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Stem Cell

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Stem Cell, Time and Space 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 stem cell, time and space related studies.
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Key words: stem cell; 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 stem cell, time and space related studies.

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

Abraham, M. A., et al. (2019). "Safety of Pediatric Peripheral Blood Stem Cell Harvest in Daycare Setting: An Institutional Experience." Anesth Essays Res **13**(1): 91-96.

Introduction: Children serving as a donor for their siblings will require anesthesia or sedation. In view of shortage of time and space in operating room setting, peripheral blood stem cell (PBSC) harvest is performed as a daycare procedure. Aim: This study aims to find out whether performing PBSC harvest in hematology blood collection area as a daycare procedure is safe or not. Settings and Design: This secondary analysis included 164 pediatric PBSC harvest (154 pediatric donors, of which 10 had repeat harvesting done) donors, performed under anesthesia, in the Department of Hematology, between January 2009 and June 2017. Materials and Methods: Donors were examined, informed consent was obtained, and adequate premedications were ensured. Induction was intravenous for cooperative donors or inhalational sevoflurane followed by intravenous maintenance infusion using either face mask or a laryngeal mask airway (LMA). During the procedure, vitals are monitored with a noninvasive monitor. Normal hemodynamics were ensured before transferring the children to the ward. Statistical Analysis: Statistical analysis was performed using SPSS 16.0 statistical software. Descriptive statistics and frequencies were used for the data description. Results: A total of 137 donors (median age of 5 years) were induced with sevoflurane and LMA was used in 84 children and face mask in 53. Twenty-seven children cooperated for intravenous induction. Various combinations of propofol, dexmedetomidine, and ketamine were used with respiratory and hemodynamic stability. The median duration of anesthesia was 250 (165-375) min. The recovery from anesthesia was smooth with a median wake-up time of 20 (5-60) min. Conclusion: This retrospective analysis demonstrates that nonoperating room anesthesia for pediatric age group for PBSC harvest can be safely and successfully accomplished outside the operation room setting by a consultant anesthesiologist.

Ahmad, T., et al. (2017). "Hybrid-spheroids incorporating ECM like engineered fragmented fibers potentiate stem cell function by improved cell/cell and cell/ECM interactions." Acta Biomater **64**: 161-175.

Extracellular matrix (ECM) microenvironment is critical for the viability, stemness, and differentiation of stem cells. In this study, we developed hybrid-spheroids of human turbinate mesenchymal stem cells (hTMSCs) by using extracellular matrix (ECM) mimicking fragmented fibers (FFs) for improvement of the viability and functions of hTMSCs. We prepared FFs with average size of 68.26 microm by partial aminolysis of poly L-lactide (PLLA) fibrous sheet (FS), which was coated with polydopamine for improved cell adhesion. The proliferation of hTMSCs within the hybrid-spheroids mixed with fragmented fibers was significantly increased as compared to that from the cell-only group. Cells and fragmented fibers were homogenously distributed with the presence of pore like empty spaces in the structure. LOX-1 staining revealed that the hybrid-spheroids improved the cell viability, which was potentially due to enhanced transport of oxygen through void space generated by engineered ECM. Transmission electron microscopy (TEM) analysis confirmed that cells within the hybrid-spheroid formed strong cell junctions and contacts with fragmented fibers. The expression of cell junction proteins including connexin 43 and E-cadherin was significantly upregulated in hybrid-spheroids by 16.53+/-0.04 and 28.26+/-0.11-fold greater than that from cell-only group. Similarly, expression of integrin alpha2, alpha5, and beta1 was significantly enhanced at the same group by 25.72+/-0.13, 27.48+/-0.49, and 592.78+/-0.06-fold, respectively. In addition, stemness markers including Oct-4, Nanog, and Sox2 were significantly upregulated in hybrid-spheroids by 96.56+/-0.06, 158.95+/-0.06, and 115.46+/-0.47-fold, respectively, relative to the cell-only group. Additionally, hTMSCs within the hybrid-spheroids showed significantly greater osteogenic differentiation under osteogenic media conditions. Taken together, our hybrid-spheroids can be an ideal approach for stem cell expansion and serve as a potential carrier for bone regeneration. STATEMENT OF SIGNIFICANCE: Cells are spatially arranged within extracellular matrix (ECM) and cell/ECM interactions are crucial for cellular functions. Here, we developed a hybrid-spheroid system incorporating engineered ECM prepared from fragmented electrospun fibers to tune stem cell functions. Conventionally prepared cell spheroids with large diameters (>200microm) is often prone to hypoxia. In contrast, the hybrid-spheroids significantly enhanced viability and proliferation of human turbinate mesenchymal stem cells (hTMSCs) as compared to spheroid prepared from cell only. Under these conditions, the presence of fragmented fibers also improved maintenance of stemness of hTMSCs for longer time cultured in growth media and demonstrated significantly greater osteogenic differentiation under osteogenic media conditions. Thus, the hybrid-spheroids can be used as a delivery carrier for stem cell based therapy or a 3D culture model for in vitro assay.

Audet, J. (2010). "Adventures in time and space: Nonlinearity and complexity of cytokine effects on stem cell fate decisions." Biotechnol Bioeng **106**(2): 173-182.

Cytokines are central factors in the control of stem cell fate decisions and, as such, they are invaluable to those interested in the manipulation of stem and progenitor cells for clinical or research purposes. In their in vivo niches or in optimized cultures, stem cells are exposed to multiple cytokines, matrix proteins and other cell types that provide individual and combinatorial signals that influence their self-renewal, proliferation and differentiation. Although the individual effects of cytokines are well-characterized in terms of increases or decreases in stem cell expansion or in the production of specific cell lineages, their interactions are often overlooked. Factorial design experiments in association with multiple linear regression is a powerful multivariate approach to derive response-surface models and to obtain a quantitative understanding of cytokine dose and interactions effects. On the other hand, cytokine interactions detected in stem cell processes can be difficult to interpret due to the fact that the cell populations examined are often heterogeneous, that cytokines can exhibit pleiotropy and redundancy and that they can also be endogenously produced. This perspective piece presents a list of possible biological mechanisms that can give rise to positive and negative two-way factor interactions in the context of in vivo and in vitro stem cell-based processes. These interpretations are based on insights provided by recent studies examining intra- and extra-cellular signaling pathways in adult and embryonic stem cells. Cytokine interactions have been classified according to four main types of molecular and cellular mechanisms: (i) interactions due to co-signaling; (ii) interactions due to sequential actions; (iii) interactions due to high-dose saturation and inhibition; and (iv) interactions due to intercellular signaling networks. For each mechanism, possible patterns of regression coefficients corresponding to the cytokine main effects, quadratic effects and two-way interactions effects are provided. Finally, directions for future mechanistic studies are presented.

