogenesis and Alizarin assays confirmed enhanced bone mineralization on PLGA-CQD. The qPCR tests based on selected mRNA expressions showed that the incorporation of CQDs significantly enhanced osteogenesis of ADSCs during all three phases of cell differentiation. The intrinsic luminescence of the composites allowed label-free monitoring of cell proliferation and bone mineralization on the scaffolds. Thus, the CQDs facilitated significant enhancements in composite processability with customized fabrication of 3D printed scaffolds, bone tissue osteoconductivity, and monitoring of cell-scaffold activities, offering multifunctional benefits for bone TE.

Nazem, A. and G. A. Mansoori (2008). "Nanotechnology solutions for Alzheimer's disease: advances in research tools, diagnostic methods and therapeutic agents." J Alzheimers Dis **13**(2): 199-223.

A century of research has passed since the discovery and definition of Alzheimer's disease (AD), the primary common dementing disorder worldwide. However, AD lacks definite diagnostic approaches and effective cure at the present. Moreover, the currently available diagnostic tools are not sufficient for an early screening of AD in order to start preventive approaches. Recently the emerging field of nanotechnology has promised new techniques to solve some of the AD challenges. Nanotechnology refers to the techniques of designing and manufacturing nanosize (1-100 nm) structures through controlled positional and/or self-assembly of atoms and molecules. In this report, we present the promises that nanotechnology brings in research on the AD diagnosis and therapy. They include its potential for the better understanding of the AD root cause molecular mechanisms, AD's early diagnoses, and effective treatment. The advances in AD research offered by the atomic force microscopy, single molecule fluorescence microscopy and NanoSIMS microscopy are examined here. In addition, the recently proposed applications of nanotechnology for the early diagnosis of AD including bio-barcode assay, localized surface plasmon resonance nanosensor, quantum dot and nanomechanical cantilever arrays are analyzed. Applications of nanotechnology in AD therapy including neuroprotections against oxidative stress and anti-amyloid therapeutics, neuroregeneration and drug delivery beyond the blood brain barrier (BBB) are discussed and analyzed. All of these applications could improve the treatment approach of AD and other neurodegenerative diseases. The complete cure of AD may become feasible by a combination of nanotechnology and some other novel approaches, like stem cell technology.

Nelson, D. A. (1990). "The biology of myelopoiesis." Clin Lab Med **10**(4): 649-659.

The myelopoietic system includes the hematopoietic cells derived from a common hematopoietic stem cell that includes erythroid, granulocytic, monocytic, and megakaryocytic lineages. The contributions of molecular genetic techniques to hematopoiesis in the past 5 years have led to quantum leaps in our understanding, but there is room for further research. Current concepts of stem cells and the hematopoietic microenvironment are discussed, as are committed progenitor cells and hematopoietic growth factors, particularly those concerning the neutrophilic, monocytic, and erythroid systems.

Ni, J. S., et al. (2020). "Nanoparticle-based Cell Trackers for Biomedical Applications." Theranostics **10**(4): 1923-1947.

The continuous or real-time tracking of biological processes using biocompatible contrast agents over a certain period of time is vital for precise diagnosis and treatment, such as monitoring tissue regeneration after stem cell transplantation, understanding the genesis, development, invasion and metastasis of cancer and so on. The rationally designed nanoparticles, including aggregation-induced emission (AIE) dots, inorganic quantum dots (QDs), nanodiamonds, superparamagnetic iron oxide nanoparticles (SPIONs), and semiconducting polymer nanoparticles (SPNs), have been explored to meet this urgent need. In this review, the development and application of these nanoparticle-based cell trackers for a variety of imaging technologies, including fluorescence imaging, photoacoustic imaging, magnetic resonance imaging, magnetic particle imaging, positron

emission tomography and single photon emission computing tomography are discussed in detail. Moreover, the further therapeutic treatments using multi-functional trackers endowed with photodynamic and photothermal modalities are also introduced to provide a comprehensive perspective in this promising research field.

Ni, X., et al. (2018). "Fluorescent Nanoparticles for Noninvasive Stem Cell Tracking in Regenerative Medicine." J Biomed Nanotechnol **14**(2): 240-256.

Stem cell-based therapies have emerged as promising platforms with the potential to treat serious diseases that are incurable by traditional medical approaches. To optimize the overall outcomes, it is important to understand the fate of transplanted stem cells (e.g., localization, migration, engraftment, survival, proliferation and differentiation). Fluorescent nanoparticles with good photostability and minimal perturbation of cell functions hold great promise for distinguishing transplanted stem cells from host tissues with high resolution, showing advantages over traditional histological methods. This review aims to summarize the recent advances in the use of fluorescent nanoparticles for the direct labelling of stem cells and the applications of such nanoparticles in stem cell tracking. The relevant fluorescent nanoparticles, including quantum dots, organic fluorogen-doped nanoparticles, fluorescent nanodiamonds, and upconversion nanoparticles are discussed. The advantages and limitations of the currently available fluorescent trackers are summarized, and perspectives on new research opportunities are discussed.

Nigam Joshi, P., et al. (2017). "Multifunctional inulin tethered silver-graphene quantum dots nanotheranostic module for pancreatic cancer therapy." Mater Sci Eng C Mater Biol Appl **78**: 1203-1211.

Cancer nanotechnology is an emerging area of cancer diagnosis and therapy. Although considerable progress has been made for targeted drug delivery systems to deliver anticancer agents to particular site of interest, new nanomaterials are frequently being developed and explored for better drug delivery efficiency. In the present work, we have explored a novel nanoformulation based on silver-graphene quantum dots (Ag-GQDs) nanocomposite for its successful implementation for pancreatic cancer specific drug delivery in wistar rats. Carboxymethyl inulin (CMI); a modified variant of natural polysaccharide inulin is tethered with the nanocomposite via carbodiimide coupling to enhance the biocompatibility of nanoformulation. Experiments are performed to investigate the cytotoxicity reduction of silver nanoparticles after inulin tethering as well as anticancer efficacy of the system using 5-Fluorouracil (5-FU) as model drug. SEM, TEM, FT-IR, UV-vis, photoluminescence and anti proliferative assays (MTT) are performed for characterisation of the nanocomposite. Hyaluronic acid (HA) is conjugated as targeting moiety for CD-44 (cancer stem cell marker) to fabricate a complete targeted drug delivery vehicle specific for pancreatic cancer. In the present work two prime objectives were achieved; mitigation the toxicity of silver nanoparticles by inulin coating and it's in vivo application for pancreatic cancer.

Nobel, P. S., et al. (1984). "Influence of Applied NaCl on Crassulacean Acid Metabolism and Ionic Levels in a Cactus, Cereus validus." Plant Physiol **75**(3): 799-803.

To determine possible physiological responses to salinity, seedlings of Cereus validus Haworth, a cactus from Salinas Grandes, Argentina, were treated with up to 600 millimolar NaCl for up to 16 days when they were about 9 months old and 100 millimeters tall. Salt stress decreased stem biomass, e.g. it was 19.7 grams for controls and 11.4 grams for plants treated with 400 millimolar NaCl for 14 days. Nocturnal CO(2) uptake in these obligate Crassulacean acid metabolism (CAM) plants was inhibited 67% upon treatment with 400 millimolar NaCl for 14 days (controls, 181 millimoles CO(2) per square meter), while nocturnal accumulation of malate was inhibited 49% (controls, 230 millimoles malate per square meter). The larger accumulation of malate as compared to uptake of atmospheric CO(2) suggests that internal CO(2) recycling occurred during the dark period. Such recycling was lower in the controls (approximately 20%) than in the NaCl-treated plants (approximately 50%). The nocturnal increase in malate and titratable acidity depended on the total daily photosynthetically active radiation available; measurements suggest a quantum requirment of 26 photons per malate. As NaCl in the medium was increased to 600 millimolar in daily increments of 50 millimolar, Na and Cl concentrations in the roots increased from about 7 to 100 millimolar, but K concentration in the cell sap remained near 26 millimolar. Concomitantly, concentrations of Na and Cl in the shoots increased from 8 to 17 millimolar and from 1 to 7 millimolar, respectively, while the K concentration increased about 16 to 60 millimolar. In plants maintained for 14 days at 500 millimolar NaCl, the root levels of Na and Cl increased to 260 millimolar, the shoot levels were about 60 millimolar, and the stem bases began to become necrotic. Such Na retention in the roots together with the special possibilities of carbon reutilization given by CAM are apparently survival mechanisms for the temporarily saline conditions experienced in its natural habitat.

Noviany, N., et al. (2020). "Structure Characterization and Biological Activity of 2-Arylbenzofurans from an

Indonesian Plant, Sesbania grandiflora (L.) Pers." Phytochem Lett **35**: 211-215.

A new 2-arylbenzofuran, sesbagrandiflorain C (1), together with four known compounds, 2-(3,4-dihydroxy-2-methoxyphenyl)-4-hydroxy-6-methoxybenzofuran-3-carbaldehyde (2), 2-(4-hydroxy-2-methoxyphenyl)-5,6-dimethoxybenzofuran-3-carboxaldehyde (3), sesbagrandiflorain A (4) and sesbagrandiflorain B (5), have been isolated from the stem bark of an Indonesian plant, Sesbania grandiflora (L.) Pers. The chemical structure of compound 1 was elucidated by UV, IR, MS, and NMR spectroscopic techniques. The proton and carbon NMR resonances of 1 were also compared with the predicted chemical shifts obtained from DFT quantum mechanical calculations with Gaussian. None of the compounds showed antibacterial activity against Bacillus subtilis, Escherichia coli, Mycobacterium smegmatis, Pseudomonas aeruginosa, and Staphylococcus aureus in an agar diffusion assay. However, sesbagrandiflorains A (4) and B (5) exhibited moderate activity against Mycobacterium tuberculosis H37Rv. In addition, compounds 1 - 5 have moderate cytotoxicity against HeLa, HepG2, and MCF-7 cancer cell lines.

Obradovic, S., et al. (2009). "Influence of intracoronary injections of bone-marrow-derived mononuclear cells on large myocardial infarction outcome: quantum of initial necrosis is the key." Vojnosanit Pregl **66**(12): 998-1004.

BACKGROUND/AIM: Autologous bone-marrow-derived intra= coronary injection of mononuclear cells (MNC) modestly improved left ventricular ejection fraction (LVEF) in the selected patients after acute ST elevation myocardial infarction (STEMI). Major determinants of stem cell therapy outcome in the subacute phase of STEMI still remain unknown. Therefore, the aim of this study was to determine modifying factors for the outcome of stem cell therapy after STEMI. METHODS: Eighteen patients in the stem cell therapy group and 24 patients in the control group with the successfully reperfused first large STEMI (LVEF < or = 40%) were enrolled in the study. The stem cell group was submitted to autologous bone-marrow-derived MNC injection between 7-12 days after MI. Left ventricular ejection fraction and infarction size at baseline and after 4 months were determined by echocardiography and scintigraphy examination. Age, pain onset to reperfusion time, admission glycemia, maximum lactate dehydrogenase (LDH) activity and C-reactive protein level, baseline LVEF and infarction size, and the number of MNC injected were compared between patients with and without significant improvement of LVEF and decrease of myocardial infarct size after 4 months. RESULTS: In the stem cell group, patients with the improvement of LVEF for more than 5.1% had significantly lower levels of LDH than patients without such improvement (1689 +/- 139 vs 2133 +/- 215 IU/L, p < 0.001) and lower baseline infarction size on scintigraphy (26.7 +/- 5.2 vs 34.9 +/- 3.7%, p < 0.001). Such dependence was not found in the control group. CONCLUSION: In the patients with first large STEMI intracoronary injection of autologous bone-marrow-derived MNC leads to the significant decrease of myocardial infarction size but not the significant improvement of LVEF after four months. Higher serum LDH levels after STEMI and very large baseline infarction size are predictors of failure of stem cell therapy in our group of STEMI patients.

Ogihara, Y., et al. (2017). "Labeling and in vivo visualization of transplanted adipose tissue-derived stem cells with safe cadmium-free aqueous ZnS coating of ZnS-AgInS2 nanoparticles." Sci Rep **7**: 40047.

The facile synthesis of ZnS-AgInS2 (ZAIS) as cadmium-free QDs and their application, mainly in solar cells, has been reported by our groups. In the present study, we investigated the safety and the usefulness for labeling and in vivo imaging of a newly synthesized aqueous ZnS-coated ZAIS (ZnS-ZAIS) carboxylated nanoparticles (ZZC) to stem cells. ZZC shows the strong fluorescence in aqueous solutions such as PBS and cell culture medium, and a complex of ZZC and octa-arginine (R8) peptides (R8-ZZC) can achieve the highly efficient labeling of adipose tissue-derived stem cells (ASCs). The cytotoxicity of R8-ZZC to ASCs was found to be extremely low in comparison to that of CdSe-based QDs, and R8-ZZC was confirmed to have no influence on the proliferation rate or the differentiation ability of ASCs. Moreover, R8-ZZC was not found to induce the production of major inflammatory cytokines (TNF-alpha, IFN-gamma, IL-12p70, IL-6 and MCP-1) in ASCs. Transplanted R8-ZZC-labeled ASCs could be quantitatively detected in the lungs and liver mainly using an in vivo imaging system. In addition, high-speed multiphoton confocal laser microscopy revealed the presence of aggregates of transplanted ASCs at many sites in the lungs, whereas individual ASCs were found to have accumulated in the liver.

Ogihara, Y., et al. (2017). "Transduction Function of a Magnetic Nanoparticle TMADM for Stem-Cell Imaging with Quantum Dots." Anal Sci **33**(2): 143-146.

We investigated the transduction function of a cationic dextran hydroxypropyltrimethyl ammonium chloride-coated magnetic iron oxide nanoparticle (TMADM-03) for transducing quantum dots (QDs) into adipose tissue-derived stem cells (ASCs). As a result, the fluorescence intensity of ASCs labeled with QDs using TMADM-03 was much higher than that of QDs only labeling. These data suggest that TMADM-03 can

be useful as a transduction agent for QDs in stem-cell imaging.

Ohyabu, Y., et al. (2009). "Stable and nondisruptive in vitro/in vivo labeling of mesenchymal stem cells by internalizing quantum dots." Hum Gene Ther **20**(3): 217-224.

Progress in stem cell research has prioritized the refinement of cell-labeling techniques for in vitro and in vivo basic and therapeutic studies. Although quantum dots, because of their optical properties, are emerging as favorable nanoparticles for bioimaging, substantial refinements or modifications that would improve their biocompatibility are still required. We report here that internalizing quantum dots (i-QDs) generated by their conjugation with an internalizing antibody against a heat shock protein-70 family stress chaperone, mortalin, offered an efficient, genetically noninvasive, nontoxic, and functionally inert way to label mesenchymal stem cells (MSCs). The i-QD-labeled MSCs underwent normal adipocyte, osteocyte, and chondrocyte differentiation in vitro and in vivo, suggesting the potential application of i-QDs in in vivo diagnostics, regenerative and therapeutic medicine.

Onoshima, D., et al. (2015). "Multifunctional quantum dots-based cancer diagnostics and stem cell therapeutics for regenerative medicine." Adv Drug Deliv Rev **95**: 2-14.

A field of recent diagnostics and therapeutics has been advanced with quantum dots (QDs). QDs have developed into new formats of biomolecular sensing to push the limits of detection in biology and medicine. QDs can be also utilized as bio-probes or labels for biological imaging of living cells and tissues. More recently, QDs has been demonstrated to construct a multifunctional nanoplatform, where the QDs serve not only as an imaging agent, but also a nanoscaffold for diagnostic and therapeutic modalities. This review highlights the promising applications of multi-functionalized QDs as advanced nanosensors for diagnosing cancer and as innovative fluorescence probes for in vitro or in vivo stem cell imaging in regenerative medicine.

Paccola Mesquita, F. C., et al. (2019). "Laminin as a Potent Substrate for Large-Scale Expansion of Human Induced Pluripotent Stem Cells in a Closed Cell Expansion System." Stem Cells Int **2019**: 9704945.

The number of high-quality cells required for engineering an adult human-sized bioartificial organ is greater than one billion. Until the emergence of induced pluripotent stem cells (iPSCs), autologous cell sources of this magnitude and with the required complexity were not available. Growing this number of cells in a traditional 2D cell culture system requires extensive time, resources, and effort and does not always meet clinical requirements. The use of a closed cell culture system is an efficient and clinically applicable method that can be used to expand cells under controlled conditions. We aimed to use the Quantum Cell Expansion System (QES) as an iPSC monolayer-based expansion system. Human iPSCs were expanded (up to 14-fold) using the QES on two different coatings (laminin 521 (LN521) and vitronectin (VN)), and a karyotype analysis was performed. The cells were characterized for spontaneous differentiation and pluripotency by RT-PCR and flow cytometry. Our results demonstrated that the QES provides the necessary environment for exponential iPSC growth, reaching 689.75 x 10(6) +/- 86.88 x 10(6) in less than 7 days using the LN521 coating with a population doubling level of 3.80 +/- 0.19. The same result was not observed when VN was used as a coating. The cells maintained normal karyotype (46-XX), expressed pluripotency markers (OCT4, NANOG, LIN28, SOX2, REX1, DPPA4, NODAL, TDGFb, TERT3, and GDF), and expressed high levels of OCT4, SOX2, NANOG, SSEA4, TRA1-60, and TRA1-81. Spontaneous differentiation into ectoderm (NESTIN, TUBB3, and NEFH), mesoderm (MSX1, BMP4, and T), and endoderm (GATA6, AFP, and SOX17) lineages was detected by RT-PCR with both coating systems. We conclude that the QES maintains the stemness of iPSCs and is a promising platform to provide the number of cells necessary to recellularize small human-sized organ scaffolds for clinical purposes.

Panda, N. K., et al. (2011). "Auditory changes in mobile users: is evidence forthcoming?" Otolaryngol Head Neck Surg **144**(4): 581-585.

OBJECTIVE: Genuine concerns are being raised as to the potential health risks posed by electromagnetic frequency exposure secondary to mobile phone usage. This study was undertaken to assess and compare potential changes in hearing function at the level of the inner ear and central auditory pathway due to chronic exposure to electromagnetic waves from both global system for mobile communications (GSM) and code division multiple access (CDMA) mobile phone usage. DESIGN: Cohort study. SETTING: Tertiary referral center. SUBJECTS AND METHODS: One hundred twenty-five subjects who were long-term mobile phone users (more than 1 year; 63 GSM and 62 CDMA) and 58 controls who had never used mobile phones underwent audiological investigations including pure tone audiometry (250-12 kHz), tympanometry, distortion product otoacoustic emissions (DPOAE), auditory brain responses (ABR), and middle latency responses (MLRs). The changes in various parameters were studied in mobile-using and non-mobile-using ears of both GSM and CDMA

subjects and corresponding ears of the controls to ascertain the effects of electromagnetic exposure. RESULTS: GSM and CDMA users were found to be at a significantly higher risk of having DPOAE absent as compared with controls (P < .05). They were found to have higher speech frequency thresholds and lower MLR wave and Na and Pa amplitudes. More than 3 years of mobile phone usage emerged as a risk factor (P < .05). The damage done was bilateral, with the quantum of damage being the same for both GSM and CDMA. CONCLUSION: Long-term and intensive GSM and CDMA mobile phone use may cause damage to cochlea as well as the auditory cortex.

Parfitt, A. M. (1984). "The cellular basis of bone remodeling: the quantum concept reexamined in light of recent advances in the cell biology of bone." Calcif Tissue Int **36 Suppl 1**: S37-45.

The cellular basis of the normal bone remodeling sequence in the human adult is discussed in relation to a cycle of five stages--quiescence, activation, resorption, reversal, formation, and return to quiescence. Normally, 80% or more of free bone surfaces are quiescent with respect to remodeling. The structure of the quiescent surface comprises 5 layers; listed in order out toward the bone marrow these are: the lamina limitans (the electron dense outer edge of the mineralized bone matrix), unmineralized connective tissue that may be confused with osteoid by light microscopy, flattened lining cells of osteoblast lineage separated by narrow gaps, more unmineralized connective tissue, and finally either the squamous sac cells of red marrow or the cytoplasm of fat cells of yellow marrow. Activation requires the recruitment of new osteoclasts derived from precursor cells of the mononuclear phagocyte system (and so ultimately from the hematopoietic stem cell), a method for precursor cells to penetrate the cellular and connective tissue barrier of the quiescent surface, and so gain access to the bone mineral, and mechanisms for their attraction and binding to the mineralized surface, possibly in response to chemotactic signals released from bone matrix or mineral. Each of these three steps is probably mediated in some way by lining cells. Resorption is carried out by osteoclasts, most of which are multinucleated. The mean life span of individual nuclei is about 12.5 days; the additional nuclei needed to sustain resorption may be derived from local as well as blood-bone precursors, but nothing is known of their fate.(ABSTRACT TRUNCATED AT 250 WORDS)

Park, J. S., et al. (2016). "Sunflower-type nanogels carrying a quantum dot nanoprobe for both superior gene delivery efficacy and tracing of human mesenchymal stem cells." Biomaterials **77**: 14-25.

Sunflower-type nanogels carrying the QD 655 nanoprobe can be used for both gene transfection and bioimaging of hMSCs. The entry of sunflower-type nanogels into hMSCs can be possibly controlled by changing the formation of QDs. The physico-chemical properties of sunflower-type nanogels internalized by hMSCs were confirmed by AFM, SEM, TEM, gel retardation, and zeta-potential analyses. The bioimaging capacity was confirmed by confocal laser microscopy, Kodak imaging, and Xenogen imaging. Specifically, we investigated the cytotoxicity of sunflower-type nanogels via SNP analysis. Internalization of sunflower-type nanogels does not cause malfunction of hMSCs.

Park, J. S., et al. (2018). "Verification of Long-Term Genetic Stability of hMSCs during Subculture after Internalization of Sunflower-Type Nanoparticles (SF-NPs)." Theranostics **8**(20): 5548-5561.

Background: For many years, researchers have sought to overcome major challenges in the use of nanoparticles as therapeutics, including issues related to intracellular delivery, biocompatibility, and activation. In particular, the genetic stability of cells treated with nanoparticles has become increasingly important in the context of stem cell therapy. Methods: Functional nanoparticles (Sunflower typed nanoparticles; SF-NPs) were fabricated by coating heparin pluronic F127 gels with quantum dot nanoparticles (QDs), and then bound the SOX9 gene to the QD nanogels. The resultant nanoparticles were transferred into stem cells, and the effect on genetic stability was monitored. To determinate gene delivery efficacy and long-term genomic stability of cells transfected with QD nanogels, hMSCs were transfected with nanogels at passage 4 (T1; Transfected cells 1) and then sub-cultured to passage of (T4). Following transplantation of transfected T1-T4 cells, the cells were monitored by in vivo imaging. The genetic stability of cells treated with nanoparticles was confirmed by chromosomal analysis, copy number variation (CNV) analysis, and mRNA profiling. Results: After 21 days of pellet culture after sub-culture from T1 to T4, hMSCs treated with QD nanogels complexed with SOX9 plasmid DNA (pDNA) significantly increased expression of specific extracellular matrix (ECM) polysaccharides and glycoproteins, as determined by Safranin O and Alcian blue staining. Moreover, the T4 hMSCs expressed higher levels of specific proteins, including collagen type II (COLII) and SOX9, than P4 hMSCs, with no evidence of DNA damage or genomic malfunction. Microarray analysis confirmed expression of genes specific to matured chondrocytes. Stem cells that internalized nanoparticles at the early stage retained genetic stability, even after passage. In in vivo studies in rats, neuronal cartilage formation was observed in damaged lesions 6 weeks after transplantation of T1 and T4 cells. The degree of differentiation into chondrocytes

in the cartilage defect area, as determined by mRNA and protein expression of COLII and SOX9, was higher in rats treated with SF-NPs. Conclusion: The QD nanogels used in this study, did not affect genome integrity during long-term subculture, and are thus suitable for multiple theranostic applications.

Park, S., et al. (2009). "Carbon nanosyringe array as a platform for intracellular delivery." Nano Lett **9**(4): 1325-1329.

We report a novel platform for intracellular delivery of genetic material and nanoparticles, based on vertically aligned carbon nanosyringe arrays (CNSAs) of controllable height. Using this technology, we have shown that plasmid and quantum dots can be efficiently delivered to the cytoplasm of cancer cells and human mesenchymal stem cells. The CNSA platform holds great promise for a myriad of applications including cell-based therapy, imaging, and tracking in vivo, and in biological studies aimed at understanding cellular function.

Park, S. W., et al. (2019). "A new regulatory mechanism for Raf kinase activation, retinoic acid-bound Crabp1." Sci Rep **9**(1): 10929.

The rapidly accelerated fibrosarcoma (Raf) kinase is canonically activated by growth factors that regulate multiple cellular processes. In this kinase cascade Raf activation ultimately results in extracellular regulated kinase 1/2 (Erk1/2) activation, which requires Ras binding to the Ras binding domain (RBD) of Raf. We recently reported that all-trans retinoic acid (atRA) rapidly (within minutes) activates Erk1/2 to modulate cell cycle progression in stem cells, which is mediated by cellular retinoic acid binding protein 1 (Crabp1). But how atRA-bound Crabp1 regulated Erk1/2 activity remained unclear. We now report Raf kinase as the direct target of atRA-Crabp1. Molecularly, Crabp1 acts as a novel atRA-inducible scaffold protein for Raf/Mek/Erk in cells without growth factor stimulation. However, Crabp1 can also compete with Ras for direct interaction with the RBD of Raf, thereby negatively modulating growth factor-stimulated Raf activation, which can be enhanced by atRA binding to Crabp1. NMR heteronuclear single quantum coherence (HSQC) analyses reveal the 6-strand beta-sheet face of Crabp1 as its Raf-interaction surface. We identify a new atRA-mimicking and Crabp1-selective compound, C3, that can also elicit such an activity. This study uncovers a new signal crosstalk between endocrine (atRA-Crabp1) and growth factor (Ras-Raf) pathways, providing evidence for atRA-Crabp1 as a novel modulator of cell growth. The study also suggests a new therapeutic strategy by employing Crabp1-selective compounds to dampen growth factor stimulation while circumventing RAR-mediated retinoid toxicity.

Patel, S. and K. B. Lee (2015). "Probing stem cell behavior using nanoparticle-based approaches." Wiley Interdiscip Rev Nanomed Nanobiotechnol **7**(6): 759-778.

Stem cells hold significant clinical potential to treat numerous debilitating diseases and injures that currently have no treatment plan. While several advances have been made in developing stem cell platforms and methods to induce their differentiation, there are two critical aspects need to be addressed: (1) efficient delivery of nucleic acids and small molecules for stem cell differentiation, and (2) effective, noninvasive, and real-time tracking of transplanted stem cells. To address this, there has been a trend of utilizing various types of nanoparticles to not only deliver biomolecules to targeted site but also track the location of transplanted stem cells in real time. Over the past decade, various types of nanoparticles, including magnetic nanoparticles, silica nanoparticles, quantum dots, and gold nanoparticles, have been developed to serve as vehicles for targeted biomolecule delivery. In addition of being biocompatible without causing adverse side effect to stem cells, these nanoparticles have unique chemical and physical properties that allow tracking and imaging in real time using different imaging instruments that are commonly found in hospitals. A summary of the landmark and progressive demonstrations that utilize nanoparticles for stem cell application is described.

Pavon, L. F., et al. (2018). "Tropism of mesenchymal stem cell toward CD133(+) stem cell of glioblastoma in vitro and promote tumor proliferation in vivo." Stem Cell Res Ther **9**(1): 310.

BACKGROUND: Previous studies have demonstrated remarkable tropism of mesenchymal stem cells (MSCs) toward malignant gliomas, making these cells a potential vehicle for delivery of therapeutic agents to disseminated glioblastoma (GBM) cells. However, the potential contribution of MSCs to tumor progression is a matter of concern. It has been suggested that CD133(+) GBM stem cells secrete a variety of chemokines, including monocytes chemoattractant protein-1 (MCP-1/CCL2) and stromal cell-derived factor-1(SDF-1/CXCL12), which could act in this tropism. However, the role in the modulation of this tropism of the subpopulation of CD133(+) cells, which initiate GBM and the mechanisms underlying the tropism of MSCs to CD133(+) GBM cells and their effects on tumor development, remains poorly defined. METHODS/RESULTS: We found that isolated and cultured MSCs (human umbilical cord blood MSCs) express CCR2 and CXCR4, the respective receptors for MCP-1/CCL2 and SDF-1/CXCL12, and demonstrated, in vitro, that MCP-1/CCL2 and SDF-1/CXC12, secreted

by CD133(+) GBM cells from primary cell cultures, induce the migration of MSCs. In addition, we confirmed that after in vivo GBM tumor establishment, by stereotaxic implantation of the CD133(+) GBM cells labeled with Qdots (705 nm), MSCs labeled with multimodal iron oxide nanoparticles (MION) conjugated to rhodamine-B (Rh-B) (MION-Rh), infused by caudal vein, were able to cross the blood-brain barrier of the animal and migrate to the tumor region. Evaluation GBM tumors histology showed that groups that received MSC demonstrated tumor development, glial invasiveness, and detection of a high number of cycling cells. CONCLUSIONS: Therefore, in this study, we validated the chemotactic effect of MCP-1/CCL2 and SDF-1/CXCL12 in mediating the migration of MSCs toward CD133(+) GBM cells. However, we observed that, after infiltrating the tumor, MSCs promote tumor growth in vivo probably by release of exosomes. Thus, the use of these cells as a therapeutic carrier strategy to target GBM cells must be approached with caution.

Peckys, D. B., et al. (2016). "Visualizing Quantum Dot Labeled ORAI1 Proteins in Intact Cells Via Correlative Light and Electron Microscopy." Microsc Microanal **22**(4): 902-912.

ORAI1 proteins are ion channel subunits and the essential pore-forming units of the calcium release-activated calcium channel complex essential for T-cell activation and many other cellular processes. In this study, we used environmental scanning electron microscopy (ESEM) with scanning transmission electron microscopy (STEM) detection to image plasma membrane expressed ORAI1 proteins in whole Jurkat T cells in the liquid state. Utilizing a stably transfected Jurkat T cell clone expressing human ORAI1 with an extracellular human influenza hemagglutinin (HA) tag we investigated if liquid-phase STEM can be applied to detect recombinant surface expressed protein. Streptavidin coated quantum dots were coupled in a one-to-one stoichiometry to ORAI1 proteins detected by biotinylated anti-HA fragmented antibody fragments. High-resolution electron microscopic images revealed the individual label locations from which protein pair distances were determined. These data were analyzed using the pair correlation function and, in addition, an analysis of cluster size and frequency was performed. ORAI1 was found to be present in hexamers in a small fraction only, and ORAI1 resided mostly in monomers and dimers.

Peckys, D. B., et al. (2014). "Correlative fluorescence and scanning transmission electron microscopy of quantum dot-labeled proteins on whole cells in liquid." Methods Cell Biol **124**: 305-322.

Correlative fluorescence microscopy combined with scanning transmission electron microscopy (STEM) of cells fully immersed in liquid is a new methodology with many application areas. Proteins, in live cells immobilized on microchips, are labeled with fluorescent quantum dot nanoparticles. In this protocol, the epidermal growth factor receptor (EGFR) is labeled. The cells are fixed after a selected labeling time, for example, 5 min as needed to form EGFR dimers. The microchip with cells is then imaged with fluorescence microscopy. Thereafter, STEM can be accomplished in two ways. The microchip with the labeled cells and one microchip with a spacer are assembled into a special microfluidic device and imaged with dedicated high-voltage STEM. Alternatively, thin edges of cells can be studied with environmental scanning electron microscopy with a STEM detector, by placing a microchip with cells in a cooled wet environment.

Peckys, D. B. and N. de Jonge (2015). "Studying the Stoichiometry of Epidermal Growth Factor Receptor in Intact Cells using Correlative Microscopy." J Vis Exp(103).

This protocol describes the labeling of epidermal growth factor receptor (EGFR) on COS7 fibroblast cells, and subsequent correlative fluorescence microscopy and environmental scanning electron microscopy (ESEM) of whole cells in hydrated state. Fluorescent quantum dots (QDs) were coupled to EGFR via a two-step labeling protocol, providing an efficient and specific protein labeling, while avoiding label-induced clustering of the receptor. Fluorescence microscopy provided overview images of the cellular locations of the EGFR. The scanning transmission electron microscopy (STEM) detector was used to detect the QD labels with nanoscale resolution. The resulting correlative images provide data of the cellular EGFR distribution, and the stoichiometry at the single molecular level in the natural context of the hydrated intact cell. ESEM-STEM images revealed the receptor to be present as monomer, as homodimer, and in small clusters. Labeling with two different QDs, i.e., one emitting at 655 nm and at 800 revealed similar characteristic results.