Bendall, S. C., et al. (2009). "An enhanced mass spectrometry approach reveals human embryonic stem cell growth factors in culture." Mol Cell Proteomics **8**(3): 421-432.

The derivation and long-term maintenance of human embryonic stem cells (hESCs) has been established in culture formats that are both dependent and independent of support (feeder) cells. However, the factors responsible for preserving the viability of hESCs in a nascent state remain unknown. We describe a mass spectrometry-based method for probing the secretome of the hESC culture microenvironment to identify potential regulating protein factors that are in low abundance. Individual samples were analyzed several times, using successive mass (m/z) and retention time-directed exclusion, without sampling the same peptide ion twice. This iterative exclusion -mass spectrometry (IE-MS) approach more than doubled protein and peptide metrics in comparison to a simple repeat analysis method on the same instrument, even after extensive sample pre-fractionation. Furthermore, implementation of the IE-MS approach was shown to enhance the performance of an older quadrupole time of flight (Q-ToF) MS. The resulting number of identified peptides approached that of a parallel repeat analysis on a newer LTQ-Orbitrap MS. The combination of the results of both instruments proved to be superior to that achieved by a single instrument in the identification of additional proteins. Using the IE-MS strategy, combined with complementary gel- and solution-based fractionation methods, the hESC culture microenvironment was extensively probed. Over 10 to 12 times more extracellular proteins were observed compared with previously published surveys. The detection of previously undetectable growth factors, present at concentrations ranging from 10(-9) to 10(-11) g/ml, highlights the depth of our profiling. The IE-MS approach provides a simple and reliable technique that greatly enhances instrument performance by increasing the effective depth of MS-based proteomic profiling. This approach should be widely applicable to any LC-MS/MS instrument platform or biological system.

Bhattacharya, D., et al. (2008). "Space-time considerations for hematopoietic stem cell transplantation." Eur J Immunol **38**(8): 2060-2067.

The mammalian blood system contains a multitude of distinct mature cell lineages adapted to serving diverse functional roles. Mutations that abrogate the development or function of one or more of these lineages can lead to profound adverse consequences, such as immunodeficiency, autoimmunity, or anemia. Replacement of hematopoietic stem cells (HSC) that carry such mutations with HSC from a healthy donor can reverse such disorders, but because the risks associated with the procedure are often more serious than the blood disorders themselves, bone marrow transplantation is generally not used to treat a number of relatively common inherited blood diseases. Aside from a number of other problems, risks associated with cytoreductive treatments that create "space" for donor HSC, and the slow kinetics with which immune competence is restored following transplantation hamper progress. This review will focus on how recent studies using experimental model systems may direct future efforts to implement routine use of HSC transplantation to cure inherited blood disorders.

Blackburn, R., et al. (2016). "Establishing an Inpatient Gym for Recipients of Stem Cell Transplantation: A Multidisciplinary Collaborative." Clin J Oncol Nurs **20**(1): 66-71.

BACKGROUND: Evidence suggests that exercise can have a profound impact on physiologic and quality-of-life outcomes for patients undergoing hematopoietic stem cell transplantation (HSCT). Despite this, implementation of a gym on inpatient HSCT units may be limited because of space, infrastructure, and budget. OBJECTIVES: This article presents the design, implementation, and evaluation of the gym and highlights its use for individual and group patient activities. METHODS: An interprofessional team at a National Cancer Institute-designated comprehensive cancer center collaborated to design and implement gym space on an inpatient HSCT unit servicing as many as 86 beds. FINDINGS: Informal feedback from patients, as well as metrics on use of the space, indicates that the gym is well received and frequently used. Limitations include the absence of a designated physical therapy technician to supervise individual activity, which may limit patient access when a staff member is unavailable. The cost associated with the implementation of such space may be offset by benefits to patients, including enhanced conditioning, quality of life, and time to discharge, as evidenced in the literature.

Bourseau-Guilmain, E., et al. (2011). "The importance of the stem cell marker prominin-1/CD133 in the uptake of transferrin and in iron metabolism in human colon cancer Caco-2 cells." PLoS One **6**(9): e25515.

As the pentaspan stem cell marker CD133 was shown to bind cholesterol and to localize in plasma membrane protrusions, we investigated a possible function for CD133 in endocytosis. Using the CD133 siRNA knockdown strategy and non-differentiated human colon cancer Caco-2 cells that constitutively over-expressed CD133, we provide for the first time

direct evidence for a role of CD133 in the intracellular accumulation of fluorescently labeled extracellular compounds. Assessed using AC133 monoclonal antibody, CD133 knockdown was shown to improve Alexa488-transferrin (Tf) uptake in Caco-2 cells but had no impact on FITC-dextran or FITC-cholera-toxin. Absence of effect of the CD133 knockdown on Tf recycling established a role for CD133 in inhibiting Tf endocytosis rather than in stimulating Tf exocytosis. Use of previously identified inhibitors of known endocytic pathways and the positive impact of CD133 knockdown on cellular uptake of clathrin-endocytosed synthetic lipid nanocapsules supported that CD133 impact on endocytosis was primarily ascribed to the clathrin pathway. Also, cholesterol extraction with methyl-beta-cyclodextrine up regulated Tf uptake at greater intensity in the CD133(high) situation than in the CD133(low) situation, thus suggesting a role for cholesterol in the inhibitory effect of CD133 on endocytosis. Interestingly, cell treatment with the AC133 antibody down regulated Tf uptake, thus demonstrating that direct extracellular binding to CD133 could affect endocytosis. Moreover, flow cytometry and confocal microscopy established that down regulation of CD133 improved the accessibility to the TfR from the extracellular space, providing a mechanism by which CD133 inhibited Tf uptake. As Tf is involved in supplying iron to the cell, effects of iron supplementation and deprivation on CD133/AC133 expression were investigated. Both demonstrated a dose-dependent down regulation here discussed to the light of transcriptional and post-transciptional effects. Taken together, these data extend our knowledge of the function of CD133 and underline the interest of further exploring the CD133-Tf-iron network.

Bratt-Leal, A. M., et al. (2011). "Magnetic manipulation and spatial patterning of multi-cellular stem cell aggregates." Integr Biol (Camb) **3**(12): 1224-1232.

The controlled assembly and organization of multi-cellular systems to mimic complex tissue structures is critical to the engineering of tissues for therapeutic and diagnostic applications. Recent advances in micro-scale technologies to control multi-cellular aggregate formation typically require chemical modification of the interface between cells and materials and lack multi-scale flexibility. Here we demonstrate that simple physical entrapment of magnetic microparticles within the extracellular space of stem cells spheroids during initial formation enables scaffold-free immobilization, translocation and directed assembly of multi-cellular aggregates across multiple length and time scales, even under dynamic suspension culture conditions. The response of aggregates to externally applied magnetic fields was a direct function of microparticle incorporation, allowing for rapid and transient control of the extracellular environment as well as separation of heterogeneous populations. In addition, spatial patterning of heterogeneous spheroid populations as well as individual multi-cellular aggregates was readily achieved by imposing temporary magnetic fields. Overall, this approach provides novel routes to examine stem cell differentiation and tissue morphogenesis with applications that encompass the creation of new model systems for developmental biology, scaffold-free tissue engineering strategies and scalable bioprocessing technologies.

Cary, C., et al. (2015). "Outcomes of postchemotherapy retroperitoneal lymph node dissection following high-dose chemotherapy with stem cell transplantation." Cancer **121**(24): 4369-4375.

BACKGROUND: Characterizing the role of postchemotherapy retroperitoneal lymph node dissection (PC-RPLND) after high-dose chemotherapy (HDCT) has been limited by small sample sizes. This study reports on survival after HDCT with stem cell support and PC-RPLND as well as histologic findings in the retroperitoneum. METHODS: The prospectively maintained testicular cancer database of Indiana University was queried for patients receiving HDCT with stem cell transplantation before PC-RPLND. The cause and date of death were obtained through patient chart review and contact with referring physicians. The Kaplan-Meier method was used to evaluate overall survival (OS). The log-rank test was used for tests of significance. A multivariate, backward, stepwise Cox regression model was built to evaluate predictors of overall mortality. RESULTS: A total of 92 patients were included in the study. In the entire cohort, the retroperitoneal (RP) histology findings at the time of PC-RPLND were necrosis (26%), teratoma (34%), and cancer (38%). Sixty-six patients (72%) harbored either a teratoma or active cancer in the RP specimen at PC-RPLND. The median follow-up for the entire cohort was 80.6 months. A total of 28 patients (30%) died during follow-up. The 5-year OS rate of the entire cohort was 70%. The most significant predictor of death was PC-RPLND performed in the desperation setting with elevated markers. CONCLUSIONS: Despite these patients being heavily pretreated with multiple cycles of chemotherapy, including HDCT, approximately three-fourths were found to have a teratoma and/or active cancer in the retroperitoneum. This underscores the importance of PC-RPLND for rendering patients free of disease and providing a potential for cure.

Cho, M., et al. (2013). "The establishment of mouse embryonic stem cell cultures on 96-well plates for high-throughput screening." Mol Cells **35**(5): 456-461.

Embryonic stem (ES) cells can be valuable for monitoring differentiation processes and for improving applications in basic developmental biology. The application of ES cells can be a useful tool for drug discovery and toxicology. Therefore, we suggest the high-throughput screening (HTS) system based on ES cells in this study. Firstly, we optimized the feeder-free condition and seeding cell number which can maintained for at least 7 days without over-confluency. We analyzed the system by cell viability, proliferation activity, RT-PCR and morphologic/immunohistochemical evaluations. The optimal cell seeding number was 30/well that was maintained the typical colonial morphology over 9 d with 1,000 U/ml LIF in the limited space. The cell in optimized condition expressed ALP, SSEA-1, Oct 4 and Nanog and the genetic expressions showed similar to protein expressions. The cell lineage marker expressions showed faint or none. The cell viability and proliferation activity were increased in time-dependent manner in our optimized HTS system. In conclusion, the novel HTS system using ES cells can by useful for developing models for drug discovery as well as toxicological screening in the near future.

Cho, S., et al. (2009). "Chip-based time-continuous monitoring of toxic effects on stem cell differentiation." Ann Anat **191**(1): 145-152.

Pesticides used to control unwanted insects are potentially toxic to humans. In assessing the risk involved in exposure to pesticides or complex chemical mixtures, an in vitro cell-based test can provide useful information regarding danger to human health. Cell differentiation is a biological process of fundamental importance in developing and adult organisms. In this paper, we propose a cell-based test system for continuous, label-free monitoring of the effect of test substances on stem cell differentiation. Using a prefabricated electrode-based chip and impedance measurement system, we investigated the influence of chlorpyrifos (a pesticide) on the differentiation of human mesenchymal stem cells (hMSCs) to adipocytes. The state of hMSCs on electrodes during adipogenic differentiation or after application of the cytotoxic substance was clearly reflected in the impedance measurement. Chlorpyrifos caused a partially uncovered electrode area with a decreased number of lipid vacuoles, thus leading to a rapid decrease in resistance in the cell layer. After removal of the chlorpyrifos, the cell layer resistance was regained due to the renewed covering of the electrodes by hMSCs. However, an increase in lipid vacuoles was not observed. From this, it was concluded that the measured resistance of hMSCs is determined by the electrical properties in the extra cellular space (e.g., cell/electrode or cell/cell gap), but not by the lipid vacuoles appearing in intracellular space during adipogenic differentiation.

Corrales, C. E., et al. (2006). "Engraftment and differentiation of embryonic stem cell-derived neural progenitor cells in the cochlear nerve trunk: growth of processes into the organ of Corti." J Neurobiol **66**(13): 1489-1500.

Hearing loss in mammals is irreversible because cochlear neurons and hair cells do not regenerate. To determine whether we could replace neurons lost to primary neuronal degeneration, we injected EYFP-expressing embryonic stem cell-derived mouse neural progenitor cells into the cochlear nerve trunk in immunosuppressed animals 1 week after destroying the cochlear nerve (spiral ganglion) cells while leaving hair cells intact by ouabain application to the round window at the base of the cochlea in gerbils. At 3 days post transplantation, small grafts were seen that expressed endogenous EYFP and could be immunolabeled for neuron-specific markers. Twelve days after transplantation, the grafts had neurons that extended processes from the nerve core toward the denervated organ of Corti. By 64-98 days, the grafts had sent out abundant processes that occupied a significant portion of the space formerly occupied by the cochlear nerve. The neurites grew in fasciculating bundles projecting through Rosenthal's canal, the former site of spiral ganglion cells, into the osseous spiral lamina and ultimately into the organ of Corti, where they contacted hair cells. Neuronal counts showed a significant increase in neuronal processes near the sensory epithelium, compared to animals that were denervated without subsequent stem cell transplantation. The regeneration of these neurons shows that neurons differentiated from stem cells have the capacity to grow to a specific target in an animal model of neuronal degeneration.