Peckys, D. B., et al. (2021). "Quantification of EGFR-HER2 Heterodimers in HER2-Overexpressing Breast Cancer Cells Using Liquid-Phase Electron Microscopy." Cells **10**(11).

Currently, breast cancer patients are classified uniquely according to the expression level of hormone receptors, and human epidermal growth factor receptor 2 (HER2). This coarse classification is insufficient to capture the phenotypic complexity and heterogeneity of the disease. A methodology was developed for absolute quantification of receptor surface density rhoR, and

molecular interaction (dimerization), as well as the associated heterogeneities, of HER2 and its family member, the epidermal growth factor receptor (EGFR) in the plasma membrane of HER2 overexpressing breast cancer cells. Quantitative, correlative light microscopy (LM) and liquid-phase electron microscopy (LPEM) were combined with quantum dot (QD) labeling. Single-molecule position data of receptors were obtained from scanning transmission electron microscopy (STEM) images of intact cancer cells. Over 280,000 receptor positions were detected and statistically analyzed. An important finding was the subcellular heterogeneity in heterodimer shares with respect to plasma membrane regions with different dynamic properties. Deriving quantitative information about EGFR and HER2 rhoR, as well as their dimer percentages, and the heterogeneities thereof, in single cancer cells, is potentially relevant for early identification of patients with HER2 overexpressing tumors comprising an enhanced share of EGFR dimers, likely increasing the risk for drug resistance, and thus requiring additional targeted therapeutic strategies.

Peckys, D. B., et al. (2019). "Visualisation of HER2 homodimers in single cells from HER2 overexpressing primary formalin fixed paraffin embedded tumour tissue." Mol Med **25**(1): 42.

BACKGROUND: HER2 is considered as one of the most important, predictive biomarkers in oncology. The diagnosis of HER2 positive cancer types such as breast- and gastric cancer is usually based on immunohistochemical HER2 staining of tumour tissue. However, the current immunohistochemical methods do not provide localized information about HER2's functional state. In order to generate signals leading to cell growth and proliferation, the receptor spontaneously forms homodimers, a process that can differ between individual cancer cells. MATERIALS AND METHODS: HER2 overexpressing tumour cells were dissociated from formalin-fixed paraffin-embedded (FFPE) patient's biopsy sections, subjected to a heat-induced antigen retrieval procedure, and immobilized on microchips. HER2 was specifically labelled via a two-step protocol involving the incubation with an Affibody-biotin compound followed by the binding of a streptavidin coated quantum dot (QD) nanoparticle. Cells with membrane bound HER2 were identified using fluorescence microscopy, coated with graphene to preserve their hydrated state, and subsequently examined by scanning transmission electron microscopy (STEM) to obtain the locations at the single molecule level. Label position data was statistically analysed via the pair correlation function, yielding information about the presence of HER2 homodimers. RESULTS: Tumour cells from two biopsies, scored HER2 3+, and a HER2 negative control sample were examined. The specific labelling protocol was first tested for a sectioned tissue sample of HER2-overexpressing tumour. Subsequently, a protocol was optimized to study HER2 homodimerization in single cells dissociated from the tissue section. Electron microscopy data showed membrane bound HER2 in average densities of 201-689 proteins/mum(2). An automated, statistical analysis of well over 200,000 of measured protein positions revealed the presence of HER2 homodimers in 33 and 55% of the analysed images for patient 1 and 2, respectively. CONCLUSIONS: We introduced an electron microscopy method capable of measuring the positions of individually labelled HER2 proteins in patient tumour cells from which information about the functional status of the receptor was derived. This method could take HER2 testing a step further by examining HER2 homodimerization directly out of tumour tissue and may become important for adjusting a personalized antibody-based drug therapy.

Peckys, D. B., et al. (2017). "The stoichiometry of the TMEM16A ion channel determined in intact plasma membranes of COS-7 cells using liquid-phase electron microscopy." J Struct Biol **199**(2): 102-113.

TMEM16A is a membrane protein forming a calcium-activated chloride channel. A homodimeric stoichiometry of the TMEM16 family of proteins has been reported but an important question is whether the protein resides always in a dimeric configuration in the plasma membrane or whether monomers of the protein are also present in its native state within in the intact plasma membrane. We have determined the stoichiometry of the human (h)TMEM16A within whole COS-7 cells in liquid. For the purpose of detecting TMEM16A subunits, single proteins were tagged by the streptavidin-binding peptide within extracellular loops accessible by streptavidin coated quantum dot (QD) nanoparticles. The labeled proteins were then imaged using correlative light microscopy and environmental scanning electron microscopy (ESEM) using scanning transmission electron microscopy (STEM) detection. The locations of 19,583 individual proteins were determined of which a statistical analysis using the pair correlation function revealed the presence of a dimeric conformation of the protein. The amounts of detected label pairs and single labels were compared between experiments in which the TMEM16A SBP-tag position was varied, and experiments in which tagged and non-tagged TMEM16A proteins were present. It followed that hTMEM16A resides in the plasma membrane as dimer only and is not present as monomer. This strategy may help to elucidate the stoichiometry of other membrane protein species within the context of the intact plasma membrane in future.

Pena-Duarte, A., et al. (2021). "Iron Quantum Dots Electro-Assembling on Vulcan XC-72R: Hydrogen Peroxide Generation for Space Applications." ACS Appl Mater Interfaces **13**(25): 29585-29601.

Highly dispersed iron-based quantum dots (QDs) onto powdered Vulcan XC-72R substrate were successfully electrodeposited by the rotating disk slurry electrodeposition (RoDSE) technique. Our findings through chemical physics characterization revealed that the continuous electron pathway interaction between the interface metal-carbon is controlled. The rotating ring-disk electrode (RRDE) and the prototype generation unit (PGU) of in-situ H2O2 generation in fuel cell experiments revealed a high activity for the oxygen reduction reaction (ORR) via two-electron pathway. These results establish the Fe/Vulcan catalyst at a competitive level for space and terrestrial new materials carriers, specifically for the in-situ H2O2 production. Transmission electron microscopy (TEM) analysis reveals the well-dispersed Fe-based quantum dots with a particle size of 4 nm. The structural and chemical-physical characterization through induced coupled plasma-optical emission spectroscopy (ICP-OES), transmission scanning electron microscopy (STEM), X-ray diffraction (XRD), Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), and X-ray absorption spectroscopy (XAS); reveals that, under atmospheric conditions, our quantum dots system is a Fe(2+/3+)/Fe(3+) combination. The QDs oxidation state tunability was showed by the applied potential. The obtention of H2O2 under the compatibility conditions of the drinking water resources available in the International Space Station (ISS) enhances the applicability of this iron- and carbon-based materials for in-situ H2O2 production in future space scenarios. Terrestrial and space abundance of iron and carbon, combined with its low toxicity and high stability, consolidates this present work to be further extended for the large-scale production of Fe-based nanoparticles for several applications.

Phan, N. D., et al. (2022). "Abietane diterpenes from Abies spectabilis and their anti-pancreatic cancer activity against the MIA PaCa-2 cell line." Bioorg Med Chem Lett **66**: 128723.

An ethanolic extract of the stem of Abies spectabilis exhibited strong cytotoxicity against MIA PaCa-2 human pancreatic cancer cells preferentially under nutrient-deprived conditions. Therefore, phytochemical investigation of this bioactive extract was carried out, and that led the isolation of ten compounds (1-10) including a new abietane-type diterpene (1). The structure of the new compound (1) was elucidated by combined spectroscopic techniques, including HRFABMS, NMR and quantum ECD calculation. All the isolated compounds were evaluated for their efficacy against MIA PaCa-2 human pancreatic cancer cell line by employing an anti-austerity strategy. Among the tested compounds, dehydroabietinol (5) displayed the most potent activity with a PC50 value of 6.6 muM. Dehydroabietinol (5) was also found to retard the MIA PaCa-2 cell migration under normal nutrient-rich conditions displaying its anti-metastatic potential. Investigation on the mechanism suggested that dehydroabietinol (5) is an inhibitor of the key cancer cell survival Akt/mTOR/autophagy signaling pathway.

Pi, Q. M., et al. (2010). "Degradation or excretion of quantum dots in mouse embryonic stem cells." BMC Biotechnol **10**: 36.

BACKGROUND: Quantum dots (QDs) have been considered as a new and efficient probe for labeling cells non-invasively in vitro and in vivo, but fairly little is known about how QDs are eliminated from cells after labeling. The purpose of this study is to investigate the metabolism of QDs in different type of cells. RESULTS: Mouse embryonic stem cells (ESCs) and mouse embryonic fibroblasts (MEFs) were labeled with QD 655. QD-labeling was monitored by fluorescence microscopy and flow cytometry for 72 hours. Both types of cells were labeled efficiently, but a quick loss of QD-labeling in ESCs was observed within 48 hours, which was not prevented by inhibiting cell proliferation. Transmission electron microscope analysis showed a dramatic decrease of QD number in vesicles of ESCs at 24 hours post-labeling, suggesting that QDs might be degraded. In addition, supernatants collected from labeled ESCs in culture were used to label cells again, indicating that some QDs were excreted from cells. CONCLUSION: This is the first study to demonstrate that the metabolism of QDs in different type of cells is different. QDs were quickly degraded or excreted from ESCs after labeling.

Pietila, M., et al. (2013). "Mortalin antibody-conjugated quantum dot transfer from human mesenchymal stromal cells to breast cancer cells requires cell-cell interaction." Exp Cell Res **319**(18): 2770-2780.

The role of tumor stroma in regulation of breast cancer growth has been widely studied. However, the details on the type of heterocellular cross-talk between stromal and breast cancer cells (BCCs) are still poorly known. In the present study, in order to investigate the intercellular communication between human mesenchymal stromal cells (hMSCs) and breast cancer cells (BCCs, MDA-MB-231), we recruited cell-internalizing quantum dots (i-QD) generated by conjugation of cell-internalizing anti-mortalin antibody and quantum dots (QD). Co-culture of illuminated and color-coded hMSCs (QD655) and BCCs (QD585) revealed the intercellular transfer of QD655 signal from hMSCs to BCCs. The amount of QD double positive

BCCs increased gradually within 48h of co-culture. We found prominent intercellular transfer of QD655 in hanging drop co-culture system and it was non-existent when hMSCs and BBCs cells were co-cultured in trans-well system lacking imminent cell-cell contact. Fluorescent and electron microscope analyses also supported that the direct cell-to-cell interactions may be required for the intercellular transfer of QD655 from hMSCs to BCCs. To the best of our knowledge, the study provides a first demonstration of transcellular crosstalk between stromal cells and BCCs that involve direct contact and may also include a transfer of mortalin, an anti-apoptotic and growth-promoting factor enriched in cancer cells.

Price, J. C., et al. (2019). "Quantum Sensing in a Physiological-Like Cell Niche Using Fluorescent Nanodiamonds Embedded in Electrospun Polymer Nanofibers." Small **15**(22): e1900455.

Fluorescent nanodiamonds (fNDs) containing nitrogen vacancy (NV) centers are promising candidates for quantum sensing in biological environments. This work describes the fabrication and implementation of electrospun poly lactic-co-glycolic acid (PLGA) nanofibers embedded with fNDs for optical quantum sensing in an environment, which recapitulates the nanoscale architecture and topography of the cell niche. A protocol that produces uniformly dispersed fNDs within electrospun nanofibers is demonstrated and the resulting fibers are characterized using fluorescent microscopy and scanning electron microscopy (SEM). Optically detected magnetic resonance (ODMR) and longitudinal spin relaxometry results for fNDs and embedded fNDs are compared. A new approach for fast detection of time varying magnetic fields external to the fND embedded nanofibers is demonstrated. ODMR spectra are successfully acquired from a culture of live differentiated neural stem cells functioning as a connected neural network grown on fND embedded nanofibers. This work advances the current state of the art in quantum sensing by providing a versatile sensing platform that can be tailored to produce physiological-like cell niches to replicate biologically relevant growth environments and fast measurement protocols for the detection of co-ordinated endogenous signals from clinically relevant populations of electrically active neuronal circuits.

Qiu, J., et al. (2016). "Effects of Graphene Quantum Dots on the Self-Renewal and Differentiation of Mesenchymal Stem Cells." Adv Healthc Mater **5**(6): 702-710.

The influence of graphene quantum dots (GQDs) on key characteristics of bone marrow derived mesenchymal stem cells (MSCs) phenotype (i.e., self-renewal, differentiation potential, and pluripotency) is systematically investigated in this work. First, the viability and impact of GQDs on the self-renewal potential of MSCs is evaluated in order to determine a threshold for the exposing dose. Second, GQDs uptake by MSCs is confirmed due to the excellent fluorescent properties of the particles. They exhibit a homogenous cytoplasmatic distribution that increases with the time and concentration. Third, the impact of GQDs on the osteogenic differentiation of MSCs is deeply characterized. An enhanced activity of alkaline phosphatase promoted by GQDs indicates early activation of osteogenesis. This is also confirmed upon GQD-induced up-regulation of phenotypically related osteogenic genes (Runx2, osteopontin, and osteocalcin) and specific biomarkers expression (osteopontin and osteocalcin). GQDs also effectively enhance the formation of calcium-rich deposits characteristics of osteoblasts. Furthermore, genes microarray results indicate that the enhanced osteogenic differentiation of MSCs by GQDs is in progress through a bone morphogenetic protein and transforming growth factor-beta relative signaling pathways. Finally, intracytoplasmatic lipid detection shows that GQDs can also promote the adipogenic differentiation of MSCs, thus confirming the prevalence of their pluripotency potential.

Quesenberry, P. J., et al. (2012). "A new stem cell biology: the continuum and microvesicles." Trans Am Clin Climatol Assoc **123**: 152-166; discussion 166.

The hierarchical models of stem cell biology have been based on work first demonstrating pluripotental spleen-colony-forming units, then showing progenitors with many differentiation fates assayed in in vitro culture; there followed the definition and separation of "stem cells" using monoclonal antibodies to surface epitopes and fluorescent-activated cell characterization and sorting (FACS). These studies led to an elegant model of stem cell biology in which primitive dormant G0 stem cells with tremendous proliferative and differentiative potential gave rise to progressively more restricted and differentiated classes of stem/progenitor cells, and finally differentiated marrow hematopoietic cells. The holy grail of hematopoietic stem cell biology became the purification of the stem cell and the clonal definition of this cell. Most recently, the long-term repopulating hematopoietic stem cell (LT-HSC) has been believed to be a lineage negative sca-1+C-kit+ Flk3- and CD150+ cell. However, a series of studies over the past 10 years has indicated that murine marrow stem cells continuously change phenotype with cell cycle passage. We present here studies using tritiated thymidine suicide and pyronin-Hoechst FACS separations indicating that the murine hematopoietic stem cell is a cycling cell. This would indicate that the hematopoietic stem cell must be

continuously changing in phenotype and, thus, could not be purified. The extant data indicate that murine marrow stem cells are continually transiting cell cycle and that the purification has discarded these cycling cells. Further in vivo BrdU studies indicate that the "quiescent" LT-HSC in G0 rapidly transits cycle. Further complexity of the marrow stem cell system is indicated by studies on cell-derived microvesicles showing that they enter marrow cells and transcriptionally alter their cell fate and phenotype. Thus, the stem cell model is a model of continuing changing potential tied to cell cycle and microvesicle exposure. The challenge of the future is to define the stem cell population, not purify the stem cell. We are at the beginning of elucidation of quantum stemomics.

Rafieerad, A., et al. (2019). "Application of Ti3 C2 MXene Quantum Dots for Immunomodulation and Regenerative Medicine." Adv Healthc Mater **8**(16): e1900569.

Inflammation is tightly linked to tissue injury. In regenerative medicine, immune activation plays a key role in rejection of transplanted stem cells and reduces the efficacy of stem cell therapies. Next-generation smart biomaterials are reported to possess multiple biologic properties for tissue repair. Here, the first use of 0D titanium carbide (Ti3 C2) MXene quantum dots (MQDs) for immunomodulation is presented with the goal of enhancing material-based tissue repair after injury. MQDs possess intrinsic immunomodulatory properties and selectively reduce activation of human CD4(+) IFN-gamma(+) T-lymphocytes (control 87.1 +/- 2.0%, MQDs 68.3 +/- 5.4%) while promoting expansion of immunosuppressive CD4(+) CD25(+) FoxP3(+) regulatory T-cells (control 5.5 +/- 0.7%, MQDs 8.5 +/- 0.8%) in a stimulated lymphocyte population. Furthermore, MQDs are biocompatible with bone marrow-derived mesenchymal stem cells and induced pluripotent stem cell-derived fibroblasts. Finally, Ti3 C2 MQDs are incorporated into a chitosan-based hydrogel to create a 3D platform with enhanced physicochemical properties for stem cell delivery and tissue repair. This composite hydrogel demonstrates increased conductivity while maintaining injectability and thermosensitivity. These findings suggest that this new class of biomaterials may help bridge the translational gap in material and stem cell-based therapies for tissue repair and treatment of inflammatory and degenerative diseases.

Raikwar, S. P., et al. (2018). "Neuro-Immuno-Gene- and Genome-Editing-Therapy for Alzheimer's Disease: Are We There Yet?" J Alzheimers Dis **65**(2): 321-344.

Alzheimer's disease (AD) is a highly complex neurodegenerative disorder and the current treatment strategies are largely ineffective thereby leading to irreversible and progressive cognitive decline in AD patients. AD continues to defy successful treatment despite significant advancements in the field of molecular medicine. Repeatedly, early promising preclinical and clinical results have catapulted into devastating setbacks leading to multi-billion dollar losses not only to the top pharmaceutical companies but also to the AD patients and their families. Thus, it is very timely to review the progress in the emerging fields of gene therapy and stem cell-based precision medicine. Here, we have made sincere efforts to feature the ongoing progress especially in the field of AD gene therapy and stem cell-based regenerative medicine. Further, we also provide highlights in elucidating the molecular mechanisms underlying AD pathogenesis and describe novel AD therapeutic targets and strategies for the new drug discovery. We hope that the quantum leap in the scientific advancements and improved funding will bolster novel concepts that will propel the momentum toward a trajectory leading to a robust AD patient-specific next generation precision medicine with improved cognitive function and excellent life quality.

Rak-Raszewska, A., et al. (2012). "Quantum dots do not affect the behaviour of mouse embryonic stem cells and kidney stem cells and are suitable for short-term tracking." PLoS One **7**(3): e32650.

Quantum dots (QDs) are small nanocrystals widely used for labelling cells in order to enable cell tracking in complex environments in vitro, ex vivo and in vivo. They present many advantages over traditional fluorescent markers as they are resistant to photobleaching and have narrow emission spectra. Although QDs have been used effectively in cell tracking applications, their suitability has been questioned by reports showing they can affect stem cell behaviour and can be transferred to neighbouring cells. Using a variety of cellular and molecular biology techniques, we have investigated the effect of QDs on the proliferation and differentiation potential of two stem cell types: mouse embryonic stem cells and tissue-specific stem cells derived from mouse kidney. We have also tested if QDs released from living or dead cells can be taken up by neighbouring cells, and we have determined if QDs affect the degree of cell-cell fusion; this information is critical in order to assess the suitability of QDs for stem cell tracking. We show here that QDs have no effect on the viability, proliferation or differentiation potential of the two stem cell types. Furthermore, we show that the extent of transfer of QDs to neighbouring cells is <4%, and that QDs do not increase the degree of cell-cell fusion. However, although the QDs have a high labelling efficiency (>85%), they are rapidly depleted from both stem cell populations. Taken together, our results suggest that

QDs are effective cell labelling probes that are suitable for short-term stem cell tracking.

Rak-Raszewska, A., et al. (2012). "Development of embryonic stem cells in recombinant kidneys." Organogenesis **8**(4): 125-136.

Embryonic stem cells (ESC) are self-renewing and can generate all cell types during normal development. Previous studies have begun to explore fates of ESCs and their mesodermal derivatives after injection into explanted intact metanephric kidneys and neonatal kidneys maturing in vivo. Here, we exploited a recently described recombinant organ culture model, mixing fluorescent quantum dot labeled mouse exogenous cells with host metanephric cells. We compared abilities of undifferentiated ESCs with ESC-derived mesodermal or non-mesodermal cells to contribute to tissue compartments within recombinant, chimeric metanephroi. ESC-derived mesodermal cells downregulated Oct4, a marker of undifferentiated cells, and, as assessed by locations of quantum dots, contributed to Wilms' tumor 1-expressing forming nephrons, synaptopodin-expressing glomeruli, and organic ion-transporting tubular epithelia. Similar results were observed when labeled native metanephric cells were recombined with host cells. In striking contrast, non-mesodermal ESC-derived cells strongly inhibited growth of embryonic kidneys, while undifferentiated ESCs predominantly formed Oct4 expressing colonies between forming nephrons and glomeruli. These findings clarify the conclusion that ESC-derived mesodermal cells have functional nephrogenic potential, supporting the idea that they could potentially replace damaged epithelia in diseased kidneys. On the other hand, undifferentiated ESCs and non-mesodermal precursors derived from ESCs would appear to be less suitable materials for use in kidney cell therapies.

Ramot, Y., et al. (2010). "Pulmonary thrombosis in the mouse following intravenous administration of quantum dot-labeled mesenchymal cells." Nanotoxicology **4**(1): 98-105.

Quantum dots (QDs) are emerging as novel diagnostic agents. Yet, only a few studies have examined the possible deleterious effects of QD-labeled stem cells. We assessed the potential toxic effects of QD-labeled human embryonic palatal mesenchymal (QD-HEPM) cells in male NOD/SCID mice for six months, following the administration of a single intravenous injection. Control animals were administered with non-labeled HEPM cells. No treatment-related clinical signs, hematological, or biochemical parameters were found in the QD-HEPM animals in comparison to control animals. Histologically, multifocal organizing thrombi were noted in the pulmonary arteries of all QD-HEPM animals from the one-week study group and in one animal from the one-month group. Additionally, increased severity of perivascular inflammation was noted at the injection sites of QD-HEPM animals from the one-week group. This is the first study reporting histopathological evidence for pro-thrombotic adverse effects mediated by QD labeling.

Ranjbarvaziri, S., et al. (2011). "Quantum dot labeling using positive charged peptides in human hematopoetic and mesenchymal stem cells." Biomaterials **32**(22): 5195-5205.

Quantum dots (QDs), as new and promising fluorescent probes, hold great potential in long term non-invasive bio-imaging, however there are many uncovered issues regarding their competency. In the present study, different QDs (525, 585 and 800 nm) were used to label CD133, CD34, CD14 and mesenchymal stem cells (MSCs) using positively charged peptides. Results demonstrated highly efficient internalization with the possible involvement of macropinocytosis. As indicated by LDH release and the TUNEL assay, no measurable effects on cell viability were detected at a concentration of 10 nM. QDs did not have any deleterious effects on normal cell functionality where both labeled CD133(+) cells and MSCs remarkably differentiated along multiple lineages with the use of the colony forming assay and adipo/osteo induction, respectively. Our results regarding QD maintenance revealed that these nano-particles are not properly stable and various excretion times have been observed depending on particle size and cell type. In vitro co-culture system and transplantation of labeled cells to an animal model showed that QDs leaked out from labeled cells and the released nano-particles were able to re-enter adjacent cells over time. These data suggest that before any utilization of QDs in bio-imaging and related applications, an efficient intra-cellular delivery technique should be considered to preserve QDs for a prolonged time as well as eliminating their leakage.

Raychaudhuri, R., et al. (2020). "Pullulan based stimuli responsive and sub cellular targeted nanoplatforms for biomedical application: Synthesis, nanoformulations and toxicological perspective." Int J Biol Macromol **161**: 1189-1205.

With growing interest in polymers of natural origin, innumerable polysaccharides have gained attention for their biomedical application. Pullulan, one of the FDA approved nutraceuticals, possesses multiple unique properties which make them highly advantageous for biomedical applications. This present review encompasses the sources, production, properties and applications of pullulan. It highlights various

pullulan based stimuli-responsive systems (temperature, pH, ultrasound, magnetic), subcellular targeted systems (mitochondria, Golgi apparatus/endoplasmic reticulum, lysosome, endosome), lipid-vesicular systems (solid-lipid nanoparticles, liposomes), polymeric nanofibres, micelles, inorganic (SPIONs, gold and silver nanoparticles), carbon-based nanoplatforms (carbon nanotubes, fullerenes, nanodiamonds) and quantum dots. This article also gives insight into different biomedical, therapeutic and diagnostic applications of pullulan viz., imaging, tumor targeting, stem cell therapy, gene therapy, vaccine delivery, cosmetic applications, protein delivery, tissue engineering, photodynamic therapy and chaperone-like activities. The review also includes the toxicological profile of pullulan which is helpful for the development of suitable delivery systems for clinical applications.

Redmond, R. W., et al. (1994). "Merocyanine dyes: effect of structural modifications on photophysical properties and biological activity." Photochem Photobiol **60**(4): 348-355.

Merocyanine derivatives were prepared by structural alterations at the barbituric acid or chalcogenazole moieties. The photophysical properties of the dyes were markedly influenced by the presence of selenium rather than sulfur as a substituent at position 2 of the barbiturate. In methanol, quantum yields of both triplet state (phi T) and singlet oxygen sensitization (phi delta) were increased by over an order of magnitude, with a concomitant decrease in fluorescence, when selenium was present in the molecule. Photoisomerization, one of the dominant deactivation pathways in the sulfur- or oxygen-containing analogues, was completely absent in the selenium-containing derivatives. Efficient triplet state formation was observed for selenium-containing derivatives incorporated into L1210 cells by diffuse reflectance laser flash photolysis. Cytotoxicity studies, carried out using clonogenic assays on L1210 leukemia cells, showed a good correlation with phi T and phi delta, measured in solution. Experimental evidence provided by this paper supports a triplet state-, and probably singlet oxygen-, mediated phototoxic mechanism. Photoisomerization or singlet state mechanisms can be discounted.

Rehni, A. K., et al. (2012). "Biocompatible nanoparticle labeling of stem cells and their distribution in brain." Methods Mol Biol **879**: 531-537.

Nanolabeling is an invaluable novel technique in biology to detect and characterize different parts of biological systems including microscopic entities, viz., cells inside the living systems. Stem cells (SCs) are multipotent cells with the potential to differentiate into bone, cartilage, fat, muscle cells, and neurons and are being investigated for their utility in cell-based transplantation therapy. Yet, adequate methods to track transplanted SCs in vivo are limited, precluding functional studies. Nanoparticles (quantum dots) offer an alternative to organic dyes and fluorescent proteins to label and track cells in vitro and in vivo. These nanoparticles are resistant to chemical and metabolic degradation, demonstrating long-term photo stability. Here, we describe the technology of labeling the stem cells with silver nitrate nanoparticles in an in vitro coculture model. This is followed by defining the procedure of administering these cells in vivo and studying the distribution pattern and resultant regenerative effects of the "tagged" stem cells.

Rizzo, M. I., et al. (2021). "Engineered mucoperiosteal scaffold for cleft palate regeneration towards the non-immunogenic transplantation." Sci Rep **11**(1): 14570.

Cleft lip and palate (CL/P) is the most prevalent craniofacial birth defect in humans. None of the surgical procedures currently used for CL/P repair lead to definitive correction of hard palate bone interruption. Advances in tissue engineering and regenerative medicine aim to develop new strategies to restore palatal bone interruption by using tissue or organ-decellularized bioscaffolds seeded with host cells. Aim of this study was to set up a new natural scaffold deriving from a decellularized porcine mucoperiosteum, engineered by an innovative micro-perforation procedure based on Quantum Molecular Resonance (QMR) and then subjected to in vitro recellularization with human bone marrow-derived mesenchymal stem cells (hBM-MSCs). Our results demonstrated the efficiency of decellularization treatment gaining a natural, non-immunogenic scaffold with preserved collagen microenvironment that displays a favorable support to hMSC engraftment, spreading and differentiation. Ultrastructural analysis showed that the micro-perforation procedure preserved the collagen mesh, increasing the osteoinductive potential for mesenchymal precursor cells. In conclusion, we developed a novel tissue engineering protocol to obtain a non-immunogenic mucoperiosteal scaffold suitable for allogenic transplantation and CL/P repair. The innovative micro-perforation procedure improving hMSC osteogenic differentiation potentially impacts for enhanced palatal bone regeneration leading to future clinical applications in humans.

Roberts, I., et al. (2012). "Scale-up of human embryonic stem cell culture using a hollow fibre bioreactor." Biotechnol Lett **34**(12): 2307-2315.

The commercialisation of human embryonic stem cell derived cell therapies for large patient populations is reliant on both minimising expensive and variable manual-handling methods whilst realising

economies of scale. The Quantum Cell Expansion System, a hollow fibre bioreactor (Terumo BCT), was used in a pilot study to expand 60 million human embryonic stem cells to 708 million cells. Further improvements can be expected with optimisation of media flow rates throughout the run to better control the cellular microenvironment. High levels of pluripotency marker expression were maintained on the bioreactor, with 97.7 % of cells expressing SSEA-4 when harvested.

Rofaani, E., et al. (2022). "Reconstituted basement membrane enables airway epithelium modeling and nanoparticle toxicity testing." Int J Biol Macromol **204**: 300-309.

Basement membrane (BM) acts as a sheet-like extracellular matrix to support and promote the formation of epithelial and endothelial cell layers. The in vitro reconstruction of the BM is however not easy due to its ultrathin membrane features. This difficulty is overcome by self-assembling type IV collagen and laminin in the porous areas of a monolayer of crosslinked gelatin nanofibers deposited on a honeycomb microframe. Herein, a method is presented to generate airway epithelium by using such an artificial basement membrane (ABM) and human-induced pluripotent stem cells (hiPSCs). Bipolar primordial lung progenitors are firstly induced from hiPSCs and then replated on the ABM for differentiation toward matured airway epithelium under submerged and air-liquid interface culture conditions. As a result, a pseudostratified airway epithelium consisting of several cell types is achieved, showing remarkable apical secretion of MUC5AC proteins and clear advantages over other types of substrates. As a proof of concept, the derived epithelium is used for toxicity test of cadmium telluride (CdTe) nanoparticles (NPs), demonstrating the applicability of ABM-based assays involving hiPSC-derived epithelial cells-based assays.

Rojewski, M. T., et al. (2013). "GMP-compliant isolation and expansion of bone marrow-derived MSCs in the closed, automated device quantum cell expansion system." Cell Transplant **22**(11): 1981-2000.

The estimated frequency of MSCs in BM is about 0.001-0.01% of total nucleated cells. Most commonly, one applied therapeutic cell dose is about 1-5 million MSCs/kg body weight, necessitating a reliable, fast, and safe expansion system. The limited availability of MSCs demands for an extensive ex vivo amplification step to accumulate sufficient cell numbers. Human platelet lysate (PL) has proven to be a safe and feasible alternative to animal-derived serum as supplement for MSC cultivation. We have investigated the functionally closed automated cell culture hollow fiber bioreactor Quantum cell expansion system as an alternative novel tool to conventional tissue flasks for efficient clinical-scale MSC isolation and expansion from bone marrow using PL. Cells expanded in the Quantum system fulfilled MSC criteria as shown by flow cytometry and adipogenic, chondrogenic, and osteogenic differentiation capacity. Cell surface expression of a variety of chemokine receptors, adhesion molecules, and additional MSC markers was monitored for several passages by flow cytometry. The levels of critical media components like glucose and lactate were analyzed. PDGF-AA, PDGF-AB/BB, bFGF, TGF-beta1, sICAM-1, sVCAM-1, RANTES, GRO, VEGF, sCD40L, and IL-6 were assessed using a LUMINEX platform. Originally optimized for the use of fetal calf serum (FCS) as supplement and fibronectin as coating reagent, we succeeded to obtain an average of more than 100x10(6) of MSCs from as little as 18.8-28.6 ml of BM aspirate using PL. We obtained similar yields of MSCs/microl BM in the FCS-containing and the xenogen-free expansion system. The Quantum system reliably produces a cellular therapeutic dose in a functionally closed system that requires minimal manipulation. Both isolation and expansion are possible using FCS or PL as supplement. Coating of the hollow fibers of the bioreactor is mandatory when loading MSCs. Fibronectin, PL, and human plasma may serve as coating reagents.

Rosen, A. B., et al. (2007). "Finding fluorescent needles in the cardiac haystack: tracking human mesenchymal stem cells labeled with quantum dots for quantitative in vivo three-dimensional fluorescence analysis." Stem Cells **25**(8): 2128-2138.