Dansey, R. D. and R. D. Baynes (2001). "Nonablative allogeneic hematopoietic stem cell transplantation." Curr Opin Oncol **13**(1): 27-32.

During the past few years there has been an explosion of knowledge in nonablative allogeneic stem cell transplantation. This approach to transplantation relies more on the creation of "immunologic space" for engraftment rather than the more traditional approach of creating "physical space" by the application of either intensive radiation or chemical therapy. Nonablative allogeneic stem cell transplantation holds the promise of allowing powerful alloimmune responses to eradicate disease processes while

minimizing the initial treatment-related morbidity and mortality, and it appears to be the necessary enabling platform by which to apply allogeneic cellular therapy. Intuitively, this approach should broaden the eligibility for potentially curative allogeneic transplantation in various disease categories, reduce initial hospitalization costs, and at the same time have a positive impact on quality of life. We review the current published data relating to this approach including the underlying principles, the preparative regimen, disease indications, preliminary results in hematologic and solid malignancies, and certain correlative immunologic evaluations.

Decimo, I., et al. (2012). "Meninges: from protective membrane to stem cell niche." Am J Stem Cells **1**(2): 92-105.

Meninges are a three tissue membrane primarily known as coverings of the brain. More in depth studies on meningeal function and ultrastructure have recently changed the view of meninges as a merely protective membrane. Accurate evaluation of the anatomical distribution in the CNS reveals that meninges largely penetrate inside the neural tissue. Meninges enter the CNS by projecting between structures, in the stroma of choroid plexus and form the perivascular space (Virchow-Robin) of every parenchymal vessel. Thus, meninges may modulate most of the physiological and pathological events of the CNS throughout the life. Meninges are present since the very early embryonic stages of cortical development and appear to be necessary for normal corticogenesis and brain structures formation. In adulthood meninges contribute to neural tissue homeostasis by secreting several trophic factors including FGF2 and SDF-1. Recently, for the first time, we have identified the presence of a stem cell population with neural differentiation potential in meninges. In addition, we and other groups have further described the presence in meninges of injury responsive neural precursors. In this review we will give a comprehensive view of meninges and their multiple roles in the context of a functional network with the neural tissue. We will highlight the current literature on the developmental feature of meninges and their role in cortical development. Moreover, we will elucidate the anatomical distribution of the meninges and their trophic properties in adult CNS. Finally, we will emphasize recent evidences suggesting the potential role of meninges as stem cell niche harbouring endogenous precursors that can be activated by injury and are able to contribute to CNS parenchymal reaction.

Ettinger, A., et al. (2017). "Specific membrane dynamics during neural stem cell division." Methods Cell Biol **137**: 143-172.

Neural stem and progenitor cells in the developing cerebral cortex, but also when grown in culture, display a range of distinct phenomena during cytokinesis. Cleavage furrow ingression in neural progenitor cells can bisect their basal processes and, later on, result in midbody formation at the apical surface. After abscission, these midbodies are released as membrane-bound particles into the extracellular space, in contrast to uptake and degradation of postabscission midbodies in other cell types. Whether these cellular dynamics are unique to neural stem cells, or more ubiquitously found, and what biological significance these processes have for cell differentiation or cell-cell communication, are open questions that require a combination of approaches. Here, we discuss techniques to study the specific membrane dynamics underlying the basal process splitting and postabscission midbody release in neural stem cells. We provide some basic concepts and protocols to isolate, enrich and stain released midbodies, and follow midbody dynamics over time. Moreover, we discuss techniques to prepare cortical sections for high-voltage electron microscopy to visualize the fine basal processes of progenitor cells.

Evarts, R. P., et al. (1993). "Activation of hepatic stem cell compartment in the rat: role of transforming growth factor alpha, hepatocyte growth factor, and acidic fibroblast growth factor in early proliferation." Cell Growth Differ **4**(7): 555-561.

We have demonstrated previously a pronounced increase in the expression of hepatocyte growth factor (HGF) (Z. Hu, R. P. Evarts, K. Fujio, E. R. Marsden, and S. S. Thorgeirsson, Am. J. Pathol., 142: 1823-1830, 1993), transforming growth factor alpha (TGF-alpha) (R. P. Evarts, H. Nakatsukasa, E. R. Marsden, Z. Hu, and S. S. Thorgeirsson, Mol. Carcinog., 5: 25-31, 1992), and acidic fibroblast growth factor (aFGF) (E. R. Marsden, Z. Hu, K. Fujio, H. Nakatsukasa, S. S. Thorgeirsson, and R. P. Evarts, Lab. Invest., 67: 427-433, 1992) that coincided with the proliferation and differentiation of putative hepatic stem cells and perisinusoidal stellate (Ito) cells. Here, we examine the earliest stages of stem cell activation in rat liver using an experimental model involving treatment with acetylaminofluorene and partial hepatectomy (R. P. Evarts, P. Nagy, E. Marsden, and S. S. Thorgeirsson, Carcinogenesis (Lond.), 8: 1737-1740, 1987). Histochemical identification of stem cell progeny and Ito cells was accomplished by OV6 and desmin antibodies, respectively. Expression of the 2.1-kilobase alpha-fetoprotein transcripts and the concomitant DNA synthesis ([3H]thymidine label)

were used as indicators for the activation of the stem cell compartment. Expression of HGF, TGF-alpha, and aFGF was analyzed at the time of partial hepatectomy and 4, 12, 24, 48, 72, and 92 h after the operation. [3H]-Thymidine-labeled OV6- and desmin-positive cells were present in the portal space and in the Glisson capsule 4 h after partial hepatectomy. (ABSTRACT TRUNCATED AT 250 WORDS)

Feigelman, J., et al. (2016). "Analysis of Cell Lineage Trees by Exact Bayesian Inference Identifies Negative Autoregulation of Nanog in Mouse Embryonic Stem Cells." Cell Syst **3**(5): 480-490 e413.

Many cellular effectors of pluripotency are dynamically regulated. In principle, regulatory mechanisms can be inferred from single-cell observations of effector activity across time. However, rigorous inference techniques suitable for noisy, incomplete, and heterogeneous data are lacking. Here, we introduce stochastic inference on lineage trees (STILT), an algorithm capable of identifying stochastic models that accurately describe the quantitative behavior of cell fate markers observed using time-lapse microscopy data collected from proliferating cell populations. STILT performs exact Bayesian parameter inference and stochastic model selection using a particle-filter-based algorithm. We use STILT to investigate the autoregulation of Nanog, a heterogeneously expressed core pluripotency factor, in mouse embryonic stem cells. STILT rejects the possibility of positive Nanog autoregulation with high confidence; instead, model predictions indicate weak negative feedback. We use STILT for rational experimental design and validate model predictions using novel experimental data. STILT is available for download as an open source framework from http://www.imsb.ethz.ch/research/claassen/Software/stilt---stochastic-inference-o n-lineage-trees.html.

Flexman, J. A., et al. (2007). "Morphological and parametric estimation of fetal neural stem cell migratory capacity in the rat brain." Conf Proc IEEE Eng Med Biol Soc **2007**: 4464-4467.

Magnetic resonance imaging (MRI) can non-invasively monitor the migratory behavior of magnetically labeled stem cells after transplantation. Signal changes associated with the clearance of the contrast agent due to cell death and leaked tracer in the interstitial space must be better understood in order to accurately interpret imaging results. In this study, fetal neural stem cells were labeled with superparamagnetic iron oxide (SPIO) particles and transplanted into the corpus callosum of the adult rat. MRI was performed on the day of transplantation and at one week. Control subjects received injections of either non-viable, labeled cells or loose SPIO particles. Two quantitative image analysis algorithms were developed to evaluate imaging results: 1) signal intensity drop-out areas were segmented and compared on a pixel-wise basis between initial and one week images; and 2) signal intensity profiles of transplanted materials at one week were parametrically modeled to estimate migration speed. Segmentation results showed that the number of pixels segmented at one week was significantly greater than the initial number of segmented pixels for subjects receiving injections of viable cells as compared to controls ($p<0.05$). The average speed of migration of viable cells along the corpus callosum was 69.2+/-41.1 microm/d and was significantly higher than controls ($p<0.05$). This study demonstrates an in vivo assay to quantitatively evaluate stem cell migration that can be used in different experimental paradigms.

Forostyak, O., et al. (2013). "Plasticity of calcium signaling cascades in human embryonic stem cell-derived neural precursors." Stem Cells Dev **22**(10): 1506-1521.

Human embryonic stem cell-derived neural precursors (hESC NPs) are considered to be a promising tool for cell-based therapy in central nervous system injuries and neurodegenerative diseases. The Ca (2+) ion is an important intracellular messenger essential for the regulation of various cellular functions. We investigated the role and physiology of Ca (2+) signaling to characterize the functional properties of CCTL14 hESC NPs during long-term maintenance in culture (in vitro). We analyzed changes in cytoplasmic Ca (2+) concentration ([Ca (2+)]i) evoked by high K (+), adenosine-5'-triphosphate (ATP), glutamate, gamma-aminobutyric acid (GABA), and caffeine in correlation with the expression of various neuronal markers in different passages (P6 through P10) during the course of hESC differentiation. We found that only differentiated NPs from P7 exhibited significant and specific [Ca (2+)]i responses to various stimuli. About 31% of neuronal-like P7 NPs exhibited spontaneous [Ca (2+)]i oscillations. Pharmacological and immunocytochemical assays revealed that P7 NPs express L- and P/Q-type Ca (2+) channels, P2X2, P2X3, P2X7, and P2Y purinoreceptors, glutamate receptors, and ryanodine (RyR1 and RyR3) receptors. The ATP- and glutamate-induced [Ca (2+)]i responses were concentration-dependent. Higher glutamate concentrations (over 100 muM) caused cell death. Responses to ATP were observed in the presence or in the absence of extracellular Ca (2+). These results emphasize the notion that with time in culture, these cells attain a transient period of operative Ca (2+) signaling that is predictive of their ability to act as stem elements.

Franquet, T., et al. (2006). "Thin-section CT findings in hematopoietic stem cell transplantation recipients with respiratory virus pneumonia." AJR Am J Roentgenol **187**(4): 1085-1090.

OBJECTIVE: The purpose of this study was to use serial thin-section CT scans to assess the incidence of respiratory viral infection and lung abnormalities in a large patient population at high risk of pulmonary complications. MATERIALS AND METHODS: The study population consisted of 26 recipients of hematopoietic stem cell transplants who had proven respiratory viral pneumonia. In all cases, thin-section CT scans were obtained before fiberoptic bronchoscopy and bronchoalveolar lavage. The study included only patients in whom bronchoalveolar lavage fluid showed no evidence of organisms other than respiratory viruses. The CT scans were assessed for the presence, extent, and anatomic distribution of ground-glass attenuation, air-space consolidation, nodules, centrilobular branching structures (tree-in-bud), thickening of the bronchovascular bundles, and pleural effusion. RESULTS: Areas of ground-glass attenuation were identified in 24 (92%) of 26 patients and were the only finding in eight patients. Multiple nodules, seen in 17 (65%) of 26 patients, measured 3-10 mm in diameter or were larger than 10 mm. The nodules had a centrilobular or random distribution. A tree-in-bud appearance was seen in six of the patients with centrilobular nodules. This pattern had a bilateral distribution and involved mainly the lower lung zones. CT revealed thickening of the bronchovascular bundles in 16 (61%) of the patients. Thickening was bilateral in 14 and unilateral in two patients. Bronchial wall thickening involved the lower lobes in six patients and had a patchy random distribution in the remaining nine patients. Air-space consolidation was present in nine (35%) of the cases. It had a lobular or subsegmental distribution in eight of the patients and a segmental distribution in one patient. Areas of consolidation were randomly distributed throughout the lungs in all cases. Less common findings included bilateral pleural effusion and bronchial dilatation. CONCLUSION: Respiratory viral infection is common among adult recipients of hematopoietic stem cell transplants, occurring over a wide time span after transplantation. The presence of respiratory viral infection must be considered in any patient with new respiratory symptoms, fever, or findings at CT such as extensive or patchy areas of ground-glass opacities or a mixture of patterns, most commonly ground-glass attenuation, thickening of the bronchial walls, and multiple small nodules.

Ghule, P. N., et al. (2008). "Staged assembly of histone gene expression machinery at subnuclear foci in the abbreviated cell cycle of human embryonic stem cells." Proc Natl Acad Sci U S A **105**(44): 16964-16969.