Stem cells show promise for repair of damaged cardiac tissue. Little is known with certainty, however, about the distribution of these cells once introduced in vivo. Previous attempts at tracking delivered stem cells have been hampered by the autofluorescence of host tissue and limitations of existing labeling techniques. We have developed a novel loading approach to stably label human mesenchymal stem cells with quantum dot (QD) nanoparticles. We report the optimization and validation of this long-term tracking technique and highlight several important biological applications by delivering labeled cells to the mammalian heart. The bright QD crystals illuminate exogenous stem cells in histologic sections for at least 8 weeks following delivery and permit, for the first time, the complete three-dimensional reconstruction of the locations of all stem cells following injection into the heart. Disclosure of potential conflicts of interest is found at the end of this article.

Rota, M., et al. (2007). "Bone marrow cells adopt the cardiomyogenic fate in vivo." Proc Natl Acad Sci U S A **104**(45): 17783-17788.

The possibility that adult bone marrow cells (BMCs) retain a remarkable degree of developmental plasticity and acquire the cardiomyocyte lineage after infarction has been challenged, and the notion of BMC transdifferentiation has been questioned. The center of the controversy is the lack of unequivocal evidence in favor of myocardial regeneration by the injection of BMCs in the infarcted heart. Because of the interest in cell-based therapy for heart failure, several approaches including gene reporter assay, genetic tagging, cell genotyping, PCR-based detection of donor genes, and direct immunofluorescence with quantum dots were used to prove or disprove BMC transdifferentiation. Our results indicate that BMCs engraft, survive, and grow within the spared myocardium after infarction by forming junctional complexes with resident myocytes. BMCs and myocytes express at their interface connexin 43 and N-cadherin, and this interaction may be critical for BMCs to adopt the cardiomyogenic fate. With time, a large number of myocytes and coronary vessels are generated. Myocytes show a diploid DNA content and carry, at most, two sex chromosomes. Old and new myocytes show synchronicity in calcium transients, providing strong evidence in favor of the functional coupling of these two cell populations. Thus, BMCs transdifferentiate and acquire the cardiomyogenic and vascular phenotypes restoring the infarcted heart. Together, our studies reveal that locally delivered BMCs generate de novo myocardium composed of integrated cardiomyocytes and coronary vessels. This process occurs independently of cell fusion and ameliorates structurally and functionally the outcome of the heart after infarction.

Roubeix, C., et al. (2015). "Intraocular pressure reduction and neuroprotection conferred by bone marrow-derived mesenchymal stem cells in an animal model of glaucoma." Stem Cell Res Ther **6**: 177.

INTRODUCTION: Glaucoma is a sight-threatening retinal neuropathy associated with elevated intraocular pressure (IOP) due to degeneration and fibrosis of the trabecular meshwork (TM). Glaucoma medications aim to reduce IOP without targeting the specific TM pathology, Bone-marrow mesenchymal stem cells (MSCs) are used today in various clinical studies. Here, we investigated the potential of MSCs therapy in an glaucoma-like ocular hypertension (OHT) model and decipher in vitro the effects of MSCs on primary human trabecular meshwork cells. METHODS: Ocular hypertension model was performed by cauterization of 3 episcleral veins (EVC) of Long-Evans male rat eyes. MSCs were isolated from rat bone marrow, amplified in vitro and tagged with quantum dot nanocrystals. Animals were distributed as 1) MSCs group receiving 5.10(5)cells/6mul Minimum Essential Medium and 2) MEM group receiving 6mul MEM (n = 10 each). Injections were performed into the anterior chamber of 20 days-hypertensive eyes and IOP was monitored twice a week for 4 weeks. At the end of experiment, cell distribution in the anterior segment was examined in confocal microscopy on flat mounted corneas. Moreover, we tested in vitro effects of MSCs conditioned medium (MSC-CM) on primary human trabecular meshwork cells (hTM cells) using Akt activation, myosin phosphorylation and TGF-beta2-dependent profibrotic phenotype in hTM cells. RESULTS: We demonstrated a rapid and long-lasting in vivo effect of MSCs transplantation that significantly reduced IOP in hypertensive eyes induced by EVC. MSCs were located to the ciliary processes and the TM. Enumeration of RGCs on whole flat-mounted retina highlighted a protective effect of MSCs on RGCs death. In vitro, MSC-CM promotes: (i) hTM cells survival by activating the antiapoptotic pathway, Akt, (ii) hTM cells relaxation as analyzed by the decrease in myosin phosphorylation and (iii) inhibition of TGF-beta2-dependent profibrotic phenotype acquisition in hTM cells. CONCLUSIONS: MSCs injection in the ocular anterior chamber in a rat model of OHT provides neuroprotective effect in the glaucoma pathophysiology via TM protection. These results demonstrate that MSCs constitute promising tool for treating ocular hypertension and retinal cell degeneration.

Ruan, Y., et al. (2012). "Detection of prostate stem cell antigen expression in human prostate cancer using quantum-dot-based technology." Sensors (Basel) **12**(5): 5461-5470.

Quantum dots (QDs) are a new class of fluorescent labeling for biological and biomedical applications. In this study, we detected prostate stem cell antigen (PSCA) expression correlated with tumor grade and stage in human prostate cancer by QDs-based immunolabeling and conventional immunohistochemistry (IHC), and evaluated the sensitivity and stability of QDs-based immunolabeling in comparison with IHC. Our data revealed that increasing levels of PSCA expression accompanied advanced tumor grade (QDs labeling, $r = 0.732$, $p < 0.001$; IHC, $r = 0.683$, $p < 0.001$) and stage (QDs labeling, $r = 0.514$, $p = 0.001$; IHC, $r = 0.432$, $p = 0.005$), and the similar tendency was detected by the two methods. In addition, by comparison between the two methods, QDs labeling was consistent with IHC in detecting the expression of PSCA in human prostate tissue correlated with different pathological types ($K = 0.845$, $p < 0.001$). During the observation time, QDs exhibited superior stability. The intensity of QDs fluorescence remained stable for two weeks ($p = 0.083$) after conjugation to the PSCA protein, and nearly 93% of positive expression with their fluorescence still could be seen after four weeks.

Ruiz, N., et al. (2019). "Control of Nitrogen Inhomogeneities in Type-I and Type-II GaAsSbN Superlattices for Solar Cell Devices." Nanomaterials (Basel) **9**(4).

Superlattice structures (SLs) with type-II (GaAsSb/GaAsN) and -I (GaAsSbN/GaAs) band alignments have received a great deal of attention for multijunction solar cell (MJSC) applications, as they present a strongly intensified luminescence and a significant external quantum efficiency (EQE), with respect to the GaAsSbN bulk layers. Despite the difficulties in characterizing the distribution of N in dilute III-V nitride alloys, in this work we have obtained N-compositional mappings before and after rapid thermal annealing (RTA) in both types of structures, by using a recent methodology based on the treatment of different scanning transmission electron microscopy (STEM) imaging configurations. Texture analysis by gray level co-occurrence matrixes (GLCM) and the measurement of the degree of clustering are used to compare and evaluate the compositional inhomogeneities of N. Comparison with the Sb maps shows that there is no spatial correlation between the N and Sb distributions. Our results reveal that a better homogeneity of N is obtained in type-I SLs, but at the expense of a higher tendency of Sb agglomeration, and the opposite occurs in type-II SLs. The RTA treatments improve the uniformity of N and Sb in both designs, with the annealed sample of type-II SLs being the most balanced structure for MJSCs.

Russell, A. L., et al. (2018). "Characterization and cost-benefit analysis of automated bioreactor-expanded mesenchymal stem cells for clinical applications." Transfusion **58**(10): 2374-2382.

BACKGROUND: Expanding quantities of mesenchymal stem cells (MSCs) sufficient to treat large numbers of patients in cellular therapy and regenerative medicine clinical trials is an ongoing challenge for cell manufacturing facilities. STUDY DESIGN AND METHODS: We evaluated options for scaling up large quantities of bone marrow-derived MSCs (BM-MSCs) using methods that can be performed in compliance with Good Manufacturing Practices (GMP). We expanded BM-MSCs from fresh marrow aspirate in alphaMEM supplemented with 5% human platelet lysate using both an automated cell expansion system (Quantum, Terumo BCT) and a manual flask-based method using multilayer flasks. We compared MSCs expanded using both methods and assessed their differentiation to adipogenic and osteogenic tissue, capacity to suppress T-cell proliferation, cytokines, and growth factor secretion profile and cost-effectiveness of manufacturing enough BM-MSCs to administer a single dose of 100 x 10(6) cells per subject in a clinical trial of 100 subjects. RESULTS: We have established that large quantities of clinical-grade BM-MSCs manufactured with an automated hollow-fiber bioreactor were phenotypically (CD73, CD90, CD105) and functionally (adipogenic and osteogenic differentiation and cytokine and growth factor secretion) similar to manually expanded BM-MSCs. In addition, MSC manufacturing costs significantly less and required less time and effort when using the Quantum automated cell expansion system over the manual multilayer flasks method. CONCLUSION: MSCs manufactured by an automated bioreactor are physically and functionally equivalent to the MSCs manufactured by the manual flask method and have met the standards required for clinical application.

Sabapathy, V. and S. Kumar (2016). "hiPSC-derived iMSCs: NextGen MSCs as an advanced therapeutically active cell resource for regenerative medicine." J Cell Mol Med **20**(8): 1571-1588.

Mesenchymal stem cells (MSCs) are being assessed for ameliorating the severity of graft-versus-host disease, autoimmune conditions, musculoskeletal injuries and cardiovascular diseases. While most of these clinical therapeutic applications require substantial cell quantities, the number of MSCs that can be obtained initially from a single donor remains limited. The utility of MSCs derived from human-induced pluripotent stem cells (hiPSCs) has been shown in recent pre-clinical studies. Since adult MSCs have limited capability regarding proliferation, the quantum of bioactive factor secretion and immunomodulation ability may be constrained. Hence, the alternate source of MSCs is being considered to replace the commonly used adult tissue-derived MSCs. The MSCs have been obtained from various adult and foetal tissues. The hiPSC-derived MSCs (iMSCs) are transpiring as an attractive source of MSCs because during reprogramming process, cells undergo rejuvination, exhibiting better cellular vitality such as survival, proliferation and differentiations potentials. The autologous iMSCs could be considered as an inexhaustible source of MSCs that could be used to meet the unmet clinical needs. Human-induced PSC-derived MSCs are reported to be superior when compared to the adult MSCs regarding cell proliferation, immunomodulation, cytokines profiles, microenvironment modulating exosomes and bioactive paracrine factors secretion. Strategies such as derivation and propagation of iMSCs in chemically defined culture conditions and use of footprint-free safer reprogramming strategies have contributed towards the development of clinically relevant cell types. In this review, the role of iPSC-derived mesenchymal stromal cells (iMSCs) as an alternate source of therapeutically active MSCs has been described. Additionally, we also describe the role of iMSCs in regenerative medical applications, the necessary strategies, and the regulatory

policies that have to be enforced to render iMSC's effectiveness in translational medicine.

Sabharwal, N., et al. (2009). "Live cell labeling of glial progenitor cells using targeted quantum dots." Ann Biomed Eng **37**(10): 1967-1973.

This study describes the development of targeted quantum dots (T-QDs) as biomarkers for the labeling of glial progenitor cells (GPCs) that over express platelet derived growth factor (PDGF) and its receptor PDGFR (GPC(PDGF)). PDGFR plays a critical role in glioma development and growth, and is also known to affect multiple biological processes such as cell migration and embryonic development. T-QDs were developed using streptavidin-conjugated quantum dots (S-QDs) with biotinylated antibodies and utilized to label the intracellular and extracellular domains of live, cultured GPC(PDGF) cells via lipofection with cationic liposomes. Confocal studies illustrate successful intracellular and extracellular targeted labeling within live cells that does not appear to impact upstream PDGFR dynamics during real-time signaling events. Further, T-QDs were nontoxic to GPC(PDGF) cells, and did not alter cell viability or proliferation over the course of 6 days. These results raise new applications for T-QDs as ultra sensitive agents for imaging and tracking of protein populations within live cells, which that will enable future mechanistic study of oncogenic signaling events in real-time.

Sanchez-Navarrete, J., et al. (2020). "Simplified modeling of E. coli mortality after genome damage induced by UV-C light exposure." Sci Rep **10**(1): 11240.

UV light is a group of high-energy waves from the electromagnetic spectrum. There are three types of UV radiations: UV-A, -B and -C. UV-C light are the highest in energy, but most are retained by the ozone layer. UV-A and -B reach the earth's surface and cause damage on living organisms, being considered as mutagenic physical agents. Numerous test models are used to study UV mutagenicity; some include special lamps, cell cultures and mathematical modeling. Mercury lamps are affordable and useful sources of UV-C light due to their emission at near the maximum absorption peak of nucleic acids. E. coli cultures are widely used because they have DNA-damage and -repairing mechanisms fairly similar to humans. In here we present two simple models that describe UV-C light incidence on a genome matrix, using fundamental quantum-mechanical concepts and considering light as a particle with a discontinuous distribution. To test the accuracy of our equations, stationary phase cultures of several E. coli strains were exposed to UV-C light in 30 s-intervals. Surviving CFUs were counted and survival/mortality curves were constructed. These graphs adjusted with high goodness of fit to the regression predictions. Results were also analyzed using three main parameters: quantum yield, specific speed and time of mortality.

Sangha, A. K., et al. (2012). "Radical coupling reactions in lignin synthesis: a density functional theory study." J Phys Chem B **116**(16): 4760-4768.

Lignin is a complex, heterogeneous polymer in plant cell walls that provides mechanical strength to the plant stem and confers resistance to degrading microbes, enzymes, and chemicals. Lignin synthesis initiates through oxidative radical-radical coupling of monolignols, the most common of which are p-coumaryl, coniferyl, and sinapyl alcohols. Here, we use density functional theory to characterize radical-radical coupling reactions involved in monolignol dimerization. We compute reaction enthalpies for the initial self- and cross-coupling reactions of these monolignol radicals to form dimeric intermediates via six major linkages observed in natural lignin. The 8-O-4, 8-8, and 8-5 coupling are computed to be the most favorable, whereas the 5-O-4, 5-5, and 8-1 linkages are less favorable. Overall, p-coumaryl self- and cross-coupling reactions are calculated to be the most favorable. For cross-coupling reactions, in which each radical can couple via either of the two sites involved in dimer formation, the more reactive of the two radicals is found to undergo coupling at its site with the highest spin density.

Santos, V. H. D., et al. (2019). "Evaluation of alginate hydrogel encapsulated mesenchymal stem cell migration in horses." Res Vet Sci **124**: 38-45.

Osteoarthritis is an incapacitating disease characterized by pain and a progressive decrease in joint mobility. The implantation of mesenchymal stem cells (MSCs) has shown promising results for its treatment. The challenge remains to keep the cells longer at the site of action, increasing their therapeutic potential. The aim of this study was to evaluate the effectiveness of the Qtracker(R) 655 nanocrystal marking on allogeneic synovial membrane (SM) MSCs, encapsulated in alginate hydrogel, evaluating the migration of these cells. The 10 radiocarpal joints were submitted to arthroscopic surgery (D0), divided into two groups. The chondral defect was treated according to the group: GA free-labelled MSCSM and GB labelled MSCSM microcapsules. Seven days after lesion induction and implantation of labelled cells, biopsies of the lesion site were performed in two animals, and fragments of SM and joint capsule also collected, which were frozen and later processed for fluorescence microscopy. The synovial fluid of the three animals was analyzed by flow cytometry three times - 3, 7 and 21days after application. The cellular marking with the nanocrystals allowed the visualization of the cells in cartilage, synovial

membrane, synovial fluid and articular capsule, but with a predilection for the synovial membrane and the lesion site was scarce. The labelled MSCSM in microcapsules were scarce in the synovial fluid and could be related to the small quantity of MSCs leaving the pores of the microcapsules, also favorable results, as the cells release paracrine effects acting for a long period until the cellular differentiation.

Sarmah, D., et al. (2017). "Stroke Management: An Emerging Role of Nanotechnology." Micromachines (Basel) **8**(9).

Stroke is among the leading causes of mortality and morbidity worldwide. Stroke incidences and associated mortality are expected to rise to 23 million and 7.8 million, respectively, by 2030. Further, the aging population, imbalanced lifestyles, and environmental factors continue to shift the rate of stroke incidence, particularly in developing countries. There is an urgent need to develop new therapeutic approaches for treating stroke. Nanotechnology is a growing field, offering an encouraging future prospect for medical research in the management of strokes. The world market for nanotechnology derived products is expected to rise manyfold in the coming decades. Different types of nanomaterials such as perfluorocarbon nanoparticles, iron oxide nanoparticles, gold nanoparticles, polymeric nanoparticles, quantum dots, nanospheres, etc. have been developed for the diagnosis as well as therapy of strokes. Today, nanotechnology has also been integrated with stem cell therapy for treating stroke. However several obstacles remain to be overcome when using such nanomaterials for treating stroke and other neurological diseases.

Satzler, K., et al. (2002). "Three-dimensional reconstruction of a calyx of Held and its postsynaptic principal neuron in the medial nucleus of the trapezoid body." J Neurosci **22**(24): 10567-10579.

The three-dimensional morphology of the axosomatic synaptic structures between a calyx of Held and a principal neuron in the medial nucleus of the trapezoid body (MNTB) in the brainstem of young postnatal day 9 rats was reconstructed from serial ultrathin sections. In the apposition zone between the calyx and the principal neuron two types of membrane specializations were identified: synaptic contacts (SCs) with active zones (AZs) and their associated postsynaptic densities (PSDs) constituted approximately 35% (n = 554) of the specializations; the remaining 65% (n = 1010) were puncta adherentia (PA). Synaptic contacts comprised approximately 5% of the apposition area of presynaptic and postsynaptic membranes. A SC had an average area of 0.100 microm(2), and the nearest neighbors were separated, on average, by 0.59 microm. Approximately one-half of the synaptic vesicles in the calyx were clustered within a distance of 200 nm of the AZ membrane area, a cluster consisting of approximately 60 synaptic vesicles (n = 52 SCs). Approximately two synaptic vesicles per SC were "anatomically docked." Comparing the geometry of the synaptic structure with its previously studied functional properties, we find that during a single presynaptic action potential (AP) (1) approximately 35% of the AZs release a transmitter quantum, (2) the number of SCs and anatomically docked vesicles is comparable with the low estimates of the readily releasable pool (RRP) of quanta, and (3) the broad distribution of PSD areas [coefficient of variation (CV) = 0.9] is likely to contribute to the large variability of miniature EPSC peaks. The geometry of the reconstructed synapse suggests that each of the hundreds of SCs is likely to contribute independently to the size and rising phase of the EPSC during a single AP.

Saulite, L., et al. (2017). "Nano-engineered skin mesenchymal stem cells: potential vehicles for tumour-targeted quantum-dot delivery." Beilstein J Nanotechnol **8**: 1218-1230.

Nanotechnology-based drug design offers new possibilities for the use of nanoparticles in imaging and targeted therapy of tumours. Due to their tumour-homing ability, nano-engineered mesenchymal stem cells (MSCs) could be utilized as vectors to deliver diagnostic and therapeutic nanoparticles into a tumour. In the present study, uptake and functional effects of carboxyl-coated quantum dots QD655 were studied in human skin MSCs. The effect of QD on MSCs was examined using a cell viability assay, Ki67 expression analysis, and tri-lineage differentiation assay. The optimal conditions for QD uptake in MSCs were determined using flow cytometry. The QD uptake route in MSCs was examined via fluorescence imaging using endocytosis inhibitors for the micropinocytosis, phagocytosis, lipid-raft, clathrin- and caveolin-dependent endocytosis pathways. These data showed that QDs were efficiently accumulated in the cytoplasm of MSCs after incubation for 6 h. The main uptake route of QDs in skin MSCs was clathrin-mediated endocytosis. QDs were mainly localized in early endosomes after 6 h as well as in late endosomes and lysosomes after 24 h. QDs in concentrations ranging from 0.5 to 64 nM had no effect on cell viability and proliferation. The expression of MSC markers, CD73 and CD90, and hematopoietic markers, CD34 and CD45, as well as the ability to differentiate into adipocytes, chondrocytes, and osteocytes, were not altered in the presence of QDs. We observed a decrease in the QD signal from labelled MSCs over time that could partly reflect QD excretion. Altogether, these data suggest that QD-labelled MSCs could be used for targeted drug delivery studies.

Saulite, L., et al. (2018). "Nanoparticle delivery to metastatic breast cancer cells by nanoengineered mesenchymal stem cells." Beilstein J Nanotechnol **9**: 321-332.

We created a 3D cell co-culture model by combining nanoengineered mesenchymal stem cells (MSCs) with the metastatic breast cancer cell line MDA-MD-231 and primary breast cancer cell line MCF7 to explore the transfer of quantum dots (QDs) to cancer cells. First, the optimal conditions for high-content QD loading in MSCs were established. Then, QD uptake in breast cancer cells was assessed after 24 h in a 3D co-culture with nanoengineered MSCs. We found that incubation of MSCs with QDs in a serum-free medium provided the best accumulation results. It was found that 24 h post-labelling QDs were eliminated from MSCs. Our results demonstrate that breast cancer cells efficiently uptake QDs that are released from nanoengineered MSCs in a 3D co-culture. Moreover, the uptake is considerably enhanced in metastatic MDA-MB-231 cells compared with MCF7 primary breast cancer cells. Our findings suggest that nanoengineered MSCs could serve as a vehicle for targeted drug delivery to metastatic cancer.

Savelli, S., et al. (2018). "Pooled human serum: A new culture supplement for bioreactor-based cell therapies. Preliminary results." Cytotherapy **20**(4): 556-563.

BACKGROUND: Bone Marrow MSCs are an appealing source for several cell-based therapies. Many bioreactors, as the Quantum Cell Expansion System, have been developed to generate a large number of MSCs under Good Manufacturing Practice conditions by using Human Platelet Lysate (HPL). Previously we isolated in the human bone marrow a novel cell population, named Mesodermal Progenitor Cells (MPCs), which we identified as precursors of MSCs. MPCs could represent an important cell source for regenerative medicine applications. As HPL gives rise to a homogeneus MSC population, limiting the harvesting of other cell types, in this study we investigated the efficacy of pooled human AB serum (ABS) to provide clinically relevant numbers of both MSCs and MPCs for regenerative medicine applications by using the Quantum System. METHODS: Bone marrow aspirates were obtained from healthy adult individuals undergoing routine total hip replacement surgery and used to generate primary cultures in the bioreactor. HPL and ABS were tested as supplements to culture medium. Morphological observations, cytofluorimetric analysis, lactate and glucose level assessment were performed. RESULTS: ABS gave rise to both heterogeneous MSC and MPC population. About 95% of cells cultured in HPL showed a fibroblast-like morphology and typical mesenchymal surface markers, but MPCs were scarcely represented. DISCUSSION: The use of ABS appeared to sustain a large scale MSC production, as well as the recovery of a subset of MPCs, and resulted a suitable alternative to HPL in the cell generation based on the Quantum System.

Schiesser, S., et al. (2013). "Deamination, oxidation, and C-C bond cleavage reactivity of 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxycytosine." J Am Chem Soc **135**(39): 14593-14599.

Three new cytosine derived DNA modifications, 5-hydroxymethyl-2'-deoxycytidine (hmdC), 5-formyl-2'-deoxycytidine (fdC) and 5-carboxy-2'-deoxycytidine (cadC) were recently discovered in mammalian DNA, particularly in stem cell DNA. Their function is currently not clear, but it is assumed that in stem cells they might be intermediates of an active demethylation process. This process may involve base excision repair, C-C bond cleaving reactions or deamination of hmdC to 5-hydroxymethyl-2'-deoxyuridine (hmdU). Here we report chemical studies that enlighten the chemical reactivity of the new cytosine nucleobases. We investigated their sensitivity toward oxidation and deamination and we studied the C-C bond cleaving reactivity of hmdC, fdC, and cadC in the absence and presence of thiols as biologically relevant (organo)catalysts. We show that hmdC is in comparison to mdC rapidly oxidized to fdC already in the presence of air. In contrast, deamination reactions were found to occur only to a minor extent. The C-C bond cleavage reactions require the presence of high concentration of thiols and are acid catalyzed. While hmdC dehydroxymethylates very slowly, fdC and especially cadC react considerably faster to dC. Thiols are active site residues in many DNA modifiying enzymes indicating that such enzymes could play a role in an alternative active DNA demethylation mechanism via deformylation of fdC or decarboxylation of cadC. Quantum-chemical calculations support the catalytic influence of a thiol on the C-C bond cleavage.

Schmuck, E. G., et al. (2016). "Biodistribution and Clearance of Human Mesenchymal Stem Cells by Quantitative Three-Dimensional Cryo-Imaging After Intravenous Infusion in a Rat Lung Injury Model." Stem Cells Transl Med **5**(12): 1668-1675.

: Cell tracking is a critical component of the safety and efficacy evaluation of therapeutic cell products. To date, cell-tracking modalities have been hampered by poor resolution, low sensitivity, and inability to track cells beyond the shortterm. Three-dimensional (3D) cryo-imaging coregisters fluorescent and bright-field microcopy images and allows for single-cell quantification within a 3D organ volume. We hypothesized that 3D cryo-imaging could be used to

measure cell biodistribution and clearance after intravenous infusion in a rat lung injury model compared with normal rats. A bleomycin lung injury model was established in Sprague-Dawley rats (n = 12). Human mesenchymal stem cells (hMSCs) labeled with QTracker655 were infused via jugular vein. After 2, 4, or 8 days, a second dose of hMSCs labeled with QTracker605 was infused, and animals were euthanized after 60, 120, or 240 minutes. Lungs, liver, spleen, heart, kidney, testis, and intestine were cryopreserved, followed by 3D cryo-imaging of each organ. At 60 minutes, 82% +/- 9.7% of cells were detected; detection decreased to 60% +/- 17% and 66% +/- 22% at 120 and 240 minutes, respectively. At day 2, 0.06% of cells were detected, and this level remained constant at days 4 and 8 postinfusion. At 60, 120, and 240 minutes, 99.7% of detected cells were found in the liver, lungs, and spleen, with cells primarily retained in the liver. This is the first study using 3D cryo-imaging to track hMSCs in a rat lung injury model. hMSCs were retained primarily in the liver, with fewer detected in lungs and spleen. SIGNIFICANCE: Effective bench-to-bedside clinical translation of cellular therapies requires careful understanding of cell fate through tracking. Tracking cells is important to measure cell retention so that delivery methods and cell dose can be optimized and so that biodistribution and clearance can be defined to better understand potential off-target toxicity and redosing strategies. This article demonstrates, for the first time, the use of three-dimensional cryo-imaging for single-cell quantitative tracking of intravenous infused clinical-grade mesenchymal stem cells in a clinically relevant model of lung injury. The important information learned in this study will help guide future clinical and translational stem cell therapies for lung injuries.

Schormann, W., et al. (2008). "Tracking of human cells in mice." Histochem Cell Biol **130**(2): 329-338.

Tracking and tracing of transplanted cells in mice is required in many fields of research. Examples are transplantation of stem cells into organs of mice to study their differentiation capacity and injection of tumor cells to examine metastatic behavior. In the present study we tested the lipid dye CM-DiI and red fluorescent nanoparticles Qdot655 for their applicability in tagging and tracing of human cells in mice. Labeling of different cell types, including MCF-7 human breast cancer cells, human cord blood derived cells, human NeoHep cells and human hepatopancreatic precursor cells, is technically easy and did not compromise further cell culture. After transplantation of CM-DiI or Qdot655 marked cells, red fluorescent structures could be detected already in unprocessed paraffin slices of the studied organs, namely liver, lung, pancreas, kidney, spleen and bone marrow. Next, we examined whether the red fluorescent structures represent the transplanted human cells. For this purpose, we established an in situ hybridization (ISH) technique that allows clear-cut differentiation between human and murine nuclei, based on simultaneous hybridization with human alu and mouse major satellite (mms) probes. We observed a high degree of coincidence between CM-DiI-marked cells and alu positive nuclei. However, also some mms positive cells contained CM-DiI, suggesting phagocytosis of the transplanted CM-DiI-marked cells. The degree of such CM-DiI-positive mouse cells depended on the cell type and route of administration. From a technical point of view it was important that CM-DiI-positive structures in paraffin slices remained fluorescent also after ISH. In contrast, Qdot655 positive structures faded during further staining procedures. In conclusion, marking of cells with CM-DiI or Qdot655 prior to transplantation facilitates recovery of human cells, since a high fraction of positive structures in the host's tissue originate from the transplanted cells. However, CM-DiI or Qdot655 positive staining of individual cells in transplanted tissues is not sufficient to prove their human origin. Additional procedures, such as ISH with alu-probes, are essential, when characterizing individual cells.

Sedlmaier, S. J., et al. (2011). "Unprecedented zeolite-like framework topology constructed from cages with 3-rings in a barium oxonitridophosphate." J Am Chem Soc **133**(31): 12069-12078.

A novel oxonitridophosphate, Ba(19)P(36)O(6+x)N(66-x)Cl(8+x) (x approximately 4.54), has been synthesized by heating a multicomponent reactant mixture consisting of phosphoryl triamide OP(NH(2))(3), thiophosphoryl triamide SP(NH(2))(3), BaS, and NH(4)Cl enclosed in an evacuated and sealed silica glass ampule up to 750 degrees C. Despite the presence of side phases, the crystal structure was elucidated ab initio from high-resolution synchrotron powder diffraction data (lambda = 39.998 pm) applying the charge flipping algorithm supported by independent symmetry information derived from electron diffraction (ED) and scanning transmission electron microscopy (STEM). The compound crystallizes in the cubic space group Fm 3c (no. 226) with a = 2685.41(3) pm and Z = 8. As confirmed by Rietveld refinement, the structure comprises all-side vertex sharing P(O,N)(4) tetrahedra forming slightly distorted 3(8)4(6)8(12) cages representing a novel composite building unit (CBU). Interlinked through their 4-rings and additional 3-rings, the cages build up a 3D network with a framework density FD = 14.87 T/1000 A(3) and a 3D 8-ring channel system. Ba(2+) and Cl(-) as extra-framework ions are located within the cages and channels of the framework. The structural model is corroborated by (31)P double-

quantum (DQ) /single-quantum (SQ) and triple-quantum (TQ) /single-quantum (SQ) 2D correlation MAS NMR spectroscopy. According to (31)P{(1)H} C-REDOR NMR measurements, the H content is less than one H atom per unit cell.

Segura, M. V. and M. J. Quiles (2015). "Involvement of chlororespiration in chilling stress in the tropical species Spathiphyllum wallisii." Plant Cell Environ **38**(3): 525-533.

Spathiphyllum wallisii plants were used to study the effect of chilling stress under high illumination on photosynthesis and chlororespiration. Leaves showed different responses that depended on root temperature. When stem, but not root, was chilled, photosystem II (PSII) was strongly photoinhibited. However, when the whole plant was chilled, the maximal quantum yield of PSII decreased only slightly below the normal values and cyclic electron transport was stimulated. Changes were also observed in the chlororespiration enzymes and PGR5. In whole plants chilled under high illumination, the amounts of NADH dehydrogenase (NDH) complex and plastid terminal oxidase (PTOX) remained similar to control and increased when only stem was chilled. In contrast, the amount of PGR5 polypeptide was higher in plants when both root and stem were chilled than in plants in which only stem was chilled. The results indicated that the contribution of chlororespiration to regulating photosynthetic electron flow is not relevant when the whole plant is chilled under high light, and that another pathway, such as cyclic electron flow involving PGR5 polypeptide, may be more important. However, when PSII activity is strongly photoinhibited in plants in which only stem is chilled, chlororespiration, together with other routes of electron input to the electron transfer chain, is probably essential.

Seleverstov, O., et al. (2006). "Quantum dots for human mesenchymal stem cells labeling. A size-dependent autophagy activation." Nano Lett **6**(12): 2826-2832.

Lately certain cytotoxicity of quantum dots (QDs) and some deleterious effects of labeling procedure on stem cells differentiation abilities were shown. In the present study we compared cytotoxicity and intracellular processing of two different-sized protein-conjugated QDs after labeling of the human mesenchymal stem cells (hMSC). An asymmetrical intracellular uptake of red (605 nm) and green (525 nm) quantum dots was observed. We describe for the first time a size-dependent activation of autophagy, caused by nanoparticles.

Sella, S., et al. (2018). "In-vitro analysis of Quantum Molecular Resonance effects on human mesenchymal stromal cells." PLoS One **13**(1): e0190082.