Human embryonic stem (hES) cells have an abbreviated G (1) phase of the cell cycle. How cells expedite G (1) events that are required for the initiation of S phase has not been resolved. One key regulatory pathway that controls G (1)/S-phase transition is the cyclin E/CDK2-dependent activation of the coactivator protein nuclear protein, ataxia-telangiectasia locus/histone nuclear factor-P (p220(NPAT)/HiNF-P) complex that induces histone gene transcription. In this study, we use the subnuclear organization of factors controlling histone gene expression to define mechanistic differences in the G (1) phase of hES and somatic cells using in situ immunofluorescence microscopy and fluorescence in situ hybridization (FISH). We show that histone gene expression is supported by the staged assembly and modification of a unique subnuclear structure that coordinates initiation and processing of transcripts originating from histone gene loci. Our results demonstrate that regulatory complexes that mediate transcriptional initiation (e.g., p220(NPAT)) and 3'-end processing (e.g., Lsm10, Lsm11, and SLBP) of histone gene transcripts colocalize at histone gene loci in dedicated subnuclear foci (histone locus bodies) that are distinct from Cajal bodies. Although appearance of CDK2-phosphorylated p220(NPAT) in these domains occurs at the time of S-phase entry, histone locus bodies are formed approximately 1 to 2 h before S phase in embryonic cells but 6 h before S phase in somatic cells. These temporal differences in the formation of histone locus bodies suggest that the G (1) phase of the cell cycle in hES cells is abbreviated in part by contraction of late G (1).

Gjorevski, N., et al. (2014). "Bioengineering approaches to guide stem cell-based organogenesis." Development **141**(9): 1794-1804.

During organogenesis, various molecular and physical signals are orchestrated in space and time to sculpt multiple cell types into functional tissues and organs. The complex and dynamic nature of the process has hindered studies aimed at delineating morphogenetic mechanisms in vivo, particularly in mammals. Recent demonstrations of stem cell-driven tissue assembly in culture offer a powerful new tool for modeling and dissecting organogenesis. However, despite the highly organotypic nature of stem cell-derived tissues, substantial differences set them apart from their in vivo counterparts, probably owing to the altered microenvironment in which they reside and the lack of mesenchymal influences. Advances in the biomaterials and microtechnology fields have, for example, afforded a high degree of spatiotemporal

control over the cellular microenvironment, making it possible to interrogate the effects of individual microenvironmental components in a modular fashion and rapidly identify organ-specific synthetic culture models. Hence, bioengineering approaches promise to bridge the gap between stem cell-driven tissue formation in culture and morphogenesis in vivo, offering mechanistic insight into organogenesis and unveiling powerful new models for drug discovery, as well as strategies for tissue regeneration in the clinic. We draw on several examples of stem cell-derived organoids to illustrate how bioengineering can contribute to tissue formation ex vivo. We also discuss the challenges that lie ahead and potential ways to overcome them.

Hambright, D., et al. (2012). "Long-term survival and differentiation of retinal neurons derived from human embryonic stem cell lines in un-immunosuppressed mouse retina." Mol Vis **18**: 920-936.

PURPOSE: To examine the potential of NIH-maintained human embryonic stem cell (hESC) lines TE03 and UC06 to differentiate into retinal progenitor cells (hESC-RPCs) using the noggin/Dkk-1/IGF-1/FGF9 protocol. An additional goal is to examine the in vivo dynamics of maturation and retinal integration of subretinal and epiretinal (vitreous space) hESC-RPC grafts without immunosuppression. METHODS: hESCs were neuralized in vitro with noggin for 2 weeks and expanded to derive neuroepithelial cells (hESC-neural precursors, NPs). Wnt (Integration 1 and wingless) blocking morphogens Dickkopf-1 (Dkk-1) and Insulin-like growth factor 1 (IGF-1) were used to direct NPs to a rostral neural fate, and fibroblast growth factor 9 (FGF9)/fibroblast growth factor-basic (bFGF) were added to bias the differentiation of developing anterior neuroectoderm cells to neural retina (NR) rather than retinal pigment epithelium (RPE). Cells were dissociated and grafted into the subretinal and epiretinal space of young adult (4-6-week-old) mice (C57BL/6J x129/Sv mixed background). Remaining cells were replated for (i) immunocytochemical analysis and (ii) used for quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis. Mice were sacrificed 3 weeks or 3 months after grafting, and the grafts were examined by histology and immunohistochemistry for survival of hESC-RPCs, presence of mature neuronal and retinal markers, and the dynamics of in vivo maturation and integration into the host retina. RESULTS: At the time of grafting, hESC-RPCs exhibited immature neural/neuronal immunophenotypes represented by nestin and neuronal class III beta-tubulin, with about half of the cells positive for cell proliferation marker Kiel University - raised antibody number 67 (Ki67), and no recoverin-positive (recoverin [+]) cells. The grafted cells expressed eye field markers paired box 6 (PAX6), retina and anterior neural fold homeobox (RAX), sine oculis homeobox homolog 6 (SIX6), LIM homeobox 2 (LHX2), early NR markers (Ceh-10 homeodomain containing homolog [CHX10], achaete-scute complex homolog 1 [MASH1], mouse atonal homolog 5 [MATH5], neurogenic differentiation 1 [NEUROD1]), and some retinal cell fate markers (brain-specific homeobox/POU domain transcription factor 3B [BRN3B], prospero homeobox 1 [PROX1], and recoverin). The cells in the subretinal grafts matured to predominantly recoverin [+] phenotype by 3 months and survived in a xenogenic environment without immunosuppression as long as the blood-retinal barrier was not breached by the transplantation procedure. The epiretinal grafts survived but did not express markers of mature retinal cells. Retinal integration into the retinal ganglion cell (RGC) layer and the inner nuclear layer (INL) was efficient from the epiretinal but not subretinal grafts. The subretinal grafts showed limited ability to structurally integrate into the host retina and only in cases when NR was damaged during grafting. Only limited synaptogenesis and no tumorigenicity was observed in grafts. CONCLUSIONS: Our studies show that (i) immunosuppression is not mandatory to xenogenic graft survival in the retina, (ii) the subretinal but not the epiretinal niche can promote maturation of hESC-RPCs to photoreceptors, and (iii) the hESC-RPCs from epiretinal but not subretinal grafts can efficiently integrate into the RGC layer and INL. The latter could be of value for long-lasting neuroprotection of retina in some degenerative conditions and glaucoma. Overall, our results provide new insights into the technical aspects associated with cell-based therapy in the retina.