Electromagnetic fields play an essential role in cellular functions interfering with cellular pathways and tissue physiology. In this context, Quantum Molecular Resonance (QMR) produces waves with a specific form at high-frequencies (4-64 MHz) and low intensity through electric fields. We evaluated the effects of QMR stimulation on bone marrow derived mesenchymal stromal cells (MSC). MSC were treated with QMR for 10 minutes for 4 consecutive days for 2 weeks at different nominal powers. Cell morphology, phenotype, multilineage differentiation, viability and proliferation were investigated. QMR effects were further investigated by cDNA microarray validated by real-time PCR. After 1 and 2 weeks of QMR treatment morphology, phenotype and multilineage differentiation were maintained and no alteration of cellular viability and proliferation were observed between treated MSC samples and controls. cDNA microarray analysis evidenced more transcriptional changes on cells treated at 40 nominal power than 80 ones. The main enrichment lists belonged to development processes, regulation of phosphorylation, regulation of cellular pathways including metabolism, kinase activity and cellular organization. Real-time PCR confirmed significant increased expression of MMP1, PLAT and ARHGAP22 genes while A2M gene showed decreased expression in treated cells compared to controls. Interestingly, differentially regulated MMP1, PLAT and A2M genes are involved in the extracellular matrix (ECM) remodelling through the fibrinolytic system that is also implicated in embryogenesis, wound healing and angiogenesis. In our model QMR-treated MSC maintained unaltered cell phenotype, viability, proliferation and the ability to differentiate into bone, cartilage and adipose tissue. Microarray analysis may suggest an involvement of QMR treatment in angiogenesis and in tissue regeneration probably through ECM remodelling.

Serke, S., et al. (1998). "Quantitative fluorescence flow cytometry: a comparison of the three techniques for direct and indirect immunofluorescence." Cytometry **33**(2): 179-187.

Three types of microbead calibrators available for quantitative fluorescence flow cytometry have been studied in parallel using a variety of monoclonal antibodies (MoAbs). The QIFI kit is designed for indirect immunofluorescence (IF), and both the Quantum Simply Cellular (QSC) assay and the Quanti-BRITE assay are designed for direct IF. Because of the different nature of the respective ligands, epitopes on cells versus F'ab-portions on QSC beads, large differences in titration curves for a large number of CD MoAbs were noted between QSC beads and cells. Use of the QSC assay and fluorescein isothiocyanate (FITC) and phycoerythrin (PE) conjugates of the same CD

reagent revealed substantially different numbers of cellular binding sites. Numbers of cellular binding sites as determined by direct IF using the Quanti-BRITE assay and by indirect IF using the QIFI kit were similar. We also found that erythrocyte (RBC)-lysing reagents cause varying and sometimes substantial reduction in the fluorescence intensity (FI) of cells stained directly with CD34 MoAb conjugates, but the RBC-lysing reagents had no effect on the FI of cells stained indirectly with the same CD34 MoAbs. This report defines a number of variables critical for standardized quantitative flow cytometry. We conclude that the choice of calibrators, fluorochrome conjugates, staining methods, and modes of sample processing can effect the determination of cellular binding sites to MoAbs. Direct immunofluorescence using the Quanti-BRITE assay and indirect IF using the QIFI kit appear to yield comparable results for the standardized determination of numbers of cellular binding sites to MoAbs.

Shah, B., et al. (2006). "Labeling and imaging of human mesenchymal stem cells with quantum dot bioconjugates during proliferation and osteogenic differentiation in long term." Conf Proc IEEE Eng Med Biol Soc **2006**: 1470-1473.

Quantum dots (QDs) are semiconductor nanocrystals that serve as promising alternatives to organic dyes for cell labeling. Because of their unique spectral, physical and chemical properties, QDs are useful for concurrently monitoring several intercellular and intracellular interactions in live normal cells and cancer cells over periods ranging from less than a second to over several days (several divisions of cells). Here, peptide CGGGRGD is immobilized on CdSe-ZnS QDs coated with carboxyl groups by cross linking with amine groups. These conjugates are directed by the peptide to bind with selected integrins on the membrane of human Mesenchymal stem cells. Upon overnight incubation with optimal concentration, QDs effectively labeled all the cells. Here, we report long-term labeling of human bone-marrow-derived mesenchymal stem cells (hMSCs) with RGD-conjugated QDs during self replication and differentiation into osteogenic cell lineages.

Shah, B. S. and J. J. Mao (2011). "Labeling of mesenchymal stem cells with bioconjugated quantum dots." Methods Mol Biol **680**: 61-75.

Quantum dots (QDs) are semiconductor nanocrystals, and recently they have been shown as effective probes for cell labeling. Due to their unique spectral, physical, and chemical properties, QDs can concurrently tag multiple intercellular and intracellular components of live cells for time ranging from seconds to months. Different color QDs can label different cell components that can be visualized with fluorescent microscopy or in vivo. Here, we provide a detailed protocol for labeling postnatal and differentiated stem/progenitor cells with bioconjugated quantum dots. For example, peptide CGGGRGD is immobilized on CdSe-ZnS QDs with free carboxyl groups. These bioconjugates label selected integrins on cell membrane of human mesenchymal stem cells (hMSCs). QD concentration and incubation time to effectively label hMSCs is optimized. We discovered that bioconjugated QDs effectively label hMSCs not only during population doubling, but also during multi-lineage differentiation into osteoblasts, chondrocytes, and adipocytes. Undifferentiated and differentiated stem cells labeled with bioconjugated QDs can be readily imaged by fluorescent microscopy. Thus, quantum dots represent an effective cell labeling probe and an alternative to organic dyes and fluorescent proteins for cell labeling and cell tracking.

Shang, W., et al. (2014). "The uptake mechanism and biocompatibility of graphene quantum dots with human neural stem cells." Nanoscale **6**(11): 5799-5806.

Cellular imaging after transplantation may provide important information to determine the efficacy of stem cell therapy. We have reported that graphene quantum dots (GQDs) are a type of robust biological labeling agent for stem cells that demonstrate little cytotoxicity. In this study, we examined the interactions of GQDs on human neural stem cells (hNSCs) with the aim to investigate the uptake and biocompatibility of GQDs. We examined the mechanism of GQD uptake by hNSCs and investigated the effects of GQDs on the proliferation, metabolic activity, and differentiation potential of hNSCs. This information is critical to assess the suitability of GQDs for stem cell tracking. Our results indicated that GQDs were taken up into hNSCs in a concentration- and time-dependent manner via the endocytosis mechanism. Furthermore, no significant change was found in the viability, proliferation, metabolic activity, and differentiation potential of hNSCs after treatment with GQDs. Thus, these data open a promising avenue for labeling stem cells with GQDs and also offer a potential opportunity to develop GQDs for biomedical applications.

Sharma, A., et al. (2022). "Emerging Trends in Mesenchymal Stem Cells Applications for Cardiac Regenerative Therapy: Current Status and Advances." Stem Cell Rev Rep **18**(5): 1546-1602.

Irreversible myocardium infarction is one of the leading causes of cardiovascular disease (CVD) related death and its quantum is expected to grow in coming years. Pharmacological intervention has been at the forefront to ameliorate injury-related morbidity and mortality. However, its outcomes are highly skewed. As an alternative, stem cell-based tissue engineering/regenerative medicine has been explored

quite extensively to regenerate the damaged myocardium. The therapeutic modality that has been most widely studied both preclinically and clinically is based on adult multipotent mesenchymal stem cells (MSC) delivered to the injured heart. However, there is debate over the mechanistic therapeutic role of MSC in generating functional beating cardiomyocytes. This review intends to emphasize the role and use of MSC in cardiac regenerative therapy (CRT). We have elucidated in detail, the various aspects related to the history and progress of MSC use in cardiac tissue engineering and its multiple strategies to drive cardiomyogenesis. We have further discussed with a focus on the various therapeutic mechanism uncovered in recent times that has a significant role in ameliorating heart-related problems. We reviewed recent and advanced technologies using MSC to develop/create tissue construct for use in cardiac regenerative therapy. Finally, we have provided the latest update on the usage of MSC in clinical trials and discussed the outcome of such studies in realizing the full potential of MSC use in clinical management of cardiac injury as a cellular therapy module.

Shi, Q., et al. (2008). "Investigation of the mechanism of the cell wall DD-carboxypeptidase reaction of penicillin-binding protein 5 of Escherichia coli by quantum mechanics/molecular mechanics calculations." J Am Chem Soc **130**(29): 9293-9303.

Penicillin-binding protein 5 (PBP 5) of Escherichia coli hydrolyzes the terminal D-Ala-D-Ala peptide bond of the stem peptides of the cell wall peptidoglycan. The mechanism of PBP 5 catalysis of amide bond hydrolysis is initial acylation of an active site serine by the peptide substrate, followed by hydrolytic deacylation of this acyl-enzyme intermediate to complete the turnover. The microscopic events of both the acylation and deacylation half-reactions have not been studied. This absence is addressed here by the use of explicit-solvent molecular dynamics simulations and ONIOM quantum mechanics/molecular mechanics (QM/MM) calculations. The potential-energy surface for the acylation reaction, based on MP2/6-31+G(d) calculations, reveals that Lys47 acts as the general base for proton abstraction from Ser44 in the serine acylation step. A discrete potential-energy minimum for the tetrahedral species is not found. The absence of such a minimum implies a conformational change in the transition state, concomitant with serine addition to the amide carbonyl, so as to enable the nitrogen atom of the scissile bond to accept the proton that is necessary for progression to the acyl-enzyme intermediate. Molecular dynamics simulations indicate that transiently protonated Lys47 is the proton donor in tetrahedral intermediate collapse to the acyl-enzyme species. Two pathways for this proton transfer are observed. One is the direct migration of a proton from Lys47. The second pathway is proton transfer via an intermediary water molecule. Although the energy barriers for the two pathways are similar, more conformers sample the latter pathway. The same water molecule that mediates the Lys47 proton transfer to the nitrogen of the departing D-Ala is well positioned, with respect to the Lys47 amine, to act as the hydrolytic water in the deacylation step. Deacylation occurs with the formation of a tetrahedral intermediate over a 24 kcal x mol(-1) barrier. This barrier is approximately 2 kcal x mol(-1) greater than the barrier (22 kcal x mol(-1)) for the formation of the tetrahedral species in acylation. The potential-energy surface for the collapse of the deacylation tetrahedral species gives a 24 kcal x mol(-1) higher energy species for the product, signifying that the complex would readily reorganize and pave the way for the expulsion of the product of the reaction from the active site and the regeneration of the catalyst. These computational data dovetail with the knowledge on the reaction from experimental approaches.

Shi, Q., et al. (2011). "A computational evaluation of the mechanism of penicillin-binding protein-catalyzed cross-linking of the bacterial cell wall." J Am Chem Soc **133**(14): 5274-5283.

Penicillin-binding protein 1b (PBP 1b) of the gram-positive bacterium Streptococcus pneumoniae catalyzes the cross-linking of adjacent peptidoglycan strands, as a critical event in the biosynthesis of its cell wall. This enzyme is representative of the biosynthetic PBP structures of the beta-lactam-recognizing enzyme superfamily and is the target of the beta-lactam antibiotics. In the cross-linking reaction, the amide between the -D-Ala-D-Ala dipeptide at the terminus of a peptide stem acts as an acyl donor toward the epsilon-amino group of a lysine found on an adjacent stem. The mechanism of this transpeptidation was evaluated using explicit-solvent molecular dynamics simulations and ONIOM quantum mechanics/molecular mechanics calculations. Sequential acyl transfer occurs to, and then from, the active site serine. The resulting cross-link is predicted to have a cis-amide configuration. The ensuing and energetically favorable cis- to trans-amide isomerization, within the active site, may represent the key event driving product release to complete enzymatic turnover.

Shi, X., et al. (2021). "Semiconducting polymer nano-radiopharmaceutical for combined radio-photothermal therapy of pancreatic tumor." J Nanobiotechnology **19**(1): 337.

BACKGROUND: Pancreatic ductal adenocarcinoma (PDAC) is a devastatingly malignant tumor with a high mortality. However, current strategies to treat PDAC generally have low efficacy and high

side-effects, therefore, effective treatment against PDAC remains an urgent need. RESULTS: We report a semiconducting polymer nano-radiopharmaceutical with intrinsic photothermal capability and labeling with therapeutic radioisotope (177)Lu ((177)Lu-SPN-GIP) for combined radio- and photothermal therapy of pancreatic tumor. (177)Lu-SPN-GIP endowed good stability at physiological conditions, high cell uptake, and long retention time in tumor site. By virtue of combined radiotherapy (RT) and photothermal therapy (PTT), (177)Lu-SPN-GIP exhibited enhanced therapeutic capability to kill cancer cells and xenograft tumor in living mice compared with RT or PTT alone. More importantly, (177)Lu-SPN-GIP could suppress the growth of the tumor stem cells and reverse epithelial mesenchymal transition (EMT), which may greatly reduce the occurrence of metastasis. CONCLUSION: Such strategy we developed could improve therapeutic outcomes over traditional RT as it is able to ablate tumor with relatively lower doses of radiopharmaceuticals to reduce its side effects.

Shim, Y. and J. M. Song (2015). "Quantum dot nanoprobe-based high-content monitoring of notch pathway inhibition of breast cancer stem cell by capsaicin." Mol Cell Probes **29**(6): 376-381.

Breast cancer is the major cause of cancer death for women worldwide. Breast cancer patients are treated with chemotherapy and radiotherapy. Although chemotherapy and radiotherapy are applied, some cancer cells still survive. These cells, called cancer stem cell (CSC), exhibit special capabilities, such as drug and radio resistance. The remaining CSC can trigger cancer recurrence. Thus, it is critical to find an effective way to target CSC. Capsaicin has been reported to affect anticancer activity in many cancers. It also has been shown that capsaicin induces apoptosis in the MCF-7 breast cancer cell line. In this study, we demonstrate that capsaicin causes dose-dependent growth disruption in breast CSC and inhibits translocation of notch intracellular membrane domain (NICD) into the nucleus. MCF-7 cells were treated with capsaicin at various concentrations (5 muM, 10 muM, and 20 muM) for 24 h. After capsaicin treatment, it was found that the number of breast CSC (%) decreased as the treatment concentration of capsaicin increased. This result was also confirmed with FACS. NICD translocation to the nucleus and apoptotic cell death of breast CSC were concurrently observed at the single breast CSC level using highly sensitive quantum dot (Qdot)-antibody nanoprobes. The control breast CSCs without the capsaicin treatment were able to translocate NICD into the nucleus. On the other hand, translocation of NICD into the nucleus was not observed in capsaicin-treated cells. In addition, apoptotic cell death was caused when the breast CSC were treated with capsaicin at more than 10 muM. Although many studies have shown that capsaicin produces anticancer activity in cancer cell lines, the present result is the first report to demonstrate that capsaicin is capable of causing breast CSC apoptotic cell death via inhibiting its notch signaling pathway.

Shim, Y. and J. M. Song (2015). "Spectral overlap-free quantum dot-based determination of benzo[a]pyrene-induced cancer stem cells by concurrent monitoring of CD44, CD24 and aldehyde dehydrogenase 1." Chem Commun (Camb) **51**(11): 2118-2121.

In this study, it was found that breast cancer stem cells (CSCs) are formed from MCF-7 cells by benzo[a]pyrene (BP)-induced mutation. The breast CSCs were detected through simultaneous monitoring of CD44, CD24 and aldehyde dehydrogenase 1 (ALDH1) by hypermulticolor cellular imaging using an acousto-optical tunable filter (AOTF) and quantum dots (Q-dots).

Shin, W. J., et al. (2017). "Cell Surface Nano-modulation for Non-invasive in vivo Near-IR Stem Cell Monitoring." ChemMedChem **12**(1): 28-32.

A stem cell tracking system is in high demand for the determination of cell destinations and for the validation of cell therapeutic efficacy in regenerative transplantation. To date, near-infrared (NIR) imaging technology has received considerable attention in cell behavior monitoring, owing to its patient compatibility, easy accessibility and cost effectiveness. Conventionally, in vivo cell tracking has been visualized by direct in-cell staining with NIR, where it may be achieved by complicated genetic engineering. Such genetic amendment techniques have suffered from serious challenges, which can destroy a cell's metabolism and can accidentally incur unexpected carcinoma. Herein we demonstrate a novel cell nano-modulation method for noninvasive stem cell monitoring. It is simply achieved by conjugating stem cells with lipid-supported, NIR-tagged, polymeric nanoparticles. These engineered cells, which are designated as NIR-labeled light-emitting stem cells (LESCs), maintain their biochemical functionality (i.e., differentiation, quantum efficacy, etc.) even after conjugation. LESCs were used for in situ stem cell monitoring at inoculation sites. It is speculated that the LESC technique could provide a new preparative methodology for in vivo cell tracking in advanced diagnostic medicine, where cell behavior is a critical issue.

Si, R., et al. (2020). "Human mesenchymal stem cells encapsulated-coacervated photoluminescent nanodots layered bioactive chitosan/collagen hydrogel matrices to indorse cardiac healing after acute myocardial infarction." J Photochem Photobiol B **206**: 111789.

Acute Myocardial Infarction (MI) is one of the foremost causes of human death worldwide and it leads to mass death of cardiomyocytes, interchanges of unfavorable biological environment and affecting electrical communications by fibrosis scar formations, and specifically deficiency of blood supply to heart which leads to heart damage and heart failure. Recently, numerous appropriate strategies have been applied to base on solve these problems wound be provide prominent therapeutic potential to cardiac regeneration after acute MI. In the present study, a combined biopolymeric conductive hydrogel was fabricated with conductive ultra-small graphene quantum dots as a soft injectable hydrogel for cardiac regenerations. The resultant hydrogel was combined with human Mesenchymal stem cells (hMSCs) to improved angiogenesis in cardiovascular tissues and decreasing cardiomyocyte necrosis of hydrogel treated acute-infarcted region has been greatly associated with the development of cardiac functions in MI models. The prepared graphene quantum dots and hydrogel groups was physico-chemically analyzed and confirmed the suitability of the materials for cardiac regeneration applications. The in vitro analyzes of hydrogels with hMSCs have established that enhanced cell survival rate, increased expressions of pro-inflammatory factors, pro-angiogenic factors and early cardiogenic markers. The results of in vivo myocardial observations and electrocardiography data demonstrated a favorable outcome of ejection fraction, fibrosis area, vessel density with reduced infarction size, implying that significant development of heart regenerative function after MI. This novel strategy of injectable hydrogel with hMSCs could be appropriate for the effective treatment of cardiac therapies after acute MI.

Siftar, Z., et al. (2010). "External quality assessment in clinical cell analysis by flow cytometry. Why is it so important?" Coll Antropol **34**(1): 207-217.

Participation in external quality assessment is an integral part of laboratory work and mandatory when the results have a clinical application, which is one of the requirements of standard 15189 for accreditation of medical laboratories. Institute of Clinical Chemistry, the first laboratory accredited for clinical cell analysis by flow cytometry in Croatia, participated in UKNEQAS for Leukocyte Immunophenotyping in 3 schemes: "Immune Monitoring", "CD34 Stem Cell Enumeration" and "Leukaemia Immunophenotyping". For sample processing on EPICS XL flow cytometer, lyse/no wash preparation technique with ammonium chloride (NH4Cl) or ImmunoPrep lysing reagent was employed. In "Immune monitoring" programme CD45/sideward light scatter (SSC) proposed gating strategy was adopted for lymphocyte subsets, while modified ISHAGE protocol was used for CD34+ cell enumeration. Absolute count determination was performed on flow cytometer using FlowCount beads solution. In the period from the beginning of 2006 until the middle of 2009 a total number of 100 stabilized whole blood samples were processed. The relative and absolute enumeration results for lymphocyte subsets were within tolerable limits, in 97.1 and 97.1% of cases, and 95 and 90% of CD34+ cell enumeration, respectively. In immune monitoring CD45/SSC proposed gating strategy is the most frequent analysis used (> 85% participants) and ISHAGE protocol for CD34+ cell determination with continuous rise from 76 to 83%. A number of participants who accept beads method for absolute count enumeration on flow cytometer get greater, 69 to 86%, while FlowCount was the second of bead-based techniques used (25 and 35%). Sample treatment in lyse/no wash technique using NH4Cl lysing solution was dominant procedure used by more than 1/3 participants, although its home made solution has replaced slowly by commercial reagents. The unacceptable results, 6 of 244, were obtained for 20 most frequently determined cell antigens in "Leukaemia Immunophenotyping" samples screened for leukaemia/lymphoma. Processing results of all participants showed that the deviation from laboratory guidelines and the use of older methods for cell identification, quantification of cell counting on haematology analyser, or usage an antibody conjugated with fluorochrome lesser fluorescence quantum often lead to an unacceptable result, although is noticeable trend to accept new referrals and protocols to reduce the inter-laboratory differences.

Singer, D. D., et al. (2013). "The effects of rat mesenchymal stem cells on injury progression in a rat model." Acad Emerg Med **20**(4): 398-402.

OBJECTIVES: Burns are common injuries that can result in significant scarring, leading to poor function and disfigurement. Unlike mechanical injuries, burns often progress both in depth and in size over the first few days after injury, possibly due to inflammation and oxidative stress. A major gap in the field of burns is the lack of an effective therapy that reduces burn injury progression. Because stem cells have been shown to improve healing in several injury models, the authors hypothesized that species-specific mesenchymal stem cells (MSCs) would reduce injury progression in a rat comb-burn model. METHODS: Using a brass comb preheated to 100 degrees C, the authors created four rectangular burns, separated by three unburned interspaces on both sides of the backs of male Sprague-Dawley rats. The interspaces represented the ischemic zones surrounding the central necrotic core. In an attempt to reduce burn injury progression, 20 rats were randomized to tail vein injections of 1 mL of rat-specific MSCs, 10(6) cells/mL (n = 10), or normal saline (n = 10), 60 minutes after injury. RESULTS: While the

authors were unable to identify any quantum dot (Q-dot)-labeled MSCs in the injured skin, at 7 days the mean percentage of the unburned interspaces that became necrotic in the MSC group was significantly less than in the control group (80% vs. 100%, $p < 0.0001$). CONCLUSIONS: Intravenous injection of rat MSCs reduced burn injury progression in a rat comb-burn model.

Sinha, S., et al. (2014). "Triazole-based Zn(2)(+)-specific molecular marker for fluorescence bioimaging." Anal Chim Acta **822**: 60-68.

Fluorescence bioimaging potential, both in vitro and in vivo, of a yellow emissive triazole-based molecular marker has been investigated and demonstrated. Three different kinds of cells, viz Bacillus thuringiensis, Candida albicans, and Techoma stans pollen grains were used to investigate the intracellular zinc imaging potential of 1 (in vitro studies). Fluorescence imaging of translocation of zinc through the stem of small herb, Peperomia pellucida, having transparent stem proved in vivo bioimaging capability of 1. This approach will enable in screening cell permeability and biostability of a newly developed probe. Similarly, the current method for detection and localization of zinc in Gram seed sprouts could be an easy and potential alternative of the existing analytical methods to investigate the efficiency of various strategies applied for increasing zinc-content in cereal crops. The probe-zinc ensemble has efficiently been applied for detecting phosphate-based biomolecules.

Sjostrom, T., et al. (2013). "2D and 3D nanopatterning of titanium for enhancing osteoinduction of stem cells at implant surfaces." Adv Healthc Mater **2**(9): 1285-1293.

The potential for the use of well-defined nanopatterns to control stem cell behaviour on surfaces has been well documented on polymeric substrates. In terms of translation to orthopaedic applications, there is a need to develop nanopatterning techniques for clinically relevant surfaces, such as the load-bearing material titanium (Ti). In this work, a novel nanopatterning method for Ti surfaces is demonstrated, using anodisation in combination with PS-b-P4VP block copolymer templates. The block copolymer templates allows for fabrication of titania nanodot patterns with precisely controlled dimensions and positioning which means that this technique can be used as a lithography-like patterning method of bulk Ti surfaces on both flat 2D and complex shaped 3D surfaces. In vitro studies demonstrate that precise tuning of the height of titania nanodot patterns can modulate the osteogenic differentiation of mesenchymal stem cells. Cells on both the 8 nm and 15 nm patterned surfaces showed a trend towards a greater number of the large, super-mature osteogenic focal adhesions than on the control polished Ti surface, but the osteogenic effect was more pronounced on the 15 nm substrate. Cells on this surface had the longest adhesions of all and produced larger osteocalcin deposits. The results suggest that nanopatterning of Ti using the technique of anodisation through a block copolymer template could provide a novel way to enhance osteoinductivity on Ti surfaces.

Slotkin, J. R., et al. (2007). "In vivo quantum dot labeling of mammalian stem and progenitor cells." Dev Dyn **236**(12): 3393-3401.

Fluorescent semiconductor nanocrystal quantum dots (QDs) are a class of multifunctional inorganic fluorophores that hold great promise for clinical applications and biomedical research. Because no methods currently exist for directed QD-labeling of mammalian cells in the nervous system in vivo, we developed novel in utero electroporation and ultrasound-guided in vivo delivery techniques to efficiently and directly label neural stem and progenitor cells (NSPCs) of the developing mammalian central nervous system with QDs. Our initial safety and proof of concept studies of one and two-cell QD-labeled mouse embryos reveal that QDs are compatible with early mammalian embryonic development. Our in vivo experiments further show that in utero labeled NSPCs continue to develop in an apparent normal manner. These studies reveal that QDs can be effectively used to label mammalian NSPCs in vivo and will be useful for studies of in vivo fate mapping, cellular migration, and NSPC differentiation during mammalian development.

Snyder, E. L., et al. (2009). "Identification of CD44v6(+)/CD24- breast carcinoma cells in primary human tumors by quantum dot-conjugated antibodies." Lab Invest **89**(8): 857-866.

Breast carcinoma cells with the CD44+/CD24(low) phenotype have been reported to exhibit 'cancer stem cell' (CSC) characteristics on the basis of their enhanced tumorigenicity and self-renewal potential in immunodeficient mice. We used immunohistochemistry to study the expression of these proteins in whole tissue sections of human breast carcinoma. We found that the fraction of CD44v6+ cells is higher in estrogen receptor-positive carcinomas after neoadjuvant chemotherapy. We also performed double immunohistochemistry for CD44v6 and for the proliferation marker Ki67. We found that the relative number of quiescent carcinoma cells is higher in the CD44v6+ population than in the CD44v6- population in specific carcinoma subtypes. We then used quantum dots and spectral imaging to increase the number of antigens that could be visualized in a single tissue section. We found that anti-CD44v6 and CD24 antibodies that were directly conjugated to quantum dots retained their ability to recognize antigen in formalin-

fixed, paraffin-embedded tissue sections. We then performed triple staining for CD44v6, CD24 and Ki67 to assess the proliferation of each sub-population of breast carcinoma cells. Our results identify differences between CD44v6-positive and CD44v6-negative breast carcinoma cells in vivo and provide a proof of principle that quantum dot-conjugated antibodies can be used to study specific sub-populations of cancer cells defined by multiple markers in a single tissue section.

Soenen, S. J., et al. (2014). "Cytotoxicity of cadmium-free quantum dots and their use in cell bioimaging." Chem Res Toxicol **27**(6): 1050-1059.

The use of quantum dots (QDots) as bright and photostable probes for long-term fluorescence imaging is gaining more interest. Thus far, (pre)clinical use of QDots remains limited, which is primarily caused by the potential toxicity of QDots. Most QDots consist of Cd2+ ions, which are known to cause high levels of toxicity. In order to overcome this problem, several strategies have been tested, such as the generation of cadmium-free QDots. In the present study, two types of cadmium-free QDots, composed of ZnSe/ZnS (QDotZnSe) and InP/ZnS (QDotInP), were studied with respect to their cytotoxicity and cellular uptake in a variety of cell types. A multiparametric cytotoxicity approach is used, where the QDots are studied with respect to cell viability, oxidative stress, cell morphology, stem cell differentiation, and neurite outgrowth. The data reveal slight differences in uptake levels for both types of QDots (maximal for QDotZnSe), but clear differences in cytotoxicity and cell functionality effects exist, with highest toxicity for QDotZnSe. Differences between cell types and between both types of QDots can be explained by the intrinsic sensitivity of certain cell types and chemical composition of the QDots. At concentrations at which no toxic effects can be observed, the functionality of the QDots for fluorescence cell visualization is evaluated, revealing that the higher brightness of QDotZnSe overcomes most of the toxicity issues compared to that of QDotInP. Comparing the results obtained with common Cd2+-containing QDots tested under identical conditions, the importance of particle functionality is demonstrated, revealing that cadmium-free QDots tested in this study are not significantly better than Cd2+-containing QDots for long-term cell imaging and that more work needs to be performed in optimizing the brightness and surface chemistry of cadmium-free QDots for them to replace currently used Cd2+-containing QDots.

Soenen, S. J., et al. (2014). "The performance of gradient alloy quantum dots in cell labeling." Biomaterials **35**(26): 7249-7258.

The interest in using quantum dots (QDots) as highly fluorescent and photostable nanoparticles in biomedicine is vastly increasing. One major hurdle that slows down the (pre)clinical translation of QDots is their potential toxicity. Several strategies have been employed to optimize common core-shell QDots, such as the use of gradient alloy (GA)-QDots. These particles no longer have a size-dependent emission wavelength, but the emission rather depends on the chemical composition of the gradient layer. Therefore, particles of identical sizes but with emission maxima spanning the entire visible spectrum can be generated. In the present study, two types of GA-QDots are studied with respect to their cytotoxicity and cellular uptake. A multiparametric cytotoxicity approach reveals concentration-dependent effects on cell viability, oxidative stress, cell morphology and cell functionality (stem cell differentiation and neurite outgrowth), where the particles are very robust against environmentally-induced breakdown. Non-toxic concentrations are defined and compared to common core-shell QDots analyzed under identical conditions. Additionally, this value is translated into a functional value by analyzing the potential of the particles for cell visualization. Interestingly, these particles result in clear endosomal localization, where different particles result in identical intracellular distributions. This is in contrast with CdTe QDots with the same surface coating, which resulted in clearly distinct intracellular distributions as a result of differences in nanoparticle diameter. The GA-QDots are therefore ideal platforms for cell labeling studies given their high brightness, low cytotoxicity and identical sizes, resulting in highly similar intracellular particle distributions which offer a lot of potential for optimizing drug delivery strategies.

Solanki, A., et al. (2008). "Nanotechnology for regenerative medicine: nanomaterials for stem cell imaging." Nanomedicine (Lond) **3**(4): 567-578.

Although stem cells hold great potential for the treatment of many injuries and degenerative diseases, several obstacles must be overcome before their therapeutic application can be realized. These include the development of advanced techniques to understand and control functions of microenvironmental signals and novel methods to track and guide transplanted stem cells. The application of nanotechnology to stem cell biology would be able to address those challenges. This review details the current challenges in regenerative medicine, the current applications of nanoparticles in stem cell biology and further potential of nanotechnology approaches towards regenerative medicine, focusing mainly on magnetic nanoparticle- and quantum dot-based applications in stem cell research.

Sonali, et al. (2018). "Nanotheranostics: Emerging Strategies for Early Diagnosis and Therapy of Brain Cancer." Nanotheranostics **2**(1): 70-86.

Nanotheranostics have demonstrated the development of advanced platforms that can diagnose brain cancer at early stages, initiate first-line therapy, monitor it, and if needed, rapidly start subsequent treatments. In brain nanotheranostics, therapeutic as well as diagnostic entities are loaded in a single nanoplatform, which can be further developed as a clinical formulation for targeting various modes of brain cancer. In the present review, we concerned about theranostic nanosystems established till now in the research field. These include gold nanoparticles, carbon nanotubes, magnetic nanoparticles, mesoporous silica nanoparticles, quantum dots, polymeric nanoparticles, upconversion nanoparticles, polymeric micelles, solid lipid nanoparticles and dendrimers for the advanced detection and treatment of brain cancer with advanced features. Also, we included the role of three-dimensional models of the BBB and cancer stem cell concept for the advanced characterization of nanotheranostic systems for the unification of diagnosis and treatment of brain cancer. In future, brain nanotheranostics will be able to provide personalized treatment which can make brain cancer even remediable or at least treatable at the primary stages.

Song, J., et al. (2019). "Polyelectrolyte-Mediated Nontoxic AgGa xIn1- xS2 QDs/Low-Density Lipoprotein Nanoprobe for Selective 3D Fluorescence Imaging of Cancer Stem Cells." ACS Appl Mater Interfaces **11**(10): 9884-9892.