Heng, B. C., et al. (2005). "Reduced mitotic activity at the periphery of human embryonic stem cell colonies cultured in vitro with mitotically-inactivated murine embryonic fibroblast feeder cells." Cell Biochem Funct **23**(2): 141-146.

This study attempted to investigate whether different levels of mitotic activity exist within different physical regions of a human embryonic stem (hES) cell colony. Incorporation of 5-bromo-2-deoxyuridine (BrdU) within newly-synthesized DNA, followed by immunocytochemical staining was used as a means of detecting mitotically-active cells within hES colonies. The results showed rather surprisingly that the highest levels of mitotic activity are primarily concentrated within the central regions of hES colonies, whereas the peripheral regions exhibited reduced levels of cellular proliferation. Two hypothetical mechanisms are

therefore proposed for hES colony growth and expansion. Firstly, it is envisaged that the less mitotically-active hES cells at the periphery of the colony are continually migrating outwards, thereby providing space for newly-divided daughter cells within the more mitotically-active central region of the hES colony. Secondly, it is proposed that the newly-divided hES cells within the central region of the colony somehow migrate to the outer periphery. This could possibly explain why the periphery of hES colonies are less mitotically-active, since there would obviously be an extended time-lag before newly-divided daughter cells are ready again for the next cell division. Further investigations need to be carried out to characterize the atypical mechanisms by which hES colonies grow and expand in size.

Hiramatsu, R., et al. (2016). "Clinicopathological analysis of allogeneic hematopoietic stem cell transplantation-related membranous glomerulonephritis." Hum Pathol **50**: 187-194.

Allogeneic hematopoietic stem cell transplantation (HSCT)-related membranous glomerulonephritis (MGN) is poorly understood. A total of 830 patients who underwent HSCT at Toranomon Hospital from 2000 to 2012 were evaluated retrospectively, including 621 patients receiving umbilical cord blood transplantation (UCBT) and 208 patients receiving unrelated bone marrow transplantation. MGN was diagnosed in 5 patients after UCBT (versus none after bone marrow transplantation) and occurred concomitantly with chronic graft-versus-host disease after cessation of immunosuppression. Light microscopy did not show any definite spikes or bubbling of the glomerular basement membrane (GBM) in all 5 patients. In 1 patient (case 5), endocapillary proliferative lesions with fibrin-like deposits were noted in addition to MGN findings. Immunofluorescence demonstrated granular deposits of immunoglobulin G (IgG; IgG1 and IgG4) along the GBM with negativity for C3, C4, and C1q in 4 patients (cases 1-4), whereas case 5 showed positivity for IgG (IgG1, IgG2, IgG3, and IgG4) as well as for C3, C4, and C1q. Electron microscopy revealed electron-dense deposits in the subepithelial space of the GBM in cases 1-4. In case 5, electron-dense deposits were present in the mesangium and the subendothelial space of the GBM, as well as in the subepithelial space. After treatment with immunosuppressants (prednisolone and/or cyclosporin) or angiotensin-converting enzyme inhibitors, complete remission with disappearance of proteinuria was achieved 12.2 months in all 5 patients, but nephrotic-range proteinuria relapsed in 2 patients during follow-up. Serum anti-PLA2R autoantibody was negative in 3 patients. HSCT-related MGN only occurred after UCBT. We believe that there were 2 morphologic patterns: early MGN and membranoproliferative pattern glomerulonephritis.

Ho, J. H., et al. (2011). "Cell contact accelerates replicative senescence of human mesenchymal stem cells independent of telomere shortening and p53 activation: roles of Ras and oxidative stress." Cell Transplant **20**(8): 1209-1220.

Mesenchymal stem cells (MSCs) are of great therapeutic potentials due to their multilineage differentiation capabilities. Before transplantation, in vitro culture expansion of MSCs is necessary to get desired cell number. We observed that cell contact accelerated replicative senescence during such process. To confirm the finding as well as to investigate the underlying mechanisms, we cultured both human bone marrow- and umbilical cord blood-derived MSCs under noncontact culture (subculture performed at 60-70% of confluence), or contact culture (cell passage performed at 100% of confluence). It was found that MSCs reached cellular senescence earlier in contact culture, and the doubling time was significantly prolonged. Marked increase of senescence-associated beta-galactosidase-positive staining was also observed as a result of cell contact. Cell cycle analysis revealed increased frequency of cell cycle arrest after contact culture. It was noted, however, that the telomere length was not altered during contact-induced acceleration of senescence. Moreover, cell cycle checkpoint regulator P53 expression was not affected by cell contact. Marked increase in intracellular reactive oxygen species (ROS) and a concomitant decrease in the activities of antioxidative enzymes were also observed during contact-induced senescence. Importantly, increased p16(INK4a) following Ras upregulation was found after contact culture. Taken together, cell contact induced accelerated senescence of MSCs, which is telomere shortening and p53 independent. ROS accumulation due to defective ROS clearance function together with Ras and p16(INK4a) upregulation play an important role in contact-induced senescence of MSCs. Overconfluence should therefore be avoided during in vitro culture expansion of MSCs in order to maintain their qualities for clinical application purposes. The contact-induced senescence model reported in this study will serve as a useful model system that allows further study of the molecular mechanisms of senescence in MSCs.

Huck, K., et al. (2006). "Three cases of renal relapse after allogeneic hematopoietic stem cell transplantation for childhood acute lymphoblastic leukemia." Haematologica **91**(5 Suppl): ECR07.

Isolated renal relapse after allogeneic hematopoietic stem cell transplantation (alloHSCT) in

children with acute lymphoblastic leukemia (ALL) is a rare condition. Generally, in ALL, the sites most frequently affected by extramedullary relapse are the central nervous system (CNS) and the testicles. Here we report on three young boys with relapsed B-precursor ALL, who underwent alloHSCT from HLA-identical siblings and suffered a histopathologically proven isolated unilateral renal relapse (two patients) or a combined renal and testicular relapse (one patient) 6, 10 and 12 months post alloHSCT. In all patients at the time of relapse bone marrow showed complete remission with complete donor hematopoiesis. They all received total body irradiation with partial shielding of the kidneys as part of their conditioning therapy, such that renal shielding could be an explanation for the observed accumulation of renal relapses. Moreover, during the past few years so called immune privilege has been postulated for frequent relapse sites such as the CNS, the testicles and the anterior chamber of the eye. Impaired accessability of these organs by cytotoxic T-cells (CTLs) with a reduced graft-versus-leukemia (GvL) effect after alloHSCT is based on a number of different molecular and cellular mechanisms. Similar mechanisms have been shown to be effective in the tubulointerstitial space of the kidney, rendering the kidney a potentially immune privileged site. Due to these observations we advocate sufficient treatment of the kidneys during conditioning therapy.