Cancer stem cells, which are a population of cancer cells sharing common properties with normal stem cells, have strong self-renewal ability and multi-lineage differentiation potential to trigger tumor proliferation, metastases, and recurrence. From this, targeted therapy for cancer stem cells may be one of the most promising strategies for comprehensive treatment of tumors in the future. We design a facile approach to establish the colon cancer stem cells-selective fluorescent probe based on the low-density lipoprotein (LDL) and the novel AgGa xIn(1- x)S2 quantum dots (AGIS QDs). The AGIS QDs with a high crystallinity are obtained for the first time via cation-exchange protocol of Ga(3+) to In(3+) starting from parent AgInS2 QDs. Photoluminescence peak of AGIS QDs can be turned from 502 to 719 nm by regulating the reaction conditions, with the highest quantum yield up to 37%. Subsequently, AGIS QDs-conjugated LDL nanocomposites (NCs) are fabricated, in which a cationic polyelectrolyte was used as a coupling reagent to guarantee the electrostatic self-assembly. The structural integrity and physicochemical properties of the LDL-QDs NCs are found to be maintained in vitro, and the NCs exhibit remarkable biocompatibility. The LDL-QDs can be selectively delivered into cancer stem cells that overexpress LDL receptor, and three-dimensional imaging of cancer stem cells is realized. The results of this study not only demonstrate the versatility of nature-derived lipoprotein nanoparticles, but also confirm the feasibility of electrostatic conjugation using cationic polyelectrolyte, allowing reseachers to design nanoarchitectures for targeted diagnosis and treatment of cancer.

Sonmez, M., et al. (2015). "SYNTHESIS AND APPLICATIONS OF Fe3O4/SiO2 CORE-SHELL MATERIALS." Curr Pharm Des **21**(37): 5324-5335.

Multifunctional nanoparticles based on magnetite/silica core-shell, consisting of iron oxides coated with silica matrix doped with fluorescent components such as organic dyes (fluorescein isothiocyanate - FITC, Rhodamine 6G) or quantum dots, have drawn remarkable attention in the last years. Due to the bi-functionality of these types of nanoparticles (simultaneously having magnetic and fluorescent properties), they are successfully used in highly efficient human stem cell labeling, magnetic carrier for photodynamic therapy, drug delivery, hyperthermia and other biomedical applications. Another application of core-shell-based nanoparticles, in which the silica is functionalized with aminosilanes, is for immobilization and separation of various biological entities such as proteins, antibodies, enzymes etc. as well as in environmental applications, as adsorbents for heavy metal ions. In vitro tests on human cancerous cells, such as A549 (human lung carcinoma), breast, human cervical cancer, THP-1 (human acute monocytic leukaemia) etc. , were conducted to assess the potential cytotoxic effects that may occur upon contact of nanoparticles with cancerous tissue. Results show that core-shell nanoparticles doped with cytostatics (cisplatin, doxorubicin, etc.), are easily adsorbed by affected tissue and in some cases lead to an inhibition of cell proliferation and induce cell death by apoptosis. The goal of this review is to summarize the advances in the field of core-shell materials, particularly those based on magnetite/silica with applicability in medicine and environmental protection. This paper briefly describes synthesis methods of silica-coated magnetite nanoparticles (Stober method and microemulsion), the method of encapsulating functional groups based on aminosilanes in silica shell, as well as applications in medicine of these types of simple or modified nanoparticles for cancer therapy, MRI, biomarker immobilization, drug delivery, biocatalysis etc., and in environmental applications (removal of heavy metal ions and catalysis).

Sporbert, A., et al. (2013). "Simple method for sub-diffraction resolution imaging of cellular structures on standard confocal microscopes by three-photon absorption of quantum dots." PLoS One **8**(5): e64023.

This study describes a simple technique that improves a recently developed 3D sub-diffraction imaging method based on three-photon absorption of commercially available quantum dots. The method combines imaging of biological samples via tri-exciton generation in quantum dots with deconvolution and spectral multiplexing, resulting in a novel approach for multi-color imaging of even thick biological samples at a 1.4 to 1.9-fold better spatial resolution. This approach is realized on a conventional confocal microscope equipped with standard continuous-wave lasers. We demonstrate the potential of multi-color tri-exciton imaging of quantum dots combined with deconvolution on viral vesicles in lentivirally transduced cells as well as intermediate filaments in three-dimensional clusters of mouse-derived neural stem cells (neurospheres) and dense microtubuli arrays in myotubes formed by stacks of differentiated C2C12 myoblasts.

Steponkiene, S., et al. (2011). "Quantum dots affect expression of CD133 surface antigen in melanoma cells." Int J Nanomedicine **6**: 2437-2444.

BACKGROUND: In novel treatment approaches, therapeutics should be designed to target cancer stem cells (CSCs). Quantum dots (QDs) are a promising new tool in fighting against cancer. However, little is known about accumulation and cytotoxicity of QDs in CSCs. METHODS: Accumulation and cytotoxicity of CdTe-MPA (mercaptopropionic acid) QDs in CSCs were assessed using flow cytometry and fluorescence-activated cell sorting techniques as well as a colorimetric cell viability assay. RESULTS: We investigated the expression of two cell surface-associated glycoproteins, CD44 and CD133, in four different cancer cell lines (glioblastoma, melanoma, pancreatic, and prostate adenocarcinoma). Only the melanoma cells were positive to both markers of CD44 and CD133, whereas the other cells were only CD44-positive. The QDs accumulated to a similar extent in all subpopulations of the melanoma cells. The phenotypical response after QD treatment was compared with the response after ionizing radiation treatment. The percentage of the CD44(high-)CD133(high) subpopulation decreased from 72% to 55%-58% for both treatments. The stem-like subpopulation CD44(high)CD133(low/-) increased from 26%-28% in the untreated melanoma cells to 36%-40% for both treatments. CONCLUSION: Treatment of melanoma cells with QDs results in an increase of stem-like cell subpopulations. The changes in phenotype distribution of the melanoma cells after the treatment with QDs are comparable with the changes after ionizing radiation.

Stern, L. D. (2013). "Alpha-1 antitrypsin deficiency, a corporate perspective." COPD **10 Suppl 1**: 54-56.

Best-in-class systems have evolved among alpha-1 antitrypsin (AAT) producers, specialty pharmacies and the Alpha-1 Foundation and AlphaNet patient groups. The Genetic Alliance, NORD and other independent parties have recognized the benefits regarding public policy, patient advocacy, medical research, medication adherence, patient identification and health outcomes. Driving the next quantum leap in disease state management for Alpha-1 patients will require strong leadership from both industry and patient groups. Six initiatives are suggested that will sustain best-in-class approaches to identify, treat and even cure Alpha-1 patients.

Steyer, G. J., et al. (2009). "Cryo-Imaging of Fluorescently-Labeled Single Cells in a Mouse." Proc SPIE Int Soc Opt Eng **7262**: 72620W-72620W72628.

We developed a cryo-imaging system to provide single-cell detection of fluorescently labeled cells in mouse, with particular applicability to stem cells and metastatic cancer. The Case cryo-imaging system consists of a fluorescence microscope, robotic imaging positioner, customized cryostat, PC-based control system, and visualization/analysis software. The system alternates between sectioning (10-40 mum) and imaging, collecting color brightfield and fluorescent block-face image volumes >60GB. In mouse experiments, we imaged quantum-dot labeled stem cells, GFP-labeled cancer and stem cells, and cell-size fluorescent microspheres. To remove subsurface fluorescence, we used a simplified model of light-tissue interaction whereby the next image was scaled, blurred, and subtracted from the current image. We estimated scaling and blurring parameters by minimizing entropy of subtracted images. Tissue specific attenuation parameters were found [u(T) : heart (267 +/- 47.6 mum), liver (218 +/- 27.1 mum), brain (161 +/- 27.4 mum)] to be within the range of estimates in the literature. "Next image" processing removed subsurface fluorescence equally well across multiple tissues (brain, kidney, liver, adipose tissue, etc.), and analysis of 200 microsphere images in the brain gave 97+/-2% reduction of subsurface fluorescence. Fluorescent signals were determined to arise from single cells based upon geometric and integrated intensity measurements. Next image processing greatly improved axial resolution, enabled high quality 3D volume renderings, and improved enumeration of single cells with connected component analysis by up to 24%. Analysis of image volumes identified metastatic cancer sites, found homing of stem cells to injury sites, and showed microsphere distribution correlated with blood flow patterns.We developed and evaluated cryo-imaging to provide single-cell detection of fluorescently labeled cells in mouse. Our cryo-imaging system provides extreme (>60GB), micron-scale, fluorescence, and

bright field image data. Here we describe our image pre-processing, analysis, and visualization techniques. Processing improves axial resolution, reduces subsurface fluorescence by 97%, and enables single cell detection and counting. High quality 3D volume renderings enable us to evaluate cell distribution patterns. Applications include the myriad of biomedical experiments using fluorescent reporter gene and exogenous fluorophore labeling of cells in applications such as stem cell regenerative medicine, cancer, tissue engineering, etc.

Straubinger, R., et al. (2017). "In Situ Thermal Annealing Transmission Electron Microscopy (TEM) Investigation of III/V Semiconductor Heterostructures Using a Setup for Safe Usage of Toxic and Pyrophoric Gases." Microsc Microanal **23**(4): 751-757.

In this study we compare two thermal annealing series of III/V semiconductor heterostructures on Si, where during the first series nitrogen is present in the in situ holder. The second, comparative, measurement is done in a tertiarybutylphosphine (TBP) environment. The sample annealed in a TBP environment shows favorable thermal stability up to 500 degrees C compared to the unstabilized sample, which begins to degrade at less than 300 degrees C. Evaporation of P from the material is tracked qualitatively by measuring the thickness of the sample during thermal annealing with and without stabilization. Finally, we investigate the in situ thermal annealing processes at atomic resolution. Here it is possible to study phase separation as well as the diffusion of As from a Ga(NAsP) quantum well in the surrounding GaP material during thermal annealing. To make these investigations possible we developed an extension for our in situ transmission electron microscopy setup for the safe usage of toxic and pyrophoric III/V semiconductor precursors. A commercially available gas cell and gas supply system were expanded with a gas mixing system, an appropriate toxic gas monitoring system and a gas scrubbing system. These components allow in situ studies of semiconductor growth and annealing under the purity conditions required for these materials.

Straubinger, R., et al. (2016). "Preparation and Loading Process of Single Crystalline Samples into a Gas Environmental Cell Holder for In Situ Atomic Resolution Scanning Transmission Electron Microscopic Observation." Microsc Microanal **22**(3): 515-519.

A reproducible way to transfer a single crystalline sample into a gas environmental cell holder for in situ transmission electron microscopic (TEM) analysis is shown in this study. As in situ holders have only single-tilt capability, it is necessary to prepare the sample precisely along a specific zone axis. This can be achieved by a very accurate focused ion beam lift-out preparation. We show a step-by-step procedure to prepare the sample and transfer it into the gas environmental cell. The sample material is a GaP/Ga(NAsP)/GaP multi-quantum well structure on Si. Scanning TEM observations prove that it is possible to achieve atomic resolution at very high temperatures in a nitrogen environment of 100,000 Pa.

Su, W., et al. (2020). "Red-Emissive Carbon Quantum Dots for Nuclear Drug Delivery in Cancer Stem Cells." J Phys Chem Lett **11**(4): 1357-1363.

Large doses of anticancer drugs entering cancer cell nuclei are found to be effective at killing cancer cells and increasing chemotherapeutic effectiveness. Here we report red-emissive carbon quantum dots, which can enter into the nuclei of not only cancer cells but also cancer stem cells. After doxorubicin was loaded at the concentration of 30 mug/mL on the surfaces of carbon quantum dots, the average cell viability of HeLa cells was decreased to only 21%, while it was decreased to 50% for free doxorubicin. The doxorubicin-loaded carbon quantum dots also exhibited a good therapeutic effect by eliminating cancer stem cells. This work provides a potential strategy for developing carbon quantum-dot-based anticancer drug carriers for effective eradication of cancers.

Subramaniam, P., et al. (2012). "Generation of a library of non-toxic quantum dots for cellular imaging and siRNA delivery." Adv Mater **24**(29): 4014-4019.

The development of non-toxic quantum dots and further investigation of their composition-dependent cytotoxicity in a high-throughput manner have been critical challenges for biomedical imaging and gene delivery. Herein, we report a rapid sonochemical synthetic methodology for generating a library of highly biocompatible ZnS-AgInS(2) (ZAIS) quantum dots for cellular imaging and siRNA delivery.

Sugaya, H., et al. (2016). "Fate of bone marrow mesenchymal stromal cells following autologous transplantation in a rabbit model of osteonecrosis." Cytotherapy **18**(2): 198-204.

BACKGROUND AIMS: Internalizing quantum dots (i-QDs) are a useful tool for tracking cells in vivo in models of tissue regeneration. We previously synthesized i-QDs by conjugating QDs with a unique internalizing antibody against a heat shock protein 70 family stress chaperone. In the present study, i-QDs were used to label rabbit mesenchymal stromal cells (MSCs) that were then transplanted into rabbits to assess differentiation potential in an osteonecrosis model. METHODS: The i-QDs were taken up by bone marrow-derived MSCs collected from the iliac of 12-week-old

Japanese white rabbits that were positive for cluster of differentiation (CD)81 and negative for CD34 and human leukocyte antigen DR. The average rate of i-QD internalization was 93.3%. At 4, 8, 12, and 24 weeks after transplantation, tissue repair was evaluated histologically and by epifluorescence and electron microscopy. RESULTS: The i-QDs were detected at the margins of the drill holes and in the necrotized bone trabecular. There was significant colocalization of the i-QD signal in transplanted cells and markers of osteoblast and mineralization at 4, 8, and 12 weeks post-transplantation, while i-QDs were detected in areas of mineralization at 12 and 24 weeks post-transplantation. Moreover, i-QDs were observed in osteoblasts in regenerated tissue by electron microscopy, demonstrating that the tissue was derived from transplanted cells. CONCLUSION: These results indicate that transplanted MSCs can differentiate into osteoblasts and induce tissue repair in an osteonecrosis model and can be tracked over the long term by i-QD labeling.

Sun, D., et al. (2010). "Study on effect of peptide-conjugated near-infrared fluorescent quantum dots on the clone formation, proliferation, apoptosis, and tumorigenicity ability of human buccal squamous cell carcinoma cell line BcaCD885." Int J Nanomedicine **5**: 401-405.

Quantum dots (QDs) have shown great development potential in noninvasive imaging and monitoring of cancer cells in vivo because of their unique optical properties. However, the key issue of whether or not QDs-labeled cancer cells affect the proliferation, apoptosis and in vivo tumorigenicity ability has not been reported. The primary issue is if the results obtained from the noninvasive visualization of QDs-labeled tumors are scientific. Here, we applied peptide-linked near-conjugated fluorescent QDs to label human buccal squamous cell carcinoma cell line (BcaCD885). We performed in vivo tumorigenicity ability assays, tumorigenic cells proliferation, and apoptotic capability assays detected by flow cytometry and plate clone formation experiment, and found that peptide-linked near-conjugated fluorescent QDs labeling did not affect the growth, proliferation, apoptosis, and tumorigenicity ability of those cancer cells. Our study provides scientific foundation to support the application of near-infrared fluorescent QDs in noninvasive imaging and monitoring of cancer cells in vivo.

Sun, S., et al. (2015). "Systematic evaluation of the degraded products evolved from the hydrothermal pretreatment of sweet sorghum stems." Biotechnol Biofuels **8**: 37.

BACKGROUND: Conversion of plant cell walls to bioethanol and bio-based chemicals requires pretreatment as a necessary step to reduce recalcitrance of cell walls to enzymatic and microbial deconstruction. In this study, the sweet sorghum stems were subjected to various hydrothermal pretreatment processes (110 degrees C to 230 degrees C, 0.5 to 2.0 h), and the focus of this work is to systematically evaluate the degraded products of polysaccharides and lignins in the liquor phase obtained during the pretreatment process. RESULTS: The maximum yield of xylooligosaccharides (52.25%) with a relatively low level of xylose and other degraded products was achieved at a relatively high pretreatment temperature (170 degrees C) for a short reaction time (0.5 h). Higher temperature (>170 degrees C) and/or longer reaction time (>0.5 h at 170 degrees C) resulted in a decreasing yield of xylooligosaccharides, but increased the concentration of arabinose and galactose. The xylooligosaccharides obtained are composed of xylopyranosyl residues, together with lower amounts of 4-O-Me-alpha-D-GlcpA units. Meanwhile, the concentrations of the degraded products (especially furfural) increased as a function of pretreatment temperature and time. Molecular weights of the water-soluble polysaccharides and lignins indicated that the degradation of the polysaccharides and lignins occurred during the conditions of harsh hydrothermal pretreatment. In addition, the water-soluble polysaccharides (rich in xylan) and water-soluble lignins (rich in beta-O-4 linkages) were obtained at 170 degrees C for 1.0 h. CONCLUSIONS: The present study demonstrated that the hydrothermal pretreatment condition had a remarkable impact on the compositions and the chemical structures of the degraded products. An extensive understanding of the degraded products from polysaccharides and lignins during the hydrothermal pretreatment will be beneficial to value-added applications of multiple chemicals in the biorefinery for bioethanol industry.

Suzuki, H., et al. (2008). "Sequestration and homing of bone marrow-derived lineage negative progenitor cells in the lung during pneumococcal pneumonia." Respir Res **9**: 25.

BACKGROUND: Bone marrow (BM)-derived progenitor cells have been shown to have the potential to differentiate into a diversity of cell types involved in tissue repair. The characteristics of these progenitor cells in pneumonia lung is unknown. We have previously shown that Streptococcus pneumoniae induces a strong stimulus for the release of leukocytes from the BM and these leukocytes preferentially sequester in the lung capillaries. Here we report the behavior of BM-derived lineage negative progenitor cells (Lin- PCs) during pneumococcal pneumonia using quantum dots (QDs),

nanocrystal fluorescent probes as a cell-tracking technique. METHODS: Whole BM cells or purified Lin- PCs, harvested from C57/BL6 mice, were labeled with QDs and intravenously transfused into pneumonia mice infected by intratracheal instillation of Streptococcus pneumoniae. Saline was instilled for control. The recipients were sacrificed 2 and 24 hours following infusion and QD-positive cells retained in the circulation, BM and lungs were quantified. RESULTS: Pneumonia prolonged the clearance of Lin- PCs from the circulation compared with control (21.7 +/- 2.7% vs. 7.7 +/- 0.9%, at 2 hours, $P < 0.01$), caused preferential sequestration of Lin- PCs in the lung microvessels (43.3 +/- 8.6% vs. 11.2 +/- 3.9%, at 2 hours, $P < 0.05$), and homing of these cells to both the lung (15.1 +/- 3.6% vs. 2.4 +/- 1.2%, at 24 hours, $P < 0.05$) and BM as compared to control (18.5 +/- 0.8% vs. 9.5 +/- 0.4%, at 24 hours, $P < 0.01$). Very few Lin- PCs migrated into air spaces. CONCLUSION: In this study, we demonstrated that BM-derived progenitor cells are preferentially sequestered and retained in pneumonic mouse lungs. These cells potentially contribute to the repair of damaged lung tissue.

Takasaki, Y., et al. (2011). "Estimation of the distribution of intravenously injected adipose tissue-derived stem cells labeled with quantum dots in mice organs through the determination of their metallic components by ICPMS." Anal Chem **83**(21): 8252-8258.

Adipose tissue-derived stem cells (ASCs) have shown promise in cell therapy because of their ability to self-renew damaged or diseased organs and easy harvest. To ensure the distribution and quantification of the ASCs injected from tail vein, several whole-body imaging techniques including fluorescence optical imaging with quantum dots (QDs) have been employed, but they may suffer from insufficient sensitivity and accuracy. Here, we report quantitative distribution of ASCs in various organs (heart, lung, liver, spleen, and kidney) of mice, which were intravenously injected with QDs-labeled ASCs (QDs-ASCs), through the detection of QDs-derived metallic components by inductively coupled plasma mass spectrometry (ICPMS). For accurate and precise determination, each organ was harvested and completely digested with a mixture of HNO(3) and H(2)O(2) in a microwave oven prior to ICPMS measurement, which was equipped with a microflow injection system and a laboratory-made capillary-attached micronebulizer. After optimization, 16 elements including major components (Cd, Se, and Te) of QDs and essential elements (Na, K, Mg, Ca, P, S, Mn, Fe, Co, Cu, Zn, Se, Sr, and Mo) were successfully determined in the organs. As compared to untreated mice, QDs-ASCs-treated mice showed significantly higher levels of Cd and Te in all organs, and as expected, the molar ratio of Cd to Te in each organ was in good agreement with the molar composition ratio in the QDs. This result indicates that the increment of Cd (or Te) can be used as a tracer for calculating the distribution of ASCs in mice organs. As a result of the calculation, 36.8%, 19.1%, 0.59%, 0.49%, and 0.25% of the total ASCs injected were estimated to be distributed in the liver, lung, heart, spleen, and kidney, respectively.

Talib, A., et al. (2015). "Synthesis of highly fluorescent hydrophobic carbon dots by hot injection method using Paraplast as precursor." Mater Sci Eng C Mater Biol Appl **48**: 700-703.

We have reported synthesis of bright blue colored hydrophobic carbon dots (hC-dots) using highly pure blend of polymers called Paraplast. We developed a hot injection method for making nearly monodispersed hC-dots with a diameter in a range: 5-30nm as confirmed by high resolution transmission electron microscopy (HRTEM). The involvement of various functional groups was confirmed by Fourier transform infra-red (FTIR) spectroscopy. These hC-dots were incubated with breast cancer stem cells in order to check the entry as well as biological imaging. The cells were analyzed using epifluorescent microscopy. hC-dots showed concentration dependent cytotoxicity (LD50: 50mg/ml) and could be used for bioimaging even at lower concentration (0.5mg/ml). hC-dots were found to be versatile agents for peeping inside the cells which could also be used for delivery of water insoluble chemotherapeutic agents to variety of solid tumors.

Tan, J., et al. (2019). "Pancreatic stem cells differentiate into insulin-secreting cells on fibroblast-modified PLGA membranes." Mater Sci Eng C Mater Biol Appl **97**: 593-601.

Diabetes mellitus is an epidemic worldwide. Pancreatic stem cells can be induced to differentiate into insulin-secreting cells, this method is an effective way to solve the shortage of islet donor. Poly (lactic acid-co-glycolic acid (PLGA) copolymer is an excellent scaffold for tissue engineering as it presents good biocompatibility and film forming properties. In this study, we adopted biological methods, using fibroblast-coated PLGA diaphragm to form a biological membrane, and then pancreatic stem cells were cultured on the fibroblast-modified PLGA membrane and the two-step induction method was utilized to induce the differentiation of pancreatic stem cells into insulin-secreting cells. The proliferation and differentiation of pancreatic stem cells on the fibroblast-modified PLGA membrane as well as the expression of genes related to the differentiation of pancreatic stem cells were examined in both normal and induced cultures to explore the potential of fibroblast-modified PLGA membrane for the transplantation to treat diabetes mellitus. The results indicated that fibroblasts can effectively improve

the cell compatibility and histocompatibility of the PLGA membrane and promote the proliferation and differentiation of pancreatic stem cells. After induction, real-time fluorescence quantitative PCR (FQRT-PCR) results showed there were more Notch receptors and its ligands expressed in the membranes of pancreatic stem cells than non-induced pancreatic stem cells or fibroblast. Semiconductor quantum dot coupled-anti-complex probe experiments revealed that induced pancreatic stem cells had higher expression levels of Notch 2 and Delta-like 1 than non-induced ones, which may regulate the expression of Neurogenin-3 (Ngn3) and Hairy/Enhancer of split-1 gene (Hes1) through Notch signaling interaction between fibroblasts and pancreatic stem cells as well as enhance the proliferation of pancreatic stem cells and their differentiation into insulin-secreting cells. Further, our study suggests that the fibroblast-modified PLGA membrane can be used as matrix material composed of pancreatic stem cells or other stem cells to construct artificial islet tissue for the treatment of diabetes mellitus.

Tan, S. K. (2003). "From genesis to genes." Ann Acad Med Singap **32**(5): 710-714.

Since the beginning of time, our ancestors have been plagued by illnesses and injuries that are not too different from today's diseases. Evidence from prehistoric times and ancient civilisations have shown man's attempts at trying to understand the nature and treatment of these conditions. It was not till the early 19th century that the scientific basis of modern medicine was firmly established when microorganisms were discovered and found to be the cause of many of these illnesses. The 20th century saw quantum leaps made in the understanding of the function of the human body and the therapeutic measures aimed at restoration of any such malfunction. The end of the last millennium was marked by historic achievements made in the Life Sciences, in particular the completion of the sequencing of the Human Genome--the code of life. The beginning of the 21st century has already seen many breakthroughs in medical sciences, especially in the fields of stem cell technology and gene therapy. The number of known illnesses directly related to genetic defects or abnormalities have increased exponentially. Many of today's scourges can be prevented or more effectively treated. Our ability to utilise this new knowledge to combat the ravages of the ageing process and its associated illnesses--degenerative diseases and cancers offer much hope for the future.

Tao, Z. W., et al. (2017). "Delivering stem cells to the healthy heart on biological sutures: effects on regional mechanical function." J Tissue Eng Regen Med **11**(1): 220-230.

Current cardiac cell therapies cannot effectively target and retain cells in a specific area of the heart. Cell-seeded biological sutures were previously developed to overcome this limitation, demonstrating targeted delivery with > 60% cell retention. In this study, both cell-seeded and non-seeded fibrin-based biological sutures were implanted into normal functioning rat hearts to determine the effects on mechanical function and fibrotic response. Human mesenchymal stem cells (hMSCs) were used based on previous work and established cardioprotective effects. Non-seeded or hMSC-seeded sutures were implanted into healthy athymic rat hearts. Before cell seeding, hMSCs were passively loaded with quantum dot nanoparticles. One week after implantation, regional stroke work index and systolic area of contraction (SAC) were evaluated on the epicardial surface above the suture. Cell delivery and retention were confirmed by quantum dot tracking, and the fibrotic tissue area was evaluated. Non-seeded biological sutures decreased SAC near the suture from 0.20 +/- 0.01 measured in sham hearts to 0.08 +/- 0.02, whereas hMSC-seeded biological sutures dampened the decrease in SAC (0.15 +/- 0.02). Non-seeded sutures also displayed a small amount of fibrosis around the sutures (1.0 +/- 0.1 mm(2)). Sutures seeded with hMSCs displayed a significant reduction in fibrosis (0.5 +/- 0.1 mm(2) , $p < 0.001$), with quantum dot-labelled hMSCs found along the suture track. These results show that the addition of hMSCs attenuates the fibrotic response observed with non-seeded sutures, leading to improved regional mechanics of the implantation region.

Teoh, G. Z., et al. (2015). "Development of resorbable nanocomposite tracheal and bronchial scaffolds for paediatric applications." Br J Surg **102**(2): e140-150.

BACKGROUND: Congenital tracheal defects and prolonged intubation following premature birth have resulted in an unmet clinical need for tracheal replacement. Advances in stem cell technology, tissue engineering and material sciences have inspired the development of a resorbable, nanocomposite tracheal and bronchial scaffold. METHODS: A bifurcated scaffold was designed and constructed using a novel, resorbable nanocomposite polymer, polyhedral oligomeric silsesquioxane poly(-caprolactone) urea urethane (POSS-PCL). Material characterization studies included tensile strength, suture retention and surface characteristics. Bone marrow-derived mesenchymal stem cells (bmMSCs) and human tracheobronchial epithelial cells (HBECs) were cultured on POSS-PCL for up to 14 days, and metabolic activity and cell morphology were assessed. Quantum dots conjugated to RGD (l-arginine, glycine and l-aspartic acid) tripeptides and anticollagen type I antibody were then employed to observe cell migration throughout the scaffold.

RESULTS: POSS-PCL exhibited good mechanical properties, and the relationship between the solid elastomer and foam elastomer of POSS-PCL was comparable to that between the cartilaginous U-shaped rings and interconnective cartilage of the native human trachea. Good suture retention was also achieved. Cell attachment and a significant, steady increase in proliferation were observed for both cell types (bmMSCs, P = 0.001; HBECs, P = 0.003). Quantum dot imaging illustrated adequate cell penetration throughout the scaffold, which was confirmed by scanning electron microscopy. CONCLUSION: This mechanically viable scaffold successfully supports bmMSC and HBEC attachment and proliferation, demonstrating its potential as a tissue-engineered solution to tracheal replacement.

Teoh, G. Z., et al. (2015). "Role of nanotechnology in development of artificial organs." Minerva Med **106**(1): 17-33.

Improvements in our understanding of the interactions between implants and cells have directed attention towards nanoscale technologies. To date, nanotechnology has played a helping hand in the development of synthetic artificial organs and regenerative medicine. This includes the production of smart nanocomposite materials; fluorescent nanoparticles like Quantum Dots (QD) and magnetic nano particles (MNP) for stem cell tracking; and carbon nanotubes (CNT) and graphene for enhancement of material properties. The scope of this paper includes the role of nanoparticles in the development of nanomaterials; the chemical surface modifications possible to improve implant function and an overview of the performance of nano-engineered organs thus far. This includes implants developed for aesthetic purposes like nasal and auricular scaffolds, plastic and reconstructive surgical constructs (i.e. dermal grafts), hollow organs for cardiothoracic applications; and last but not least, orthopedic implants. The five-year outlook for nano-enhanced artificial organs is also discussed, highlighting the key research and development areas, available funds and the hurdles we face in accomplishing progression from prototypes on the laboratory bench to off-the-shelf products for the consumer market. Ultimately, this review aims to delineate the advantages of incorporating nanotechnology, as an individual entity or as a part of a construct for the development of tissue engineering scaffolds and/or artificial organs, and unravel the mechanisms of tissue cell-biomaterial interactions at the nanoscale, allowing for better progress in the development and optimization of unique nanoscale surface features for a wide range of applications.

Terrovitis, J. V., et al. (2010). "Assessment and optimization of cell engraftment after transplantation into the heart." Circ Res **106**(3): 479-494.

Myocardial regeneration using stem and progenitor cell transplantation in the injured heart has recently become a major goal in the treatment of cardiac disease. Experimental studies and clinical applications have generally been encouraging, although the functional benefits that have been attained clinically are modest and inconsistent. Low cell retention and engraftment after myocardial delivery is a key factor limiting the successful application of cell therapy, irrespective of the type of cell or the delivery method. To improve engraftment, accurate methods for tracking cell fate and quantifying cell survival need to be applied. Several laboratory techniques (histological methods, real-time quantitative polymerase chain reaction, radiolabeling) have provided invaluable information about cell engraftment. In vivo imaging (nuclear medicine modalities, bioluminescence, and MRI) has the potential to provide quantitative information noninvasively, enabling longitudinal assessment of cell fate. In the present review, we present several available methods for assessing cell engraftment, and we critically discuss their strengths and limitations. In addition to providing insights about the mechanisms mediating cell loss after transplantation, these methods can evaluate techniques for augmenting engraftment, such as tissue engineering approaches, preconditioning, and genetic modification, allowing optimization of cell therapies.

Thakkar, U. G., et al. (2016). "Infusion of autologous adipose tissue derived neuronal differentiated mesenchymal stem cells and hematopoietic stem cells in post-traumatic paraplegia offers a viable therapeutic approach." Adv Biomed Res **5**: 51.

BACKGROUND: Spinal cord injury (SCI) is not likely to recover by current therapeutic modalities. Stem cell (SC) therapy (SCT) has promising results in regenerative medicine. We present our experience of co-infusion of autologous adipose tissue derived mesenchymal SC differentiated neuronal cells (N-Ad-MSC) and hematopoietic SCs (HSCs) in a set of patients with posttraumatic paraplegia. MATERIALS AND METHODS: Ten patients with posttraumatic paraplegia of mean age 3.42 years were volunteered for SCT. Their mean age was 28 years, and they had variable associated complications. They were subjected to adipose tissue resection for in vitro generation of N-Ad-MSC and bone marrow aspiration for generation of HSC. Generated SCs were infused into the cerebrospinal fluid (CSF) below injury site in all patients. RESULTS: Total mean quantum of SC infused was 4.04 ml with a mean nucleated cell count of 4.5 x 10(4)/muL and mean CD34+ of 0.35%, CD45-/90+ and CD45-/73+ of 41.4%, and 10.04%, respectively. All of them expressed

transcription factors beta-3 tubulin and glial fibrillary acid protein. No untoward effect of SCT was noted. Variable and sustained improvement in Hauser's index and American Spinal Injury Association score was noted in all patients over a mean follow-up of 2.95 years. Mean injury duration was 3.42 years against the period of approximately 1-year required for natural recovery, suggesting a positive role of SCs. CONCLUSION: Co-infusion of N-Ad-MSC and HSC in CSF is safe and viable therapeutic approach for SCIs.

Tirughana, R., et al. (2018). "GMP Production and Scale-Up of Adherent Neural Stem Cells with a Quantum Cell Expansion System." Mol Ther Methods Clin Dev **10**: 48-56.

Cell-based therapies hold great promise for a myriad of clinical applications. However, as these therapies move from phase I to phase II and III trials, there is a need to improve scale-up of adherent cells for the production of larger good manufacturing practice (GMP) cell banks. As we advanced our neural stem cell (NSC)-mediated gene therapy trials for glioma to include dose escalation and multiple treatment cycles, GMP production using cell factories (CellStacks) generated insufficient neural stem cell (NSC) yields. To increase yield, we developed an expansion method using the hollow fiber quantum cell expansion (QCE) system. Seeding of 5.2 x 10(7) NSCs in a single unit yielded up to 3 x 10(9) cells within 10 days. These QCE NSCs showed genetic and functional stability equivalent to those expanded by conventional flask-based methods. We then expanded the NSCs in 7 units simultaneously to generate a pooled GMP-grade NSC clinical lot of more than 1.5 x 10(10) cells in only 9 days versus 8 x 10(9) over 6 weeks in CellStacks. We also adenovirally transduced our NSCs within the QCE. We found the QCE system enabled rapid cell expansion and increased yield while maintaining cell properties and reducing process time, labor, and costs with improved efficiency and reproducibility.

Toita, S., et al. (2008). "Protein-conjugated quantum dots effectively delivered into living cells by a cationic nanogel." J Nanosci Nanotechnol **8**(5): 2279-2285.

Quantum dots (QDs) have attracted attention for their potential as a cell imaging regent. However, the development of effective intracellular delivery system for QDs is needed to apply various cell lines without affecting cellular function. We reported here new QDs delivery system by using cationic nanogel consisting of cholesterol-bearing pullulan modified with an amino group (CHPNH2). The uptake of hybrid nanoparticles into HeLa cells was followed by flow cytometry, and confocal laser scanning fluorescence microscopy. Protein-conjugated QDs were effectively internalized into cells by the nanogel compared with a cationic liposome system. The hybrid nanoparticle was used to stain rabbit mesenchymal stem cells (MSCs) so as to evaluate their effect on cell function. CHPNH2-QD hybrid nanoparticles remained detectable inside MSCs for at least 2 weeks of culture and had little effect on the in vitro chondrogenic ability of MSCs. The hybrid nanoparticles are a promising candidate as a cell tracer in tissue engineering.

Tong, H., et al. (2013). "Polyethylenimine600-beta-cyclodextrin: a promising nanopolymer for nonviral gene delivery of primary mesenchymal stem cells." Int J Nanomedicine **8**: 1935-1946.

Genetically modified mesenchymal stem cells (MSCs) have great potential in the application of regenerative medicine and molecular therapy. In the present manuscript, we introduce a nanopolymer, polyethylenimine600-beta-cyclodextrin (PEI600-beta-CyD), as an efficient polyplex-forming plasmid delivery agent with low toxicity and ideal transfection efficiency on primary MSCs. PEI600-beta-CyD causes significantly less cytotoxicity and apoptosis on MSCs than 25 kDa high-molecular-weight PEI (PEI25kDa). PEI600-beta-CyD also exhibits similar transfection efficiency as PEI25kDa on MSCs, which is higher than that of PEI600Da. Quantum dot-labeled plasmids show that PEI600-beta-CyD or PEI25kDa delivers the plasmids in a more scattered manner than PEI600Da does. Further study shows that PEI600-beta-CyD and PEI25kDa are more capable of delivering plasmids into the cell lysosome and nucleus than PEI600Da, which correlates well with the results of their transfection-efficiency assay. Moreover, among the three vectors, PEI600-beta-CyD has the most capacity of enhancing the alkaline phosphatase activity of MSCs by transfecting bone morphogenetic protein 2, 7, or special AT-rich sequence-binding protein 2. These results clearly indicate that PEI600-beta-CyD is a safe and effective candidate for the nonviral gene delivery of MSCs because of its ideal inclusion ability and proton sponge effect, and the application of this nanopolymer warrants further investigation.

Tong, W. F., et al. (2002). "Somatic cell nuclear transfer (cloning): implications for the medical practitioner." Singapore Med J **43**(7): 369-376.

The current century will bring tremendous changes to the science and the practice of medicine. This century will be acknowledged as the century of Biology as the fusion of molecular genetics and experimental embryology pushes the barriers of science beyond perimeters that we have thought existed, as much as the past century was the century of Physics, with all the exact scientific calculations and predictions, resulting in electricity, nuclear power and quantum physics. The first major breakthrough has been the pioneering work

of Wilmut and Campbell, first with the birth of Megan and Moran in 1995 (1), followed by the birth of Dolly the sheep, the first reported mammalian clone from a fully differentiated adult cell, reported in July 1996 (2). However, current cloning techniques are an extension of over 40 years of research using nuclei derived from non-human embryonic and fetal cells. However, following the birth of Dolly, the prospects of cloning technology have extended to ethically hazier areas of human cloning and embryonic stem cell research. This review hopes to bring the reader closer to the science and the ethics of this new technology, and what the implications are for the medical practitioner.

Tsai, C. L., et al. (2015). "Differentiation of Stem Cells From Human Exfoliated Deciduous Teeth Toward a Phenotype of Corneal Epithelium In Vitro." Cornea **34**(11): 1471-1477.

PURPOSE: The aim of this study was to characterize stem cells from human exfoliated deciduous teeth (SHED) and to investigate the potential of SHED to differentiate toward corneal epithelium-like cells in vitro. METHODS: Mesenchymal and embryonic stem cell markers were analyzed by flow cytometry. The SHED was cocultured in either a transwell noncontact system or in a mixed culture system with immortalized human corneal epithelial (HCE-T) cells to induce the epithelial transdifferentiation. Expression of the mature corneal epithelium-specific marker cytokeratin 3 (CK3) and corneal epithelial progenitor marker cytokeratin 19 (CK19) were detected by immunofluorescence and the reverse transcription-polymerase chain reaction, respectively. RESULTS: SHED strongly expressed a set of mesenchymal stromal cell markers and pluripotency markers including NANOG and OCT-4. Seven days after the transwells were cocultured with HCE-T cells, SHED successfully upregulated epithelial lineage markers CK3 (16.6 +/- 7.9%) and CK19 (10.0 +/- 4.3%) demonstrating the potential for epithelial transdifferentiation, whereas CK3 and CK19 were barely expressed in SHED when cultured alone. Expression of transcript levels of CK3 and CK19 were significantly upregulated when SHED were transwell cocultured or mixed cultured with HCE-T cells by 7, 14, and 21 days. CONCLUSIONS: We have demonstrated that SHED retain the potential for transdifferentiation to corneal epithelium-like cells by in vitro coculture with immortal corneal epithelium cells. Thus, exfoliated teeth may be an alternative tissue resource for providing stem cells for potential clinical applications in ocular surface regeneration.

Tsuchiya, S., et al. (2013). "Rat bone marrow stromal cell-conditioned medium promotes early osseointegration of titanium implants." Int J Oral Maxillofac Implants **28**(5): 1360-1369.

PURPOSE: To enhance the stability of titanium (Ti) implants using conditioned medium (CM) derived from rat bone marrow stromal cell (BMSC). MATERIALS AND METHODS: BMSCs were isolated from rat femurs and grown in culture, and the culture medium was used as CM. The CM was immobilized on the surface of Ti implants with calcifying solution. The topology of the Ti implants after immobilization of CM was observed by scanning electron microscopy (SEM). The Ti-immobilized CM was analyzed by liquid chromatography with tandem mass spectrometry. The adhesiveness and the osteogenic differentiation of BMSCs grown on CM-coated discs were analyzed by reverse-transcription polymerase chain reaction. Ti implants with specimen-immobilized CM labeled with quantum dots (QDs) were placed into rat femurs. The localization of the CM was detected by in vivo imaging at 1, 7, 14, and 28 days after implantation. The removal torque test and histologic bone implant contact (BIC) were also analyzed. RESULTS: Rat BMSC-CM was successfully immobilized on Ti implants. The immobilized CM contained about 2000 proteins, including collagen type I, bone sialoprotein, fibronectin, and vascular endothelial growth factor that are important in new bone formation. CM promoted cell adhesion and osteocalcin gene expression of rat BMSCs. The labeled CM remained associated with the Ti implant at 1, 7, 14, and 28 days postimplantation. The removal torque value and BIC of Ti implants with immobilized CM were higher than those of control implants on days 1, 7, and 14 after implantation. CONCLUSION: Immobilized CM components on the surface of Ti implants promoted integration into bone during an early stage.

Ulusoy, M., et al. (2016). "Evaluation of CdTe/CdS/ZnS core/shell/shell quantum dot toxicity on three-dimensional spheroid cultures." Toxicol Res (Camb) **5**(1): 126-135.

In this work, three-dimensional (3D) spheroid cultures of human adipose-derived mesenchymal stem cells (hAD-MSCs), with tissue-mimetic morphology through well developed cell-cell and cell-matrix interactions and distinct diffusion/transport characteristics, were assessed for dose-dependent toxic effects of red-emitting CdTe/CdS/ZnS quantum dots (Qdots). Morphological investigations and time-resolved microscopy analysis in addition to cell metabolic activity studies revealed that 3D spheroid cultures are more resistant to Qdot-induced cytotoxicity in comparison to conventional 2D cultures. The obtained results suggest the presence of two distinct cell populations in 2D cultures with different sensitivity to Qdots, however that effect wasn't observed in 3D spheroids. Our investigations were aimed to improve the prediction of nanotoxicity of Qdot on tissue-level and provide the essential screening steps prior to any in vivo

application. Moreover, penetration ability of highly fluorescent Qdots to densely-packed spheroids will fortify the biological application of developed Qdots in tissue-like structures.

Vaez Ghaemi, R., et al. (2019). "Brain Organoids: A New, Transformative Investigational Tool for Neuroscience Research." Adv Biosyst **3**(1): e1800174.

Brain organoids are self-assembled, three-dimensionally structured tissues that are typically derived from pluripotent stem cells. They are multicellular aggregates that more accurately recapitulate the tissue microenvironment compared to the other cell culture systems and can also reproduce organ function. They are promising models for evaluating drug leads, particularly those that target neurodegeneration, since they are genetically and phenotypically stable over prolonged durations of culturing and they reasonably reproduce critical physiological phenomena such as biochemical gradients and responses by the native tissue to stimuli. Beyond drug discovery, the use of brain organoids could also be extended to investigating early brain development and identifying the mechanisms that elicit neurodegeneration. Herein, the current state of the fabrication and use of brain organoids in drug development and medical research is summarized. Although the use of brain organoids represents a quantum leap over existing investigational tools used by the pharmaceutical industry, they are nonetheless imperfect systems that could be greatly improved through bioengineering. To this end, some key scientific challenges that would need to be addressed in order to enhance the relevance of brain organoids as model tissue are listed. Potential solutions to these challenges, including the use of bioprinting, are highlighted thereafter.

Vanikar, A. V., et al. (2010). "Cotransplantation of adipose tissue-derived insulin-secreting mesenchymal stem cells and hematopoietic stem cells: a novel therapy for insulin-dependent diabetes mellitus." Stem Cells Int **2010**: 582382.

Aims. Insulin dependent diabetes mellitus (IDDM) is believed to be an autoimmune disorder with disturbed glucose/insulin metabolism, requiring life-long insulin replacement therapy (IRT), 30% of patients develop end-organ failure. We present our experience of cotransplantation of adipose tissue derived insulin-secreting mesenchymal stem cells (IS-AD-MSC) and cultured bone marrow (CBM) as IRT for these patients. Methods. This was a prospective open-labeled clinical trial to test efficacy and safety of IS-AD-MSC+CBM co-transplantation to treat IDDM, approved by the institutional review board after informed consent in 11 (males : females: 7 : 4) patients with 1-24-year disease duration, in age group: 13-43 years, on mean values of exogenous insulin requirement of 1.14 units/kg BW/day, glycosylated hemoglobin (Hb1Ac): 8.47%, and c-peptide levels: 0.1 ng/mL. Intraportal infusion of xenogeneic-free IS-AD-MSC from living donors, subjected to defined culture conditions and phenotypically differentiated to insulin-secreting cells, with mean quantum: 1.5 mL, expressing Pax-6, Isl-1, and pdx-1, cell counts: 2.1 x 10(3)/muL, CD45(-)/90(+)/73(+):40/30.1%, C-Peptide level:1.8 ng/mL, and insulin level: 339.3 IU/mL with CBM mean quantum: 96.3 mL and cell counts: 28.1 x 10(3)/muL, CD45(-)/34(+):0.62%, was carried out. Results. All were successfully transplanted without any untoward effect. Over mean followup of 23 months, they had a decreased mean exogenous insulin requirement to 0.63 units/kgBW/day, Hb1Ac to 7.39%, raised serum c-peptide levels to 0.38 ng/mL, and became free of diabetic ketoacidosis events with mean 2.5 Kg weight gain on normal vegetarian diet and physical activities. Conclusion. This is the first report of treating IDDM with insulin-secreting-AD-MSC+CBM safely and effectively with relatively simple techniques.

Vanikar, A. V., et al. (2016). "Stem cell therapy emerging as the key player in treating type 1 diabetes mellitus." Cytotherapy **18**(9): 1077-1086.

Type 1 diabetes mellitus (T1DM) is an autoimmune disease causing progressive destruction of pancreatic beta cells, ultimately resulting in loss of insulin secretion producing hyperglycemia usually affecting children. Replacement of damaged beta cells by cell therapy can treat it. Currently available strategies are insulin replacement and islet/pancreas transplantation. Unfortunately these offer rescue for variable duration due to development of autoantibodies. For pancreas/islet transplantation a deceased donor is required and various shortfalls of treatment include quantum, cumbersome technique, immune rejection and limited availability of donors. Stem cell therapy with assistance of cellular reprogramming and beta-cell regeneration can open up new therapeutic modalities. The present review describes the history and current knowledge of T1DM, evolution of cell therapies and different cellular therapies to cure this condition.

Vibin, M., et al. (2011). "Fluorescence imaging of stem cells, cancer cells and semi-thin sections of tissues using silica-coated CdSe quantum dots." J Fluoresc **21**(4): 1365-1370.

Trioctylphosphine oxide capped cadmium selenide quantum dots, synthesized in organic media were rendered water soluble by silica overcoating. Silanisation was done by a simple reverse microemulsion method using aminopropyl silane as the silica precursor. Further, the strong photoluminescence

of the silica-coated CdSe quantum dots has been utilized to visualize rabbit adipose tissue-derived mesenchymal stem cells (RADMSCs) and Daltons lymphoma ascites (DLA) cancerous cells in vitro. Subsequently the in vivo fluorescence behaviours of QDs in the tissues were also demonstrated by intravenous administration of the QDs in Swiss albino mice. The fluorescence microscopic images in the stem cells, cancer cells and semi-thin sections of mice organs proved the strong luminescence property of silica-coated quantum dots under biological systems. These results establish silica-coated CdSe QDs as extremely useful tools for molecular imaging and cell tracking to study the cell division and metastasis of cancer and other diseases.

Villa, C., et al. (2021). "Treatment with ROS detoxifying gold quantum clusters alleviates the functional decline in a mouse model of Friedreich ataxia." Sci Transl Med **13**(607).

Friedreich ataxia (FRDA) is caused by the reduced expression of the mitochondrial protein frataxin (FXN) due to an intronic GAA trinucleotide repeat expansion in the FXN gene. Although FRDA has no cure and few treatment options, there is research dedicated to finding an agent that can curb disease progression and address symptoms as neurobehavioral deficits, muscle endurance, and heart contractile dysfunctions. Because oxidative stress and mitochondrial dysfunctions are implicated in FRDA, we demonstrated the systemic delivery of catalysts activity of gold cluster superstructures (Au8-pXs) to improve cell response to mitochondrial reactive oxygen species and thereby alleviate FRDA-related pathology in mesenchymal stem cells from patients with FRDA. We also found that systemic injection of Au8-pXs ameliorated motor function and cardiac contractility of YG8sR mouse model that recapitulates the FRDA phenotype. These effects were associated to long-term improvement of mitochondrial functions and antioxidant cell responses. We related these events to an increased expression of frataxin, which was sustained by reduced autophagy. Overall, these results encourage further optimization of Au8-pXs in experimental clinical strategies for the treatment of FRDA.

Wahab, R., et al. (2019). "Gold quantum dots impair the tumorigenic potential of glioma stem-like cells via beta-catenin downregulation in vitro." Int J Nanomedicine **14**: 1131-1148.

BACKGROUND: Over the past several decades, the incidence of solid cancers has rapidly increased worldwide. Successful removal of tumor-initiating cells within tumors is essential in the field of cancer therapeutics to improve patient disease-free survival rates. The biocompatible multivarient-sized gold nanoparticles (MVS-GNPs) from quantum dots (QDs, <10 nm) to nanosized (up to 50 nm) particles have vast applications in various biomedical areas including cancer treatment. The role of MVS-GNPs for inhibition of tumorigenic potential and stemness of glioma was investigated in this study. METHODS: Herein, MVS-GNPs synthesized and characterized by means of X-ray diffraction pattern (XRD) and transmission electron microscopy (TEM) techniques. Afterwards, interaction of these GNPs with glioma stem-cell like cells along with cancer cells were evaluated by MTT, cell motility, self-renewal assays and biostatistics was also applied. RESULTS: Among these GNPs, G-QDs contributed to reduce metastatic events and spheroid cell growth, potentially blocking the self-renewal ability of these cells. This study also uncovers the previously unknown role of the inhibition of CTNNB1 signaling as a novel candidate to decrease the tumorigenesis of glioma spheroids and subsequent spheroid growth. The accurate and precise biostatistics results were obtained at quantify level. CONCLUSION: In summary, G-QDs may exhibit possible contribution on suppressing the growth of tumor-initiating cells. These data reveal a unique therapeutic approach for the elimination of residual resistant stem-like cells during cancer treatment.

Wang, G., et al. (2015). "Biocompatibility of quantum dots (CdSe/ZnS) in human amniotic membrane-derived mesenchymal stem cells in vitro." Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub **159**(2): 227-233.

BACKGROUND AND AIM: Amniotic membrane-derived mesenchymal stem cells (hAM-dMSCs) are a potential source of mesenchymal stem cells which could be used to repair skin damage. The use of mesenchymal stem cells to repair skin damage requires safe, effective and biocompatible agents to evaluate the effectiveness of the result. Quantum dots (QDs) composed of CdSe/ZnS are semiconductor nanocrystals with broad excitation and narrow emission spectra, which have been considered as a new chemical and fluorescent substance for non-invasively labeling different cells in vitro and in vivo. This study investigated the cytotoxic effects of QDs on hAM-dMSCs at different times following labeling. METHODS: Using 0.75, 1.5 and 3.0 muL between quantum dots, labeled human amniotic mesenchymal stem cells were collected on days 1, 2 and 4 and observed morphological changes, performed an MTT cell growth assay and flow cytometry for mesenchymal stem cells molecular markers. RESULTS: Quantum dot concentration 0.75 mug/mL labeled under a fluorescence microscope, cell morphology was observed, The MTT assay showed cells in the proliferative phase. Flow cytometry expression CD29, CD31, CD34, CD44, CD90, CD105 and CD106. CONCLUSIONS: Within a certain range of concentrations between quantum dots labeled human

amniotic mesenchymal stem cells has good biocompatibility.

Wang, H. C., et al. (2010). "Transplantation of quantum dot-labelled bone marrow-derived stem cells into the vitreous of mice with laser-induced retinal injury: survival, integration and differentiation." Vision Res **50**(7): 665-673.

Accidental laser exposure to the eyes may result in serious visual impairment due to retina degeneration. Currently limited treatment is available for laser eye injury. In the current study, we investigated the therapeutic potential of bone marrow-derived stem cells (BMSCs) for laser-induced retinal trauma. Lineage negative bone marrow cells (Lin(-) BMCs) were labelled with quantum dots (Qdots) to track the cells in vivo. Lin(-) BMCs survived well after intravitreal injection. In vivo bromodeoxyuridine (BrdU) labelling showed these cells continued to proliferate and integrate into injured retinas. Furthermore, they expressed markers that distinguished retinal pigment epithelium (RPE), endothelium, pericytes and photoreceptors. Our results suggest that BMSCs participate in the repair of retinal lesions by differentiating into retinal cells. Intravitreal transplantation of BMSCs is a potential treatment for laser-induced retinal trauma.

Wang, H. H., et al. (2011). "Fluorescent gold nanoclusters as a biocompatible marker for in vitro and in vivo tracking of endothelial cells." ACS Nano **5**(6): 4337-4344.

We have been investigating the fluorescent property and biocompatibility of novel fluorescent gold nanoclusters (FANC) in human aortic endothelial cells (HAEC) and endothelial progenitor cells (EPC). FANC (50-1000 nmol/L) was delivered into cells via the liposome complex. The fluorescence lasted for at least 28 days with a half-life of 9 days in vitro. Examination of 12 transcripts regulating the essential function of endothelial cells after a 72 h delivery showed that only the vascular cell adhesion molecule 1 and the vascular endothelial cadherin were down-regulated at high concentration (500 nmol/L). In addition, no activation of caspase 3 or proliferating cell nuclear antigens was detected. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay demonstrated that, unlike the markedly suppressed viability in cells treated with quantum dots, FANC had minimal effect on the viability, unless above 500 nmol/L, at which level a minor reduction of viability mainly caused by liposome was found. Tube formation assay showed no impaired angiogenesis in the EPC treated with FANC. In vivo study using hindlimb ischemic mice with an intramuscular injection of FANC-labeled human EPC showed that the cells preserved an angiogenic potential and exhibited traceable signals after 21 days. These findings demonstrated that FANC is a promising biocompatible fluorescent probe.

Wang, J., et al. (2014). "Uptake, translocation, and transformation of quantum dots with cationic versus anionic coatings by Populus deltoides x nigra cuttings." Environ Sci Technol **48**(12): 6754-6762.

Manipulation of the organic coatings of nanoparticles such as quantum dots (QDs) to enhance specific applications may also affect their interaction and uptake by different organisms. In this study, poplar trees (Populus deltoides x nigra) were exposed hydroponically to 50-nM CdSe/CdZnS QDs coated with cationic polyethylenimine (PEI) (35.3 +/- 6.6 nm) or poly(ethylene glycol) of anionic poly(acrylic acid) (PAA-EG) (19.5 +/- 7.2 nm) to discern how coating charge affects nanoparticle uptake, translocation, and transformation within woody plants. Uptake of cationic PEI-QDs was 10 times faster despite their larger hydrodynamic size and higher extent of aggregation (17 times larger than PAA-EG-QDs after 11-day incubation in the hydroponic medium), possibly due to electrostatic attraction to the negatively charged root cell wall. QDs cores aggregated upon root uptake, and their translocation to poplar shoots (negligible for PAA-EG-QDs and 0.7 ng Cd/mg stem for PEI-QDs) was likely limited by the endodermis. After 2-day exposure, PEI and PAA-EG coatings were likely degraded from the internalized QDs inside the plant, leading to the aggregation of the metallic cores and a "red-shift" of fluorescence. The fluorescence of PEI-QD aggregates was stable inside the roots through the 11-day exposure period. In contrast, the PAA-EG-QD aggregates lost fluorescence inside the plant after 11 days probably due to destabilization of the coating, even though these QDs were stable in the hydroponic solution. Overall, these results highlight the importance of coating properties in the rate and extent to which nanoparticles are assimilated by plants and potentially introduced into food webs.

Wang, J., et al. (2019). "Molecular beacon immobilized on graphene oxide for enzyme-free signal amplification in electrochemiluminescent determination of microRNA." Mikrochim Acta **186**(3): 142.

An electrochemiluminescence (ECL) based biosensor is described for determination of microRNAs in the A549 cell line. Firstly, graphene oxide (GO) is dripped onto a glassy carbon electrode surface to form an interface to which one end of the capture probe (with a stem-loop structure) can be anchored through pi-interaction via dangling unpaired bases. The other end of the capture probe is directed away from the GO surface to make it stand upright. Target microRNAs can open the hairpin structure to form a double-stranded DNA-RNA structure. Two auxiliary probes, generating

a hybridization chain reaction, are used to elongate the DNA duplex. Finally, doxorubicin-modified cadmium telluride quantum dot nanoparticles (Dox-CdTe QD) are intercalated into the base pairs of the hybrid duplexes to act as signalling molecules. The ECL signal of the Dox-CdTe QD increases proportionally with the concentration of microRNAs, specifically for microRNA-21. The assay covers a wide linear range (1 fM to 0.1 nM), has a low detection limit for microRNA-21 (1 fM), and is selective, reproducible, and stable. Graphical abstract An enzyme-free amplification electrochemiluminescent assay is described to quantitative detection of microRNA in the A549 cell line. Graphene oxide was used to immobilize capture probes obviating the special modification. Doxorubicin-modified cadmium telluride quantum dot nanoparticles are intercalated into the base pairs of the hybrid duplexes to act as signalling molecules.

Wang, L., et al. (2017). "Fluorescent Poly(glycerol-co-sebacate) Acrylate Nanoparticles for Stem Cell Labeling and Longitudinal Tracking." ACS Appl Mater Interfaces **9**(11): 9528-9538.

The stable presence of fluorophores within the biocompatible and biodegradable elastomer poly(glycerol-co-sebacate) acrylate (PGSA) is critical for monitoring the transplantation, performance, and degradation of the polymers in vivo. However, current methods such as physically entrapping the fluorophores in the polymer matrix or providing a fluorescent coating suffer from rapid leakage of fluorophores. Covalent conjugation of fluorophores with the polymers and the subsequent core-cross-linking are proposed here to address this challenge. Taking rhodamine as the model dye and PGSA nanoparticles (NPs) as the model platform, we successfully showed that the synthesized rhodamine-conjugated PGSA (PGSAR) NPs only released less than 30% rhodamine at day 28, whereas complete release of dye occurred for rhodamine-encapsulated PGSA (PGSA-p-R) NPs at day 7 and 57.49% rhodamine was released out for the un-cross-linked PGSAR NPs at day 28. More excitingly, PGSAR NPs showed a strong quantum yield enhancement (26.24-fold) of the fluorophores, which was due to the hydrophobic environment within PGSAR NPs and the restricted rotation of (6-diethylamino-3H-xanthen-3-ylidene) diethyl group in rhodamine after the conjugation and core-cross-linking. The stable presence of dye in the NPs and enhanced fluorescence allowed a longitudinal tracking of stem cells both in vitro and in vivo for at least 28 days.

Wang, Q., et al. (2019). "Experimental and simulation studies of strontium/fluoride-codoped hydroxyapatite nanoparticles with osteogenic and antibacterial activities." Colloids Surf B Biointerfaces **182**: 110359.

Multiple ions codoping may effectively modulate physicochemical and biological properties of hydroxyapatite (HA) for diverse biomedical applications. This study synthesized strontium (Sr)-, fluorine (F)- doped, and Sr/F-codoped HA nanoparticles by a hydrothermal method, and investigated the effect of ion doping on characteristics of HA, including crystallinity, crystal size, lattice parameters, and substitution sites by experiments and simulation with density functional theory (DFT) methods. It was found that, Sr doping increased the lattice parameters of HA whereas F doping decreased these parameters. Additionally, F doping enhanced the structural stability of the Sr-doped HA. F doping created excellent antibacterial properties to effectively inhibit growth of Streptococcus mutans. An appropriate Sr doping level endowed HA with optimum osteogenic ability to promote osteoblastic differentiation of bone marrow stem cells. These suggest that, Sr/F codoping is an effective approach to synthesizing HA-based materials with both antibacterial and osteogenic properties. More broadly, HA nanomaterials with specific characteristics may be designed for meeting diverse requirements from biomedical applications.

Wang, Y., et al. (2013). "Commercial nanoparticles for stem cell labeling and tracking." Theranostics **3**(8): 544-560.

Stem cell therapy provides promising solutions for diseases and injuries that conventional medicines and therapies cannot effectively treat. To achieve its full therapeutic potentials, the homing process, survival, differentiation, and engraftment of stem cells post transplantation must be clearly understood. To address this need, non-invasive imaging technologies based on nanoparticles (NPs) have been developed to track transplanted stem cells. Here we summarize existing commercial NPs which can act as contrast agents of three commonly used imaging modalities, including fluorescence imaging, magnetic resonance imaging and photoacoustic imaging, for stem cell labeling and tracking. Specifically, we go through their technologies, industry distributors, applications and existing concerns in stem cell research. Finally, we provide an industry perspective on the potential challenges and future for the development of new NP products.

Wang, Z., et al. (2022). "rBMSC osteogenic differentiation enhanced by graphene quantum dots loaded with immunomodulatory layered double hydroxide nanoparticles." Biomed Mater **17**(2).

Bone tissue defects caused by disease, trauma, aging or genetic factors emerged as one of the main factors that endanger human health. At present, advanced development of bone tissue engineering and regenerative medicine focused on the biomaterials

regulated stem cell for responsive differentiation.In vivotransplantation of allogeneic bone materials has the needs of both osteogenic and immune regulation function. In this study, we utilized the extensively proved biocompatible layered double hydroxide (LDH) nanoparticles as the nanocarrier of graphene quantum dots (GQD), the functional loading was validated by characteristics analysis of scanning electron microscopy, surface zeta potential, X-ray diffraction and fourier transform infrared spectroscopy. Further, we investigated the cellular uptake of nanoparticles in rat bone marrow derived mesenchymal stem cells, the significant enhanced endocytosis was occurred in LDH-GQD treated groups. The enhanced osteogenic differentiation abilities of LDH-GQD were systematically investigated through alkaline phosphatase staining, alizarin red staining and qPCR analysis. In addition, the anti-inflammatory regulation of LDH facilitated the phenotypic transition of macrophage in LDH-GQD nanocomposites. Overall, the successful construction and functional validation of nanomaterials in this study will provide clinical therapeutic potential in bone defects regeneration.

Wen, S., et al. (2020). "Amylase-Protected Ag Nanodots for in vivo Fluorescence Imaging and Photodynamic Therapy of Tumors." Int J Nanomedicine **15**: 3405-3414.

BACKGROUND: Fluorescent metallic nanodots (NDs) have become a promising nanoprobe for a wide range of biomedical applications. Because Ag NDs have a high tendency to be oxidized, their synthesis and storage are a big challenge. Thus, the method for preparing stable Ag NDs is urgently needed. Surface modification and functionalization can enrich the capability of Ag NDs. METHODS: In this work, fluorescent Ag NDs were synthesized in deoxygenated water by using porcine pancreatic alpha-amylase (PPA) as the stabilizing/capping agent. The absorption and fluorescence of PPA-protected Ag NDs (PPA@AgNDs) were measured with a spectrophotometer and a spectrofluorometer, respectively. The morphology of PPA@AgNDs was characterized by high-angle annular dark-field (HAADF) scanning transmission electron microscopy (STEM). The biocompatibility of PPA@AgNDs was evaluated by tetrazolium (MTT)-based assay. PolyLys-Cys-SH (sequence: KKKKKKC) peptides were conjugated to PPA@AgNDs via heterobifunctional crosslinkers. PolyLys-Cys-linked PPA@AgNDs absorbed 5-aminolevulinic acid (ALA) by electrostatic interaction at physiological pH. The capability of tumor targeting was evaluated by intravenously injecting PPA@AgND-ALA into 4T1 breast cancer xenograft mouse models. Photodynamic therapy (PDT) against tumors was performed under 635 nm laser irradiation. RESULTS: PPA@AgNDs emitted at 640 nm with quantum yield of 2.1%. The Ag NDs exhibited strong photostability over a long period and a fluorescence lifetime of 5.1 ns. PPA@AgNDs easily entered the cells to stain the nuclei, showing the capabilities of living cell imaging with negligible cytotoxicity. ALA-loaded PPA@AgNDs (PPA@AgND-ALA) presented the superiority of passive tumor targeting via the enhanced permeability and retention (EPR) effect. Tumors were visualized in the near-infrared (NIR) region with reduced background noise. ALA molecules released from PPA@AgND-ALA was converted into the photosensitizer (PS) of protoporphyrin IX (PpIX) intracellularly and intratumorally, which greatly improved the PDT efficacy. CONCLUSION: Our approach opens a new way to design a novel theranostic nanoplatform of PPA@AgND-ALA for effective tumor targeting and fluorescence image-guided PDT.

Wen, X., et al. (2014). "In vivo monitoring of neural stem cells after transplantation in acute cerebral infarction with dual-modal MR imaging and optical imaging." Biomaterials **35**(16): 4627-4635.

Stem cell therapies are promising strategies for the treatment of stroke. However, their clinical translation has not been fully realized due, in part, to insufficient ability to track stem cell migration and survival longitudinally over long periods of time in vivo. In this work, we synthesized a new class of manometer-sized cationic polymersomes loaded with superparamagnetic iron oxide nanoparticles and quantum dots for in vivo dual-modal imaging of stem cells. The results demonstrated that the synthesized cationic polymersomes can act as an effective and safety carrier to transfer image labels into neural stem cells, upon which the distribution and migration of grafted stem cells could be monitored by MR imaging up to 6 weeks and by fluorescence imaging within 4 weeks in the context of ischaemic brain injury. Cationic polymersomes hold great promise in the longitudinal monitoring of transplanted stem cells by using dual-modal MRI and optical imaging.

Williams, A., et al. (2010). "Technologies and methods used for the detection, enrichment and characterization of cancer stem cells." Natl Med J India **23**(6): 346-350.

Cancer stem cells (CSCs) represent a subclass of tumour cells with the ability for self-renewal, production of differentiated progeny, prolonged survival, resistance to damaging therapeutic agents, and anchorage-independent survival, which together make this population effectively equipped to metastasize, invade and colonize secondary tissues in the face of therapeutic intervention. In recent years, investigators have increasingly focused on the characterization of CSCs to better understand the mechanisms that govern malignant disease progression in an effort to develop

more effective, targeted therapeutic agents. The primary obstacle to the study of CSCs, however, is their rarity. Thus, the study of CSCs requires the use of sensitive and efficient technologies for their enrichment and detection. This review discusses technologies and methods that have been adapted and used to isolate and characterize CSCs to date, as well as new potential directions for the enhanced enrichment and detection of CSCs. While the technologies used for CSC enrichment and detection have been useful thus far for their characterization, each approach is not without limitations. Future studies of CSCs will depend on the enhanced sensitivity and specificity of currently available technologies, and the development of novel technologies for increased detection and enrichment of CSCs.

Winter, R. L., et al. (2018). "Growth and function of equine endothelial colony forming cells labeled with semiconductor quantum dots." BMC Vet Res **14**(1): 247.

BACKGROUND: Endothelial progenitor cells (EPCs) contribute to neovascularization and vascular repair in vivo and are attractive for clinical use in ischemic disease. Tracking of stem and progenitor cells is essential to determine engraftment after administration. Semiconductor quantum dots (QD) are promising for cell labeling due to their ease of uptake by many cell lines and their continued presence after many cell generations. The purpose of this study was to evaluate function and growth of equine EPCs after QD labeling. Additionally, this study evaluated the duration of QD label retention and mechanisms of QD label loss. RESULTS: Endothelial colony forming cells (ECFCs) from adult horses (N = 3) were employed for this study, with QD labeled and unlabeled ECFCs tested from each horse. Cell proliferation of ECFCs labeled with QD at 20 nM was quantified by comparing the number of cell doublings per day (NCD) and the population doubling time (PDT) in labeled and unlabeled cells. Function of labeled and unlabeled ECFCs was assessed by comparing uptake of acetylated low-density lipoprotein (DiO-Ac-LDL) and tubule formation on growth factor containing matrix. Cell proliferation was not impacted by QD labeling; both NCD (p = 0. 95) and PDT (P = 0. 91) did not differ between unlabeled and QD labeled cells. Function of ECFCs assessed by DiO-Ac-LDL and tubule formation was also not different between unlabeled and QD labeled cells (P = 0. 33 and P = 0. 52, respectively). ECFCs retained their QD labeling over 7 passages with both 5 nM and 20 nM label concentrations. Reduction in label intensity was observed over time, and the mechanism was determined to be cell division. CONCLUSIONS: Equine ECFCs are effectively labeled with QD, and QD concentrations up to 20 nM do not affect cell growth or function. QD label loss is a result of cell division. The use of QD labeling with equine EPCs may be an ideal way to track engraftment of EPCs for in vivo applications.

Winter, R. L., et al. (2020). "Cell engraftment, vascularization, and inflammation after treatment of equine distal limb wounds with endothelial colony forming cells encapsulated within hydrogel microspheres." BMC Vet Res **16**(1): 43.

BACKGROUND: Endothelial colony forming cells (ECFCs) may be useful therapeutically in conditions with poor blood supply, such as distal limb wounds in the horse. Encapsulation of ECFCs into injectable hydrogel microspheres may ensure cell survival and cell localization to improve neovascularization and healing. Autologous ECFCs were isolated from 6 horses, labeled with quantum nanodots (QD), and a subset were encapsulated in poly(ethylene) glycol fibrinogen microspheres (PEG-Fb MS). Full-thickness dermal wounds were created on each distal limb and injected with empty PEG-Fb MS, serum, ECFCs, or ECFCs encapsulated into PEG- Fb MS (ECFC/MS). Analysis included wound surface area (WSA), granulation tissue scoring (GS), thermography, collagen density staining, and immunohistochemical staining for endothelial and inflammatory cells. The purpose of this study was to track cell location and evaluate wound vascularization and inflammatory response after injection of ECFC/MS or naked ECFCs in equine distal limb wounds. RESULTS: ECFCs were found near and within newly formed blood vessels up to 3 weeks after injection. ECFC and ECFC/MS groups had the greatest blood vessel quantity at week 1 in the wound periphery. Wounds treated with ECFCs and ECFC/MS had the lowest density of neutrophils and macrophages at week 4. There were no significant effects of ECFC or ECFC/MS treatment on other measured parameters. CONCLUSIONS: Injection of microsphere encapsulated ECFCs was practical for clinical use and well-tolerated. The positive ECFC treatment effects on blood vessel density and wound inflammation warrant further investigation.

Wittmann, C. and H. Pfanz (2007). "Temperature dependency of bark photosynthesis in beech (Fagus sylvatica L.) and birch (Betula pendula Roth.) trees." J Exp Bot **58**(15-16): 4293-4306.

Temperature dependencies of stem dark respiration (R(d)) and light-driven bark photosynthesis (A(max)) of two temperate tree species (Fagus sylvatica and Betula pendula) were investigated to estimate their probable influence on stem carbon balance. Stem R(d) was found to increase exponentially with increasing temperatures, whereas A(max) levelled off or decreased at the highest temperatures chosen (35-40 degrees C). Accordingly, a linear relationship between respiratory and assimilatory metabolism was only found at

moderate temperatures (10-30 degrees C) and the relationship between stem R(d) and A(max) clearly departed from linearity at chilling (5 degrees C) and at high temperatures (35-40 degrees C). As a result, the proportional internal C-refixation rate also decreased non-linearly with increasing temperature. Temperature response of photosystem II (PSII) photochemistry was also assessed. Bark photochemical yield (Delta F/F(m)') followed the same temperature pattern as bark CO(2) assimilation. Maximum quantum yield of PSII (F(v)/F(m)) decreased drastically at freezing temperatures (-5 degrees C), while from 30 to 40 degrees C only a marginal decrease in F(v)/F(m) was found. In in situ measurements during winter months, bark photosynthesis was found to be strongly reduced. Low temperature stress induced an active down-regulation of PSII efficiency as well as damage to PSII due to photoinhibition. All in all, the benefit of bark photosynthesis was negatively affected by low (<5 degrees C) as well as high temperatures (>30 degrees C). As the carbon balance of tree stems is defined by the difference between photosynthetic carbon gain and respiratory carbon loss, this might have important implications for accurate modelling of stem carbon balance.

Witty, N. (2013). "Quantum leap year." Stem Cell Reports **1**(1): 3-4.

Wu, S. Q., et al. (2016). "Penetrating Peptide-Bioconjugated Persistent Nanophosphors for Long-Term Tracking of Adipose-Derived Stem Cells with Superior Signal-to-Noise Ratio." Anal Chem **88**(7): 4114-4121.

Reliable long-term in vivo tracking of stem cells is of great importance in stem cell-based therapy and research. Fluorescence imaging with in situ excitation has significant autofluorescence background, which results in poor signal-to-noise ratio (SNR). Here we report TAT penetrating peptide-bioconjugated long persistent luminescence nanoparticles (LPLNP-TAT) for long-term tracking of adipose-derived stem cells (ASC) without constant external excitation. LPLNP-TAT exhibits near-infrared emitting, red light renewable capability, and superior in vivo imaging depth and SNR compared with conventional organic dye and quantum dots. Our findings show that LPLNP-TAT can successfully label ASC without impairing their proliferation and differentiation and can effectively track ASC in skin-regeneration and tumor-homing models. We believe that LPLNP-TAT represents a new generation of cell tracking probes and will have broad application in diagnosis and therapy.

Wu, Y., et al. (2016). "Functional quantum dot-siRNA nanoplexes to regulate chondrogenic differentiation of mesenchymal stem cells." Acta Biomater **46**: 165-176.

SOX9 plays an important role in mesenchymal condensations during the early development of embryonic skeletons. However, its function in the chondrogenic differentiation of adult mesenchymal stem cells (MSCs) has not been fully investigated because SOX9 RNA interference in adult MSCs has seldom been studied. This study used SOX9 gene as the target gene and the quantum dot (QD)-based nanomaterial QD-NH2 (ZnS shell and poly-ethylene glycol (PEG) coating) with a fluorescent tracer function as the gene carrier to transfect siSOX9 into MSCs after sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC) activation in vitro and in vivo. The results showed that QD-SMCC could effectively bind and deliver siRNAs into the MSCs, followed by efficient siRNA escape from the endosomes. The siRNAs released from QD-SMCC retained their structural integrity and could effectively inhibit the targeted gene expression, leading to reduced chondrogenic differentiation of MSCs and delayed cartilage repair. QDs were excreted from living cells instead of dead cells, and the ZnS shell and PEG coating layer greatly reduced the cytotoxicity of the QDs. The transfection efficiency of QD-SMCC was superior to that of polyethylenimine (PEI). In addition, QD-SMCC has an intrinsic signal for noninvasive imaging of siRNA transport. The results indicate that SOX9 is imperative for the chondrogenesis of MSCs and QD-SMCC has great potential for real-time tracking of transfection. STATEMENT OF SIGNIFICANCE: In this study, we developed functional quantum dot (QD) nanoplexes by sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC) activation of PEG-coated CdSe/ZnS QDs as the gene carrier of siRNA to study the effect of SOX9 RNA interference on the chondrogenic differentiation of MSCs. This study confirmed the importance of SOX9 in chondrogenesis, as evidenced by the findings that SOX9 knockdown significantly inhibited the expression of cartilage-specific markers including acan and col2a1 in MSCs and further delayed cartilage repair. Moreover, QD-SMCC has an intrinsic signal for noninvasive imaging of siRNA transport. The results indicate that SOX9 is imperative for the chondrogenesis of MSCs and QD-SMCC has great potential for real-time tracking of transfection.

Xiong, R., et al. (2016). "Cytosolic Delivery of Nanolabels Prevents Their Asymmetric Inheritance and Enables Extended Quantitative in Vivo Cell Imaging." Nano Lett **16**(10): 5975-5986.

Long-term in vivo imaging of cells is crucial for the understanding of cellular fate in biological processes in cancer research, immunology, or in cell-

based therapies such as beta cell transplantation in type I diabetes or stem cell therapy. Traditionally, cell labeling with the desired contrast agent occurs ex vivo via spontaneous endocytosis, which is a variable and slow process that requires optimization for each particular label-cell type combination. Following endocytic uptake, the contrast agents mostly remain entrapped in the endolysosomal compartment, which leads to signal instability, cytotoxicity, and asymmetric inheritance of the labels upon cell division. Here, we demonstrate that these disadvantages can be circumvented by delivering contrast agents directly into the cytoplasm via vapor nanobubble photoporation. Compared to classic endocytic uptake, photoporation resulted in 50 and 3 times higher loading of fluorescent dextrans and quantum dots, respectively, with improved signal stability and reduced cytotoxicity. Most interestingly, cytosolic delivery by photoporation prevented asymmetric inheritance of labels by daughter cells over subsequent cell generations. Instead, unequal inheritance of endocytosed labels resulted in a dramatic increase in polydispersity of the amount of labels per cell with each cell division, hindering accurate quantification of cell numbers in vivo over time. The combined benefits of cell labeling by photoporation resulted in a marked improvement in long-term cell visibility in vivo where an insulin producing cell line (INS-1E cell line) labeled with fluorescent dextrans could be tracked for up to two months in Swiss nude mice compared to 2 weeks for cells labeled by endocytosis.

Yan, C., et al. (2020). "Photoluminescent functionalized carbon quantum dots loaded electroactive Silk fibroin/PLA nanofibrous bioactive scaffolds for cardiac tissue engineering." J Photochem Photobiol B **202**: 111680.

Tissue engineering and stem cell rehabilitation are the hopeful aspects that are being investigated for the management of Myocardial Infarction (MI); cardiac patches have been used to start myocardial rejuvenation. In this study, we engineered p-phenylenediamine surface functionalized (modif-CQD) into the Silk fibroin/PLA (SF/PLA) nanofibrous bioactive scaffolds with improved physico-chemical abilities, mechanical and cytocompatibility to cardiomyocytes. The micrograph results visualized the morphological improved spherical modif-CQD have been equivalently spread throughout the SF/PLA bioactive cardiac scaffolds. The fabricated CQD@SF/PLA nanofibrous bioactive scaffolds were highly porous with fully consistent pores; effectively improved young modulus and swelling asset for the suitability and effective implantation efficacy. The scaffolds were prepared with rat cardiomyocytes and cultured for up to 7days, without electrical incentive. After 7days of culture, the scaffold pores all over the construct volume were overflowing with cardiomyocytes. The metabolic activity and viability of the cardiomyocytes in CQD@SF/PLA scaffolds were significantly higher than cardiomyocytes in Silk fibroin /PLA scaffolds. The integration of CQD also influenced greatly and increases the expression of cardiac-marker genes. The results of the present investigations evidently recommended that well-organized cardiac nanofibrous scaffold with greater cardiac related mechanical abilities and biocompatibilities for cardiac tissue engineering and nursing care applications.

Yan, J., et al. (2015). "Simultaneously targeted imaging cytoplasm and nucleus in living cell by biomolecules capped ultra-small GdOF nanocrystals." Biomaterials **59**: 21-29.

Simultaneously targeted imaging cytoplasm and nucleus in living cell by just one photoluminescent nanocrystals has been a giant challenge in nanobiotechnology and nanomedicine. Herein we report a novel Arg-Gly-Asp peptide (RGD) or cysteine (Cys) functionalized ultra-small GdOF nanocrystals for simultaneously targeted imaging cell cytoplasm and nucleus. As-prepared RGD@GdOF and Cys@GdOF nanocrystals possessed high water dispersibility, ultra-small size (about 5 nm) and double emissions (545 nm and 587 nm) with high quantum yield. Such functionalized nanocrystals presented high cellular biocompatibility and were successfully used to label living cells with very high signal to noise ratio. The living cells cytoplasm and nucleus (cancer cells and stem cells) could be imaged simultaneously through the mergence of green and red emission of nanocrystals, based on mechanism of fluorescent intensity difference. These functionalized nanocrystals also exhibited significantly higher photostability and brightness as compared to dyes. Such the ultra-small size, high photostability and intensity, double emissions, excellent biocompatibility and targeted ability, make as-prepared functionalized nanocrystals particularly promising for cellular and molecular-level bioimaging applications.

Yan, J., et al. (2018). "The effect of surface charge on the cytotoxicity and uptake of carbon quantum dots in human umbilical cord derived mesenchymal stem cells." Colloids Surf B Biointerfaces **171**: 241-249.

Carbon quantum dots (CQDs) are emerging as an ideal agent for efficient stem cell labeling. In current study, we synthesized a series of CQDs carrying different surface charges by changing the mass ratio of diammonium citrate (DC) and spermidine (Spd), and evaluated the effects of different surface charges on the cytotoxicity, cellular uptake, stability in human umbilical cord derived mesenchymal stem cells (hUCMSCs). We ascertained the optimal labeling time

(24 h) and subtoxic concentration (50 mug/mL) of all different charged CQDs. Our results demonstrated that, although positively charged CQDs are more cytotoxic and have lower photoluminescence (PL) compared to negative CQDs, they still have higher labeling efficiency for their higher uptake capacity. We found that relatively weak positive surface charges enabled CQDs to possess good biocompatibility and labeling efficiency in hUCMSCs. This work will helpfully contribute to the design and optimization of CQDs for tracking stem cells and further benefit to clinical research and application.

Yan, R. H., et al. (2010). "[Magnetic/luminescent quantum dots bifunctional nanoparticles labeling of rat bone mesenchymal stem cells]." Zhonghua Yi Xue Za Zhi **90**(1): 56-60.

OBJECTIVE: To evaluate the dual-labeling efficiency of magnetic and luminescent quantum dots bifunctional nanoparticles to rat bone mesenchymal stem cells (BMSC). METHODS: Rat BMSC were isolated, purified, and expanded. Magnetic/luminescent bifunctional nanoparticles (Fe(3)O(4)@CdTe@SiO(2)), were prepared by using silicon dioxide (SiO(2)) to encapsulate simultaneously Fe(3)O(4) and CdTe quantum dots. BMSC were incubated with the Fe(3)O(4)@CdTe@SiO(2) nanoparticles which iron concentration was 25 microg/ml. Fluorescence microscope was used to detect the fluorescence of the intracellular nanoparticles. The dual-labeled BMSC with various concentration underwent ex vivo MR imaging with T(1)WI, T(2)WI and T(2)(*)WI sequences. To show the intracellular iron of labeled cells, prussian blue stain was performed. Spectrophotometer was used to detect the iron concentration in the cells. RESULTS: Intracellular red fluorescence of Fe(3)O(4)@CdTe@SiO(2) can be observed via fluorescent microscopy. Rat BMSC could be effectively dual-labeled with approximately 90% efficiency. The MR images with T(2)WI and T(2)(*)WI sequences, especially with T(2)(*)WI sequence, showed that the signals of the dual-labeled BMSC were lower than those of the unlabeled cells. Cellular total iron is 14.05 + or - 4.15 pg per cell. Iron containing intracytoplasmic vesicles could be observed with Prussian blue staining. CONCLUSION: Rat BMSC can be dual-labeled successfully with Fe(3)O(4)@CdTe@SiO(2) magnetic/luminescent bifunctional nanoparticles successfully, and might serve as a tool for magnetic resonance imaging and in vivo optical imaging.

Yang, C., et al. (2019). "Seeing the fate and mechanism of stem cells in treatment of ionizing radiation-induced injury using highly near-infrared emissive AIE dots." Biomaterials **188**: 107-117.

Ionizing radiation-induced skin injury is a common and severe side effect of radiotherapy suffered by cancer patients. Although the therapy using stem cells has been demonstrated to be effective, fully grasping their role in the repair of radiation-induced skin damage remains challenging owing to the lack of highly reliable cell trackers. Herein, we report the design and synthesis of a highly near-infrared emissive organic nanodots with aggregation-induced emission (AIE) characteristic, which give excellent performance in seeing the fate and regenerative mechanism of adipose-derived stem cells (ADSCs) in treatment of radiation-induced skin injury. The resultant AIE dots show a rather high quantum yield of 33% in aqueous media, prominent retention ability in ADSCs without leakage, good biocompatibility during the ADSC differentiation and proliferation as well as excellent injury relief capability on radiation-induced endothelial cells injury. In vivo studies reveal that the AIE dots are capable of serving as an effective fluorescent cell tracker to precisely trace the behavior of the transplanted ADSCs in radiation-induced skin injury-bearing mice and help to understand the ADSCs therapeutic mechanism for at least one month. This study will provide new materials and insights into the stem cell therapy of radiation-induced injury.

Yang, F., et al. (2017). "Endogenous MicroRNA-Triggered and Real-Time Monitored Drug Release via Cascaded Energy Transfer Payloads." Anal Chem **89**(19): 10239-10247.

It is a great challenge to design a drug delivery system with a controlled manner, especially one triggered by an exclusive endogenous disease marker and with an easily tracked release process. Herein, we developed a drug delivery platform of carbon dots which were connected to a stem-loop molecular beacon loaded with doxorubicin and polyethylene glycol modified folic acid. Such a platform enables one to release drugs on demand under the stimuli of endogenous microRNA-21, and turn on the fluorescence of carbon dots and doxorubicin, which allows one to monitor the drug release process. The intracellular experiment indicated that folic acid could mediate endocytosis of the nanocarrier, and the overexpressed endogenous microRNA-21 served as a unique key to unlock the drug nanocarrier by competitive hybridization with the molecular beacon, which finally resulted in fluorescence recovery and realized a chemotherapeutic effect within human breast cancer cells. The nanocarrier may have potential application in personalized treatment of different cancer subtypes in which the corresponding miRNAs are overexpressed.

Yang, H. N., et al. (2014). "The effect of quantum dot size and poly(ethylenimine) coating on the efficiency of gene delivery into human mesenchymal stem cells." Biomaterials **35**(29): 8439-8449.

Quantum dot (QDs) have been employed as bioimaging agents and delivery vehicles for gene therapeutics in several types of cells. In this study, we fabricated multiple QD bundled nanoparticles (NPs) to investigate the effect of QD size and poly(ethylenimine) (PEI) coating on the efficiency of gene delivery into human mesenchymal stem cells (hMSCs). Several types of QDs, which exhibit different ranges of particle size and fluorescence when employed, were coated with PEI to alter their negative charges and to enable them to be bundled into larger particles. Using specific wavelengths of QDs for bioimaging, gene-complexed QD bundled NPs were easily detected in the hMSCs using several different methods such as fluorescence-activated cell sorter, confocal laser scanning microscopy, and in vivo optical imaging. These PEI-coated, bundled QD NPs exhibited significantly higher gene transfection efficacy than single-type QDs. Particularly, the largest QD bundled NPs examined, QD655, had a much higher uptake capability and greater gene expression ability than the other QD NPs (QD525, QD565, and QD605). We believe that our findings help to enrich knowledge of design considerations that will aid in the engineering of QD NPs for stem cell application in the future.

Yang, H. N., et al. (2012). "Transfection of VEGF(165) genes into endothelial progenitor cells and in vivo imaging using quantum dots in an ischemia hind limb model." Biomaterials **33**(33): 8670-8684.

Endothelial progenitor cells (EPCs) were transfected with fluorescently labeled quantum dot nanoparticles (QD NPs) with or without VEGF(165) plasmid DNA (pDNA) to probe the EPCs after in vivo transplantation and to test whether they presented as differentiated endothelial cells (ECs). Bare QD NPs and QD NPs coated with PEI or PEI + VEGF(165) genes were characterized by dynamic light scattering, scanning electron microscopy, and atomic force microscopy. Transfection of EPCs with VEGF(165) led to the expression of specific genes and proteins for mature ECs. A hind limb ischemia model was generated in nude mice, and VEGF(165) gene-transfected EPCs were transplanted intramuscularly into the ischemic limbs. At 28 days after transplantation, the VEGF(165) gene-transfected EPCs significantly increased the number of differentiated ECs compared with the injection of medium or bare EPCs without VEGF(165) genes. Laser Doppler imaging revealed that blood perfusion levels were increased significantly by VEGF(165) gene-transfected EPCs compared to EPCs without VEGF(165). Moreover, the transplantation of VEGF(165) gene-transfected EPCs increased the specific gene and protein expression levels of mature EC markers and angiogenic factors in the animal model.

Yang, Q., et al. (2020). "Quantum dots are conventionally applicable for wide-profiling of wall polymer distribution and destruction in diverse cells of rice." Talanta **208**: 120452.

Plant cell walls represent enormous biomass resources for biofuels, and it thus becomes important to establish a sensitive and wide-applicable approach to visualize wall polymer distribution and destruction during plant growth and biomass process. Despite quantum dots (QDs) have been applied to label biological specimens, little is reported about its application in plant cell walls. Here, semiconductor QDs (CdSe/ZnS) were employed to label the secondary antibody directed to the epitopes of pectin or xylan, and sorted out the optimal conditions for visualizing two polysaccharides distribution in cell walls of rice stem. Meanwhile, the established QDs approach could simultaneously highlight wall polysaccharides and lignin co-localization in different cell types. Notably, this work demonstrated that the QDs labeling was sensitive to profile distinctive wall polymer destruction between alkali and acid pretreatments with stem tissues of rice. Hence, this study has provided a powerful tool to characterize wall polymer functions in plant growth and development in vivo, as well as their distinct roles during biomass process in vitro.

Yang, X., et al. (2022). "Probing the Intracellular Delivery of Nanoparticles into Hard-to-Transfect Cells." ACS Nano **16**(6): 8751-8765.

Hard-to-transfect cells are cells that are known to present special difficulties in intracellular delivery of exogenous entities. However, the special transport behaviors underlying the special delivery problem in these cells have so far not been examined carefully. Here, we combine single-particle motion analysis, cell biology studies, and mathematical modeling to investigate nanoparticle transport in bone marrow-derived mesenchymal stem cells (BMSCs), a technologically important type of hard-to-transfect cells. Tat peptide-conjugated quantum dots (QDs-Tat) were used as the model nanoparticles. Two different yet complementary single-particle methods, namely, pair-correlation function and single-particle tracking, were conducted on the same cell samples and on the same viewing stage of a confocal microscope. Our results reveal significant differences in each individual step of transport of QDs-Tat in BMSCs vs a commonly used model cell line, HeLa cells. Single-particle motion analysis demonstrates that vesicle escape and cytoplasmic diffusion are dramatically more difficult in BMSCs than in HeLa cells. Cell biology studies show that BMSCs use different biological pathways for the cellular uptake, vesicular transport, and exocytosis of QDs-Tat than HeLa cells. A reaction-diffusion-advection model is employed to mathematically integrate the individual

steps of cellular transport and can be used to predict and design nanoparticle delivery in BMSCs. This work provides dissective, quantitative, and mechanistic understandings of nanoparticle transport in BMSCs. The investigative methods described in this work can help to guide the tailored design of nanoparticle-based delivery in specific types and subtypes of hard-to-transfect cells.

Yang, X., et al. (2019). "Effects of graphene oxide and graphene oxide quantum dots on the osteogenic differentiation of stem cells from human exfoliated deciduous teeth." Artif Cells Nanomed Biotechnol **47**(1): 822-832.

Graphene and its derivatives, graphene oxide (GO) and graphene oxide quantum dots (GOQDs), have recently attracted much attention as bioactive factors in differentiating stem cells towards osteoblastic lineage. The stem cells from human exfoliated deciduous teeth (SHEDs) possess the properties of self-renewal, extensive proliferation, and multiple differentiation potential, and have gradually become one of the most promising mesenchymal stem cells (MSCs) in bone tissue engineering. The purpose of this study was to explore the effects of GO and GOQDs on the osteogenic differentiation of SHEDs. In this study, GO and GOQDs facilitated SHED proliferation up to 7 days in vitro at the concentration of 1 mug/ml. Because of their excellent fluorescent properties, GOQD uptake by SHEDs was confirmed and distributed in the SHED cytoplasm. Calcium nodules formation, alkaline phosphatase (ALP) activity, and RNA and protein expression increased significantly in SHEDs treated with osteogenic induction medium containing GOQDs but decreased with osteogenic induction medium containing GO. Interestingly, the Wnt/beta-catenin signaling pathway appeared to be involved in osteogenic differentiation of SHEDs induced with GOQDs. In summary, GO and GOQDs at the concentration of 1 mug/ml promoted SHED proliferation. GOQDs induced the osteogenic differentiation of SHEDs, whilst GO slightly inhibited it.

Yang, Y., et al. (2019). "Visualizing the Fate of Intra-Articular Injected Mesenchymal Stem Cells In Vivo in the Second Near-Infrared Window for the Effective Treatment of Supraspinatus Tendon Tears." Adv Sci (Weinh) **6**(19): 1901018.

Mesenchymal stem cells (MSCs) are capable of exerting strong therapeutic potential for the treatment of supraspinatus tendon tear. However, MSC therapy remains underutilized and perhaps underrated due to the limited evidence of dynamic visualization of cellular behavior in vivo. Here, second near-infrared fluorescence imaging with biocompatible PbS quantum dots (QDs) provides a cellular migration map and information on the biodistribution and clearance processes of three densities of intra-articularly injected, labeled MSCs to treat supraspinatus tendon tear in mice. Intra-articular injection avoids entrapment of MSCs by filter organs and reduces the QD-induced organ toxicity. Notably, the MSCs share a similar migration direction, but the moderate density group is somewhat more efficient, showing the longest residence time and highest cell retention rate around the footprint during the repair stage. Furthermore, quantitative kinetic investigation demonstrates that labeled MSCs are cleared by feces and urine. Histomorphometric analysis demonstrates that the moderate density group achieves maximum therapeutic effect and labeled MSCs do not induce any injury or inflammation to major organs, which suggests that administration of too many or few MSCs may decrease their effectiveness. Such an imaging approach provides spatiotemporal evidence for response to MSC therapy in vivo, facilitating the optimization of MSC therapy.

Yi, D. K., et al. (2017). "Recent progress in nanotechnology for stem cell differentiation, labeling, tracking and therapy." J Mater Chem B **5**(48): 9429-9451.

Stem cells offer great potential for regenerative medicine due to their excellent capability to differentiate into a specialized cell type of the human body. Recently, nanomaterial based scaffolds (e.g. graphene), biodegradable polymers (e.g. PLGA: poly-d,l-lactic-co-glycolic acid), and inorganic nanoparticles (NPs, e.g. metallic, magnetic, upconversion) have made considerable advances in controlling the differentiation of stem cells. Some of the notable advances include the development of a variety of NPs such as gold, silica, selenium and graphene quantum dots (QDs) for the controlled differentiation of stem cells - human mesenchymal stem cells (hMSCs), and magnetic core-shell NPs (e.g. ZnFe2O4-Au) for the control of neural stem cells (NSCs). Multimodal imaging (MR, optical, ultrasound, photoacoustic) of stem cells provides opportunities for probing the fate of implanted cells, thereby determining the therapeutic efficacy. Novel multifunctional NPs have been developed over the years, and probed using the aforementioned imaging techniques for stem cell research. This review article underscores the recent progress in nanotechnology for stem cell differentiation, labeling, tracking and therapy. Nano/biomaterial assisted stem cell therapies for bone, heart, and liver regeneration are also delineated.

Yin, L., et al. (2022). "KRT13 promotes stemness and drives metastasis in breast cancer through a plakoglobin/c-Myc signaling pathway." Breast Cancer Res **24**(1): 7.

BACKGROUND: Keratins (KRTs) are intermediate filament proteins that interact with multiple regulatory proteins to initiate signaling cascades. Keratin 13 (KRT13) plays an important role in breast

cancer progression and metastasis. The objective of this study is to elucidate the mechanism by which KRT13 promotes breast cancer growth and metastasis. METHODS: The function and mechanisms of KRT13 in breast cancer progression and metastasis were assessed by overexpression and knockdown followed by examination of altered behaviors in breast cancer cells and in xenograft tumor formation in mouse mammary fat pad. Human breast cancer specimens were examined by immunohistochemistry and multiplexed quantum dot labeling analysis to correlate KRT13 expression to breast cancer progression and metastasis. RESULTS: KRT13-overexpressing MCF7 cells displayed increased proliferation, invasion, migration and in vivo tumor growth and metastasis to bone and lung. Conversely, KRT13 knockdown inhibited the aggressive behaviors of HCC1954 cells. At the molecular level, KRT13 directly interacted with plakoglobin (PG, gamma-catenin) to form complexes with desmoplakin (DSP). This complex interfered with PG expression and nuclear translocation and abrogated PG-mediated suppression of c-Myc expression, while the KRT13/PG/c-Myc signaling pathway increased epithelial to mesenchymal transition and stem cell-like phenotype. KRT13 expression in 58 human breast cancer tissues was up-regulated especially at the invasive front and in metastatic specimens (12/18) ($p < 0.05$). KRT13 up-regulation in primary breast cancer was associated with decreased overall patient survival. CONCLUSIONS: This study reveals that KRT13 promotes breast cancer cell growth and metastasis via a plakoglobin/c-Myc pathway. Our findings reveal a potential novel pathway for therapeutic targeting of breast cancer progression and metastasis.

Yiu, H. H., et al. (2010). "Preparation and characterization of iron oxide-silica composite particles using mesoporous SBA-15 silica as template and their internalization into mesenchymal stem cell and human bone cell lines." IEEE Trans Nanobioscience **9**(3): 165-170.

A new procedure for preparing iron oxide-silica nanocomposite particles using SBA-15 mesoporous silica as a template is described. These composite materials retained the 2-D hexagonal structure of the SBA-15 template. Transmission electron micrograms of the particles depicted the formation of iron oxide nanocrystals inside the mesochannels of SBA-15 silica framework. Powder x-ray diffraction showed that the iron oxide core of the composite particles consists of a mixture of maghemite (gamma-Fe(2)O(3)) and heamatite (alpha-Fe(2)O(3)), which is the predominant component. Superconducting quantum interference device (SQUID) magnetometry studies showed that these iron oxide-silica composite materials exhibit superparamagnetic properties. On increasing the iron oxide content, the composite particles exhibited a stronger response to magnetic fields but a less homogeneous core, with some large iron oxide particles which were thought to be formed outside the mesochannels of the SBA-15 template. Internalization of these particles into human cell lines (mesenchymal stem cells and human bone cells), which indicates their potential in medicine and biotechnology, is also discussed.

Yong, K. T., et al. (2009). "Imaging pancreatic cancer using bioconjugated InP quantum dots." ACS Nano **3**(3): 502-510.

In this paper, we report the successful use of non-cadmium-based quantum dots (QDs) as highly efficient and nontoxic optical probes for imaging live pancreatic cancer cells. Indium phosphide (core)-zinc sulfide (shell), or InP/ZnS, QDs with high quality and bright luminescence were prepared by a hot colloidal synthesis method in nonaqueous media. The surfaces of these QDs were then functionalized with mercaptosuccinic acid to make them highly dispersible in aqueous media. Further bioconjugation with pancreatic cancer specific monoclonal antibodies, such as anticlaudin 4 and antiprostate stem cell antigen (anti-PSCA), to the functionalized InP/ZnS QDs, allowed specific in vitro targeting of pancreatic cancer cell lines (both immortalized and low passage ones). The receptor-mediated delivery of the bioconjugates was further confirmed by the observation of poor in vitro targeting in nonpancreatic cancer based cell lines which are negative for the claudin-4-receptor. These observations suggest the immense potential of InP/ZnS QDs as non-cadmium-based safe and efficient optical imaging nanoprobes in diagnostic imaging, particularly for early detection of cancer.

Yoo, J. S., et al. (2011). "Evidence for an additional metastatic route: in vivo imaging of cancer cells in the primo-vascular system around tumors and organs." Mol Imaging Biol **13**(3): 471-480.

PURPOSE: Researchers have been studying the mechanisms by which metastasis can be prevented via blocking the hematogenous and the lymphatic routes for a long time now. However, metastasis is still the single most challenging obstacle for successful cancer management. In a new twist that may require some retooling of this established approach, we investigated the hypothesis that tumor metastases can occur via an independent fluid-conducting system called the primo-vascular system. PROCEDURES: The dissemination and growth of near-infrared quantum dot (NIR QD)-electroporated cancer cells in metastatic sites were investigated using in vivo multispectral imaging techniques. RESULTS: Our results show that the NIR QD-labeled cancer cells were able to migrate through

not only the blood vascular and lymphatic systems but also the primo-vascular system extending from around the tumor to inside the abdominal cavity. Furthermore, the NIR QD-labeled cancer cells, which had been seeded intraperitoneally, specifically infiltrated the primo-vascular system in the omentum and in the gonadal fat. CONCLUSIONS: These findings strongly suggest that the primo-vascular system may be an additional metastasis route, complementing the lymphatic and hematogenous routes, which facilitate the dissemination and colonization of cancer cells at secondary sites.

Yoo, M., et al. (2014). "Analysis of human embryonic stem cells with regulatable expression of the cell adhesion molecule l1 in regeneration after spinal cord injury." J Neurotrauma **31**(6): 553-564.

Cell replacement therapy is one potential avenue for central nervous system (CNS) repair. However, transplanted stem cells may not contribute to long-term recovery of the damaged CNS unless they are engineered for functional advantage. To fine tune regenerative capabilities, we developed a human neural cell line expressing L1, a regeneration-conducive adhesion molecule, under the control of a doxycycline regulatable Tet-off promoter. Controlled expression of L1 is desired because overexpression after regenerative events may lead to adverse consequences. The regulated system was tested in several cell lines, where doxycycline completely eliminated green fluorescent protein or L1 expression by 3-5 days in vitro. Increased colony formation as well as decreased proliferation were observed in H9NSCs without doxycycline (hL1-on). To test the role of L1 in vivo after acute compression spinal cord injury of immunosuppressed mice, quantum dot labeled hL1-on or hL1-off cells were injected at three sites: lesion; proximal; and caudal. Mice transplanted with hL1-on cells showed a better Basso Mouse Scale score, when compared to those with hL1-off cells. As compared to the hL1-off versus hL1-on cell transplanted mice 6 weeks post-transplantation, expression levels of L1, migration of transplanted cells, and immunoreactivity for tyrosine hydroxylase were higher, whereas expression of chondroitin sulfate proteoglycans was lower. Results indicate that L1 expression is regulatable in human stem cells by doxycycline in a nonviral engineering approach. Regulatable expression in a prospective nonleaky Tet-off system could hold promise for therapy, based on the multifunctional roles of L1, including neuronal migration and survival, neuritogenesis, myelination, and synaptic plasticity.

Yoshioka, T., et al. (2011). "Fate of bone marrow mesenchymal stem cells following the allogeneic transplantation of cartilaginous aggregates into osteochondral defects of rabbits." J Tissue Eng Regen Med **5**(6): 437-443.

The purpose of this study was to track mesenchymal stem cells (MSCs) labelled with internalizing quantum dots (i-QDs) in the reparative tissues, following the allogeneic transplantation of three-dimensional (3D) cartilaginous aggregates into the osteochondral defects of rabbits. QDs were conjugated with a unique internalizing antibody against a heat shock protein-70 (hsp70) family stress chaperone, mortalin, which is upregulated and expressed on the surface of dividing cells. The i-QDs were added to the culture medium for 24 h. Scaffold-free cartilaginous aggregates formed from i-QD-labelled MSCs (i-MSCs), using a 3D culture system with chondrogenic supplements for 1 week, were transplanted into osteochondral defects of rabbits. At 4, 8 and 26 weeks after the transplantation, the reparative tissues were evaluated macroscopically, histologically and fluoroscopically. At as early as 4 weeks, the defects were covered with a white tissue resembling articular cartilage. In histological appearance, the reparative tissues resembled hyaline cartilage on safranin-O staining throughout the 26 weeks. In the deeper portion, subchondral bone and bone marrow were well remodelled. On fluoroscopic evaluation, QDs were tracked mainly in bone marrow stromata, with some signals detected in cartilage and the subchondral bone layer. We showed that the labelling of rabbit MSCs with anti-mortalin antibody-conjugated i-QDs is a tolerable procedure and provides a stable fluorescence signal during the cartilage repair process for up to 26 weeks after transplantation. The results suggest that i-MSCs did not inhibit, and indeed contributed to, the regeneration of osteochondral defects.

Yow, S. Z., et al. (2009). "Collagen-based fibrous scaffold for spatial organization of encapsulated and seeded human mesenchymal stem cells." Biomaterials **30**(6): 1133-1142.

Living tissues consist of groups of cells organized in a controlled manner to perform a specific function. Spatial distribution of cells within a three-dimensional matrix is critical for the success of any tissue-engineering construct. Fibers endowed with cell-encapsulation capability would facilitate the achievement of this objective. Here we report the synthesis of a cell-encapsulated fibrous scaffold by interfacial polyelectrolyte complexation (IPC) of methylated collagen and a synthetic terpolymer. The collagen component was well distributed in the fiber, which had a mean ultimate tensile strength of 244.6+/-43.0 MPa. Cultured in proliferating medium, human mesenchymal stem cells (hMSCs) encapsulated in the fibers showed higher proliferation rate than those seeded on the scaffold. Gene expression analysis revealed the maintenance of multipotency for both encapsulated and seeded samples up to 7 days as evidenced by Sox 9, CBFA-1, AFP, PPARgamma2, nestin, GFAP, collagen

I, osteopontin and osteonectin genes. Beyond that, seeded hMSCs started to express neuronal-specific genes such as aggrecan and MAP2. The study demonstrates the appeal of IPC for scaffold design in general and the promise of collagen-based hybrid fibers for tissue engineering in particular. It lays the foundation for building fibrous scaffold that permits 3D spatial cellular organization and multi-cellular tissue development.

Yu, G., et al. (2014). "CLAVATA3 dodecapeptide modified CdTe nanoparticles: a biocompatible quantum dot probe for in vivo labeling of plant stem cells." PLoS One **9**(2): e89241.

CLAVATA3 (CLV3) dodecapeptides function in plant stem cell maintenance, but CLV3 function in cell-cell communication remains less clear. Here, we coupled CLV3 dodecapeptides to synthesized CdTe nanoparticles to track their bioactivity on stem cells in the root apical meristem. To achieve this, we first synthesized CdTe quantum dots (QDs) using a one-pot method, and then evaluated the cytotoxicity of the QDs in BY-2 cells. The results showed that QDs in plant cells must be used at low concentrations and for short treatment time. To make biocompatible probes to track stem cell fate, we conjugated CLV3 dodecapeptides to the QDs by the zero-coupling method; this modification greatly reduced the cytotoxicity of the QDs. Furthermore, we detected CLV3-QDs localized on the cell membrane, consistent with the known localization of CLV3. Our results indicate that using surface-modified QDs at low concentrations and for short time treatment can improve their utility for plant cell imaging.

Yu, L., et al. (2019). "Oral administration of hydroxylated-graphene quantum dots induces intestinal injury accompanying the loss of intestinal stem cells and proliferative progenitor cells." Nanotoxicology **13**(10): 1409-1421.

Graphene quantum dots (GQDs) have gained significant attention in various biomedical applications. The physicochemical properties of these nanoparticles, including toxic effects, are largely determined by their surface modifications. Previous studies have demonstrated high in vitro cytotoxicity of the hydroxylated GQDs (OH-GQDs). The focus of this study was on the intestinal toxicity of OH-GQDs. Briefly, C57BL/6J mice were given daily oral gavage of 0.05, 0.5 or 5 mg/kg OH-GQD for 7 days, and the indices of intestinal damage were evaluated. Higher doses of the OH-GQDs caused significant intestinal injuries, such as enhanced intestinal permeability, shortened villi and crypt loss. The number of Lgr5(+) intestinal stem cells also decreased dramatically upon OH-GQDs exposure, which also inhibited the Ki67(+) proliferative progenitor cells. In addition, an increased number of crypt cells harboring the oxidized DNA base 8-OHdG and gammaH2AX foci were also detected in the intestines of OH-GQD-treated mice. Mechanistically, the OH-GQDs up-regulated both total and phosphorylated p53. Consistent with this, the average number of TUNEL(+) and cleaved caspase-3(+) apoptotic intestinal epithelial cells were significantly increased after OH-GQDs treatment. Finally, a 3-dimensional organoid culture was established using isolated crypts, and OH-GQDs treatment significantly reduced the size of the surviving intestinal organoids. Taken together, the intestinal toxicity of the OH-GQDs should be taken into account during biomedical applications.

Yuan, R., et al. (2018). "Quantum dot-based fluorescent probes for targeted imaging of the EJ human bladder urothelial cancer cell line." Exp Ther Med **16**(6): 4779-4783.

QDs are a type of inorganic nanoparticle with unique optical properties. As a fluorescent label, QDs are widely used in biomedical fields. In the present study, fluorescent probes of quantum dots (QDs) conjugated with a prostate stem cell antigen (PSCA) monoclonal antibody (QD-PSCA) were prepared to study the targeted imaging of QD-PSCA probes in EJ human bladder urothelial cancer cells and analyze the feasibility of QD-based non-invasive tumor-targeted imaging in vivo. QDs with an emission wavelength of 605 nm (QD605) were conjugated with PSCA to prepare QD605-PSCA fluorescent probes by chemical covalent coupling. The optical properties of the probes coupled and uncoupled with PSCA were measured and assessed using an ultraviolet spectrophotometer and a fluorescence spectrophotometer. Direct immune-fluorescent labeling was utilized to detect and analyze imaging of the probes for EJ cells. The results revealed that QD605-PSCA probes retained the fluorescent properties of QD605 and the immunogenicity of the PSCA protein. The probes were able to specifically recognize the PSCA protein expressed in bladder cancer cells, while fluorescence was stable and had a long duration. The present study suggests that QD-PSCA fluorescent probes may be useful for specific targeted labeling and imaging in bladder urothelial cancer cells. Furthermore, the probes possess good optical stability and may be useful for research into non-invasive targeted imaging, early diagnosis and targeted in vivo tumor therapy.

Yuan, Y., et al. (2020). "In vivo dynamic cell tracking with long-wavelength excitable and near-infrared fluorescent polymer dots." Biomaterials **254**: 120139.

Development of cell-based therapeutic systems has attracted great interest in biomedicine. In vivo cell tracking by fluorescence provides indispensable

information for further advancing cell therapy in clinical applications. However, it is still challenging in many cases because of the limited light penetration depth as well as the variations in fluorescent probes, cell lines, and labeling brightness. Here, we designed highly fluorescent polymer dots (Pdots) with far-red-light absorption and near-infrared (NIR) emission for cell tracking. The Pdots consisted of a donor-acceptor polymer blending system where intra-particle energy transfer yielded a narrow-band emission at 800 nm with a high quantum yield of ~0.22. We investigated biocompatibility and cell labeling brightness of the Pdots coated with cell penetrating peptides. Flow cytometry indicated that the cell-labeling brightness of both stem cells and cancer cells increased as much as ~4 orders of magnitude comparing the intensity measurements of labeled cells and controls. Yet, in vivo cell tracking results revealed distinctive fluorescence distribution for the same number of cells that were administered into mice through the tail vein. The stem cells initially accumulated in the lung and remained for seven days, whereas the cancer cells tended to be cleared by the liver in four days. The difference is likely due to the fact that cancer cells are easily attacked by the immune system, whereas stem cells have low immunogenicity. Results obtained herein confirm that NIR-fluorescent Pdots are promising platforms for in vivo cell tracking in small animals.

Yukawa, H. and Y. Baba (2017). "In Vivo Fluorescence Imaging and the Diagnosis of Stem Cells Using Quantum Dots for Regenerative Medicine." Anal Chem **89**(5): 2671-2681.

Yukawa, H. and Y. Baba (2018). "In Vivo Imaging Technology of Transplanted Stem Cells Using Quantum Dots for Regenerative Medicine." Anal Sci **34**(5): 525-532.

Quantum dots (QDs) have excellent fluorescence properties in comparison to traditional fluorescence probes. Thus, the optical application of QDs is rapidly expanding to each field of analytical chemistry. In this review paper, we reviewed the application of QDs to regenerative medicine, especially stem cell transplantation therapy. The labeling of stem cells using QDs composed of semiconductor materials in combination with a chemical substance, poly-cationic liposome and cell penetrating peptide is reported. In addition, the influence of QD labeling on the pluripotency of stem cells is also reported. Finally, the in vivo imaging of transplanted stem cells in mice by QDs emitting fluorescence in the near-infrared region, which can be detected by in vivo fluorescence imaging systems such as IVIS and SAI-1000, is described. The future prospects for stem cell imaging technology by QDs are also discussed.

Yukawa, H., et al. (2010). "Quantum dots labeling using octa-arginine peptides for imaging of adipose tissue-derived stem cells." Biomaterials **31**(14): 4094-4103.

Quantum dots (QDs) have been used to study the effects of fluorescent probes for biomolecules and cell imaging. Adipose tissue-derived stem cells, which carry a relatively lower donor site morbidity, while yielding a large number of stem cells at harvest, were transduced with QDs using the octa-arginine peptide (R8) cell-penetrating peptide (CPP). The concentration ratio of QDs:R8 of 1 x 10(4) was optimal for delivery into ASCs. No cytotoxicity was observed in ASCs transduced with less than 16 nM of QDs655. In addition, >80% of the cells could be labeled within 1 h and the fluorescent intensity was maintained at least for 2 weeks. The ASCs transduced with QDs using R8 could be differentiated into both adipogenic and osteogenic cells, thus suggesting that the cells maintained their stem cell potency. The ASCs labeled with QDs using R8 were further transplanted subcutaneously into the backs of mice or into mice through the tail vein. The labeled ASCs could be imaged with good contrast using the Maestro in vivo imaging system. These data suggested that QD labeling using R8 could be utilized for the imaging of ASCs.

Yukawa, H., et al. (2009). "Quantum dots for labeling adipose tissue-derived stem cells." Cell Transplant **18**(5): 591-599.

Adipose tissue-derived stem cells (ASCs) have a self-renewing ability and can be induced to differentiate into various types of mesenchymal tissue. Because of their potential for clinical application, it has become desirable to label the cells for tracing transplanted cells and for in vivo imaging. Quantum dots (QDs) are novel inorganic probes that consist of CdSe/ZnS-core/shell semiconductor nanocrystals and have recently been explored as fluorescent probes for stem cell labeling. In this study, negatively charged QDs655 were applied for ASCs labeling, with the cationic liposome, Lipofectamine. The cytotoxicity of QDs655-Lipofectamine was assessed for ASCs. Although some cytotoxicity was observed in ASCs transfected with more than 2.0 nM of QDs655, none was observed with less than 0.8 nM. To evaluate the time dependency, the fluorescent intensity with QDs655 was observed until 24 h after transfection. The fluorescent intensity gradually increased until 2 h at the concentrations of 0.2 and 0.4 nM, while the intensity increased until 4 h at 0.8 nM. The ASCs were differentiated into both adipogenic and osteogenic cells with red fluorescence after transfection with QDs655, thus suggesting that the cells retain their potential for differentiation even after transfected with QDs655.

These data suggest that QDs could be utilized for the labeling of ASCs.

Yukawa, H., et al. (2013). "Induced Pluripotent Stem Cell Labeling Using Quantum Dots." Cell Med **6**(1-2): 83-90.

Induced pluripotent stem (iPS) cells have received remarkable attention as the cell sources for clinical applications of regenerative medicine including stem cell therapy. Additionally, labeling technology is in high demand for tracing transplanted cells used in stem cell therapy. In this study, we used quantum dots (QDs), which have distinct fluorescence abilities in comparison with traditional probes, as the labeling materials and investigated whether iPS cells could be labeled with QDs with no cytotoxicity. iPS cells could not be labeled with QDs alone but required the use of cell-penetrating peptides such as octaarginine (R8). No significant cytotoxicity to iPS cells was confirmed by up to 8 nM QDs, and the iPS cells labeled with QDs maintained their undifferentiated state and pluripotency. These data suggest that QDs can be used for fluorescence labeling of iPS cells.

Yukawa, H., et al. (2012). "Monitoring transplanted adipose tissue-derived stem cells combined with heparin in the liver by fluorescence imaging using quantum dots." Biomaterials **33**(7): 2177-2186.

Adipose tissue-derived stem cell (ASC) transplantation, when used in combination with heparin, has proven to be an effective treatment for acute liver failure in mice. However, the behavior and organ-specific accumulation of transplanted ASCs alone or in combination with heparin is poorly understood. In this paper, we investigated whether quantum dots (QDs) labeling using octa-arginine peptide (R8) for ASCs could be applied for in vivo fluorescence imaging in mice with acute liver failure, and analyzed the behavior and organ-specific accumulation of ASCs that were transplanted alone or in combination with heparin using an IVIS((R)) Spectrum analysis. Almost all of the transplanted ASCs were observed to accumulate in the lungs within 10 min without heparin. However, when heparin was used in combination with the ASCs, the accumulation of the transplanted ASCs was found not only in the lungs but also in the liver. The region of interest (ROI) analysis of ex vivo fluorescence imaging showed that the accumulation rate of transplanted ASCs in the liver increased to about 30%. In the time course analysis, the accumulation rate of ASCs in the liver was about 10% in 1 day and was maintained at that level for at least 2 day. We observed that heparin was effective for increasing the accumulation of transplanted ASCs in the liver using fluorescence imaging technology. We suggest that fluorescence imaging by means of QDs labeling using R8 can be useful for tracing the transplanted cells.

Zhang, H. (2004). Quantum dot-A10 RNA aptamer-doxorubicin conjugate. Molecular Imaging and Contrast Agent Database (MICAD). Bethesda (MD).

Prostate-specific membrane antigen (PSMA) is a type II membrane glycoprotein with a molecular weight of ~100 kDa (1). PSMA is composed of several domains, including a potential phosphorylation site in the cytoplasmic tail (amino acids 1-18), a highly hydrophobic alpha-helix in the transmembrane region (amino acids 19-43), and catalytic sites in the extensive extracellular domain (amino acids 44-750). Two unique enzymatic functions are found in PSMA: N-acetylated, alpha-linked, dipeptidase (NAALADase) activity and folate hydrolase activity. As a prostate cancer cell marker, PSMA expression is primarily prostate-specific, with very low levels (~1,000-fold less) in the brain, salivary glands, and small intestine. PSMA has become an excellent target for imaging and therapy prostate cancer. Aptamers (from the Latin aptus, to fit, and the Greek meros, part or region) are single-stranded or double-stranded oligonucleotides (RNA or DNA, respectively) that are modified to bind a variety of targets with high binding affinity and specificity (2). Aptamers range in size from 20 to 80 base pairs (~6-26 kDa) with dissociation constants in the range of 10 pM to 10 nM (3). Unlike linear oligonucleotides, which contain genetic information or antisense oligonucleotides that interrupt the transcription of genetic information, aptamers are globular molecules with a shape similar to tRNA and bind to target proteins specifically (4). A10 RNA aptamer (Apt) is a nuclease-stabilized 2'-fluoropyrimidine RNA molecule of 57 base pairs with a molecular weight of 18.5 kDa (5). Its 2'-fluoro-modified ribose on all pyrimidines and 3'-inverted deoxythymidine cap provide significant resistance to nuclease in blood (6). Apt has a single 5'-CG-3' sequence in its predicted double-stranded stem region, which is a preferred binding site for the anthracycline class of anticancer drugs such as doxorubicin (Dox) (7). Dox intercalates within the GC pair in Apt to form physical conjugate Apt(Dox) at molar ratio of 1.11:1 (dissociation constant = 600 nM) and emit fluorescence simultaneously. Because Dox possesses high efficacy against a range of neoplasms, including acute lymphoblastic and myeloblastic leukemias, malignant lymphomas, soft tissue and bone sarcomas, and breast, ovarian, prostate bladder, gastric, and bronchogenic carcinomas (8), this complex can be used as a PSMA-specific drug carrier to deliver Dox to prostate cancer cells. Quantum dots (QDs) are semiconductor nanocrystals 2 to 10 nm in diameter (200-10,000 atoms) that possess a quantum confinement effect (hence the name "quantum dots") caused by the

restriction of electrons and holes in all three dimensions (9, 10). Like classic semiconductors that are composed of two types of atoms from the II/VI or III/V group elements in the periodic table, these nanocrystals have a valence band and a conduction band separated by an energy gap (band gap). Upon excitation, an electron is promoted from the filled valence band to the largely empty conduction band, which creates a positive vacancy "hole" in the valence band. The spatial separation (Bohr radius) of this electron-hole pair ("exciton") is typically 1-10 nm for most semiconductors (10). The quantum confinement arises when one of the dimensions in the nanocrystals becomes comparable to its Bohr radius, at which time these valence/conduction bands are quantized with an energy value that is directly related to the nanocrystal size. Thus, the excitons are confined in a manner similar to a particle-in-the-box problem, leading to a finite band gap and discretization of energy levels. When the electron fills the vacancy in the valence band, light of a certain wavelength is emitted, which corresponds to the respective band gap energy that is a function of nanocrystal size. For instance, the emission wavelength is 550 nm for 3-nm CdSe QDs and 650 nm for 7-nm CdSe QDs (11, 12). For biological applications, QDs are generally encapsulated with biocompatible polymers, functionalized for various bioconjugations, and widely used to label molecules for optical imaging. The QD-Apt(Dox) conjugate is an PSMA-specific agent used for optical imaging of the delivery of the anticancer drug Dox (13). QD-Apt(Dox) consists of three components: PSMA-specific Apt covalently attached to the core surface as targeting molecule and drug carrier, an anthracycline class of anticancer drug Dox as a therapeutic agent and optical sensor, and a carboxyl core-CdSe/ZnS shell QD (QD490) as a carrier of A10 and Dox and as the second optical sensor. The two sensors (Dox and QD) generate an optical signal via formation of bi-molecular fluorescence resonance energy transfer (Bi-FRET) complex. FRET is a near-field dipole-dipole interaction that involves energy transfer between two molecules in close proximity (3-6 nm). In QD-Apt(Dox), there is a donor-acceptor relationship between QDs and Dox. The fluorescence of both QD and Dox is quenched ("OFF") when QD-Apt is loaded with Dox, and fluorescence is restored upon the release of Dox ("ON") after the uptake of QD-Apt(Dox) by prostate cancer cells. Thus, QD-Apt(Dox) can be used to image drug delivery to prostate cancer cells.

Zhang, H., et al. (2015). "Molecular image-guided theranostic and personalized medicine 2014." Biomed Res Int **2015**: 258612.

Zhang, J., et al. (2011). "Labeling primary nerve stem cells with quantum dots." J Nanosci Nanotechnol **11**(11): 9536-9542.

The primary nerve stem cells (NSCs) were labeled with the carboxyl QDs by passive loading. The studies on QDs' effect on the NSCs showed that with a proper concentration, QDs have little effect on NSCs growth and proliferation within a week, and the QDs did not affect either differentiation potential of NSCs or the protein expression of neuron and astrocyte derived from NSCs. The results suggested that this labeling method is appropriate for labeling studies in vitro. Combined with the unique optical properties of QDs, it is possibly to fulfill NSCs fate-tracking in vivo.

Zhao, W., et al. (2013). "Targeting human embryonic stem cells with quantum dot-conjugated phages." Sci Rep **3**: 3134.

Targeting embryonic stem cells (ESCs) is important for ESC labeling, drug delivery and cell fate control. In this study, we identified twenty-two phage clones that bind specifically to the hESC cell line X-01, which was derived from human blastocysts of Chinese origin. One phage (H178), which displays the sequence VGGEAWSSPTDL, showed higher binding affinity to hESCs than to a monkey ES cell line (RS366.4) and two mouse ES cell lines (R1 and E14). Using quantum dots (QDs) conjugated to the H178 phage, we demonstrate that the phage can specifically bind to hESCs in vitro. Our results suggest a possible interaction between the selected peptide and the stem cell extracellular matrix (ECM). The selection method described here allows rapid and efficient screening of unique phage clones and targeting cells. The phages displaying peptides identified by this study have potential applications for cargo delivery and receptor studies.

Zhao, W., et al. (2010). "Isolation and initial application of a novel peptide that specifically recognizes the neural stem cells derived from rhesus monkey embryonic stem cells." J Biomol Screen **15**(6): 687-694.

The search for new receptor ligands is important in the study of embryonic stem (ES) cell differentiation processes. In this study, a novel peptide (HGEVPRFHAVHL) with a specific ability to bind with neural stem cells derived from rhesus monkey ES cells was successfully screened out using a Ph.D-12 peptide phage display library. High affinity and specificity of the HGE phage were shown in an enzyme-linked immunosorbent assay. The binding ability of the phage could be matched with that of a chemically synthesized peptide with a sequence identical to that displayed by the phage, indicating that this binding capability manifests a peptide sequence. Combined with quantum dots, the HGE peptide can be used as a direct tool to show optical imaging of specific binding on a single cell membrane. Further results of Western blot showed that the HGE

peptide interacted with 48/34-kDa proteins on the membrane of neural stem cells. This work is the first time that a phage display technique has been applied in ES cell differentiation studies. The findings extend the utilization of a targeting agent for neural stem cells and can also be used as a research tool in studying other cell lineages derived from ES cells.

Zheng, J., et al. (2006). "Cellular imaging and surface marker labeling of hematopoietic cells using quantum dot bioconjugates." Lab Hematol **12**(2): 94-98.

Semiconductor quantum dots (qdots) are emerging as a new class of fluorescent labels. The unique optical properties of qdots make them appealing in laboratory diagnosis; however, qdot-based probes remain to be developed and evaluated for clinical laboratory applications. In this study, 2 different approaches were employed to label hematopoietic cells with qdots. The first was based on a generalized intracellular delivery of qdots using qdot-transferrin conjugates through receptor-mediated endocytosis. Hematopoietic cells from umbilical cord blood or bone marrow were successfully labeled with qdot-transferrin in cell cultures. The fluorescence signal of qdot-transferrin was detected in the cytoplasmic location. The second approach was to use qdot-antibodies for labeling cell surface markers. The monoclonal antibodies to CD5, CD19, and CD45 surface antigens were conjugated to qdots with distinct emission spectra. The qdot-linked antibodies were shown to bind successfully to specific cell markers on lymphocytes. The signal obtained from the labeling of cells was detectable by using fluorescence microscopy and flow cytometry. The qdot signals were shown to be target specific, bright, and photo stable. The results of this study demonstrated the feasibility of using qdots for cell labeling and surface marker analysis of hematopoietic cells. Given the superior optical properties of qdots as compared to conventional fluorescence dyes, the qdot-based probe offers a promising tool for hematology analysis in clinical laboratories.

Zhong, Y., et al. (2012). "Induction of brain CYP2E1 by chronic ethanol treatment and related oxidative stress in hippocampus, cerebellum, and brainstem." Toxicology **302**(2-3): 275-284.

Ethanol is one of the most commonly abused substances, and oxidative stress is an important causative factor in ethanol-induced neurotoxicity. Cytochrome P450 2E1 (CYP2E1) is involved in ethanol metabolism in the brain. This study investigates the role of brain CYP2E1 in the susceptibility of certain brain regions to ethanol neurotoxicity. Male Wistar rats were intragastrically treated with ethanol (3.0 g/kg, 30 days). CYP2E1 protein, mRNA expression, and catalytic activity in various brain regions were respectively assessed by immunoblotting, quantitative quantum dot immunohistochemistry, real-time RT-PCR, and LC-MS. The generation of reactive oxygen species (ROS) was analyzed using a laser confocal scanning microscope. The hippocampus, cerebellum, and brainstem were selectively damaged after ethanol treatment, indicated by both lactate dehydrogenase (LDH) activity and histopathological analysis. Ethanol markedly increased the levels of CYP2E1 protein, mRNA expression, and activity in the hippocampus and cerebellum. CYP2E1 protein and activity were significantly increased by ethanol in the brainstem, with no change in mRNA expression. ROS levels induced by ethanol paralleled the enhanced CYP2E1 proteins in the hippocampus, granular layer and white matter of cerebellum as well as brainstem. Brain CYP2E1 activity was positively correlated with the damage to the hippocampus, cerebellum, and brainstem. These results suggest that the selective sensitivity of brain regions to ethanol neurodegeneration may be attributed to the regional and cellular-specific induction of CYP2E1 by ethanol. The inhibition of CYP2E1 levels may attenuate ethanol-induced oxidative stress via ROS generation.

Zhou, J., et al. (2018). "Effects of lead stress on the growth, physiology, and cellular structure of privet seedlings." PLoS One **13**(3): e0191139.

In this study, we investigated the effects of different lead (Pb) concentrations (0, 200, 600, 1000, 1400 mg kg-1 soil) on the growth, ion enrichment in the tissues, photosynthetic and physiological characteristics, and cellular structures of privet seedlings. We observed that with the increase in the concentrations of Pb, the growth of privet seedlings was restricted, and the level of Pb ion increased in the roots, stem, and leaves of the seedlings; however, most of the ions were concentrated in the roots. Moreover, a decreasing trend was observed for chlorophyll a, chlorophyll b, total chlorophyll, net photosynthesis (Pn), transpiration rate (Tr), stomatal conductance (Gs), sub-stomatal CO2 concentration (Ci), maximal photochemical efficiency (Fv/Fm), photochemical quenching (qP), and quantum efficiency of photosystem II (PhiPSII). In contrast, the carotene levels, minimum fluorescence (F0), and non-photochemical quenching (qN) showed an increasing trend. Under Pb stress, the chloroplasts were swollen and deformed, and the thylakoid lamellae were gradually expanded, resulting in separation from the cell wall and eventual shrinkage of the nucleus. Using multiple linear regression analysis, we found that the content of Pb in the leaves exerted the maximum effect on the seedling growth. We observed that the decrease in photosynthetic activation energy, increase in pressure because of the excess activation energy, and decrease in the transpiration rate could result in maximum effect on the photosynthetic abilities of the seedlings under Pb stress. Our results should help in better understanding of the effects of heavy metals on plants and in assessing their potential for use in bioremediation.

Zhou, X., et al. (2013). "Solution-processable graphene quantum dots." Chemphyschem **14**(12): 2627-2640.

This minireview describes recent progress in solution-processable graphene quantum dots (SGQDs). Advances in the preparation, modification, properties, and applications of SGQDs are highlighted in detail. As one of emerging nanostructured materials, possible ongoing research related to the precise control of the lateral size, edge structure and surface functionality; the manipulation and characterization; the relationship between the properties and structure; and interfaces with biological systems of SGQDs have been speculated upon.

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3/22/2023