Ilmarinen, T., et al. (2015). "Ultrathin Polyimide Membrane as Cell Carrier for Subretinal Transplantation of Human Embryonic Stem Cell Derived Retinal Pigment Epithelium." PLoS One **10**(11): e0143669.

In this study, we investigated the suitability of ultrathin and porous polyimide (PI) membrane as a carrier for subretinal transplantation of human embryonic stem cell (hESC) -derived retinal pigment epithelial (RPE) cells in rabbits. The in vivo effects of hESC-RPE cells were analyzed by subretinal suspension injection into Royal College of Surgeons (RCS) rats. Rat eyes were analyzed with electroretinography (ERG) and histology. After analyzing the surface and permeability properties of PI, subretinal PI membrane transplantations with and without hESC-RPE were performed in rabbits. The rabbits were followed for three months and eyes analyzed with fundus photography, ERG, optical coherence tomography (OCT), and histology. Animals were immunosuppressed with cyclosporine the entire follow-up time. In dystrophic RCS rats, ERG and outer nuclear layer (ONL) thickness showed some rescue after hESC-RPE injection. Cells positive for human antigen were found in clusters under the retina 41 days post-injection but not anymore after 105 days. In rabbits, OCT showed good placement of the PI. However, there was loss of pigmentation on the hESC-RPE-PI over time. In the eyes with PI alone, no obvious signs of inflammation or retinal atrophy were observed. In the presence of hESC-RPE, mononuclear cell infiltration and retinal atrophy were observed around the membranes. The porous ultrathin PI membrane was well-tolerated in the subretinal space and is a promising scaffold for RPE transplantation. However, the rejection of the transplanted cells seems to be a major problem and the given immunosuppression was insufficient for reduction of xenograft induced inflammation.

Isa, A., et al. (2010). "Impaired cell surface expression of HLA-B antigens on mesenchymal stem cells and muscle cell progenitors." PLoS One **5**(5): e10900.

HLA class-I expression is weak in embryonic stem cells but increases rapidly during lineage progression. It is unknown whether all three classical HLA class-I antigens follow the same developmental program. In the present study, we investigated allele-specific expression of HLA-A, -B, and -C at the mRNA and protein levels on human mesenchymal stem cells from bone marrow and adipose tissue as well as striated muscle satellite cells and lymphocytes. Using multicolour flow cytometry, we found high cell surface expression of HLA-A on all stem cells and PBMC examined. Surprisingly, HLA-B was either undetectable or very weakly expressed on all stem cells protecting them from complement-dependent cytotoxicity (CDC) using relevant human anti-B and anti-Cw sera. IFNgamma stimulation for 48-72 h was required to induce full HLA-B protein expression. Quantitative real-time RT-PCR showed that IFNgamma induced a 9-42 fold increase of all six HLA-A,-B,-C gene transcripts. Interestingly, prior to stimulation, gene transcripts for all but two alleles were present in similar amounts suggesting that post-transcriptional mechanisms regulate the constitutive expression of HLA-A,-B, and -C. Locus-restricted expression of HLA-A, -B and -C challenges our current understanding of the function of these molecules as regulators of CD8(+) T-cell and NK-cell function and should lead to further inquiries into their expression on other cell types.

Ito, H., et al. (2004). "Local irradiation enhances congenic donor pluripotent hematopoietic stem cell engraftment similarly in irradiated and nonirradiated sites." Blood **103**(5): 1949-1954.

Long-term multilineage chimerism is achieved in CD45 congenic mice receiving high bone marrow doses with or without mediastinal irradiation (MI). Increased donor chimerism results in MI-treated compared with nonirradiated animals, suggesting that

MI makes "space" for engraftment of donor pluripotent hematopoietic stem cells (PHSCs). We have now examined whether space is systemic or whether increased engraftment of donor marrow in locally irradiated mice is confined to the irradiated bones. While increased donor chimerism was observed in irradiated bones compared with nonirradiated bones of MI-treated animals 4 weeks following bone marrow transplantation (BMT), these differences were minimal by 40 weeks. MI-treated chimeras contained more adoptively transferable donor PHSCs in the marrow of both irradiated and distant bones compared with non-MI-treated chimeras. Similar proportions of donor PHSCs were present in irradiated and nonirradiated bones of locally irradiated mice at both 4 and 40 weeks. Irradiated bones contained more donor short-term repopulating cells than distant bones at 4 weeks, but not 40 weeks, after BMT. Our study suggests that local proliferation of donor PHSCs in mice receiving local irradiation rapidly leads to a systemic increase in donor PHSC engraftment.

Iyer, D., et al. (2015). "Robust derivation of epicardium and its differentiated smooth muscle cell progeny from human pluripotent stem cells." Development **142**(8): 1528-1541.

The epicardium has emerged as a multipotent cardiovascular progenitor source with therapeutic potential for coronary smooth muscle cell, cardiac fibroblast (CF) and cardiomyocyte regeneration, owing to its fundamental role in heart development and its potential ability to initiate myocardial repair in injured adult tissues. Here, we describe a chemically defined method for generating epicardium and epicardium-derived smooth muscle cells (EPI-SMCs) and CFs from human pluripotent stem cells (HPSCs) through an intermediate lateral plate mesoderm (LM) stage. HPSCs were initially differentiated to LM in the presence of FGF2 and high levels of BMP4. The LM was robustly differentiated to an epicardial lineage by activation of WNT, BMP and retinoic acid signalling pathways. HPSC-derived epicardium displayed enhanced expression of epithelial- and epicardium-specific markers, exhibited morphological features comparable with human foetal epicardial explants and engrafted in the subepicardial space in vivo. The in vitro-derived epicardial cells underwent an epithelial-to-mesenchymal transition when treated with PDGF-BB and TGFbeta1, resulting in vascular SMCs that displayed contractile ability in response to vasoconstrictors. Furthermore, the EPI-SMCs displayed low density lipoprotein uptake and effective lowering of lipoprotein levels upon treatment with statins, similar to primary human coronary artery SMCs. Cumulatively, these findings suggest that HPSC-derived epicardium and EPI-SMCs could serve as important tools for studying human cardiogenesis, and as a platform for vascular disease modelling and drug screening.

Jindal, N., et al. (2017). "Alteration of Neurotrophic Factors After Transplantation of Bone Marrow Derived Lin-ve Stem Cell in NMDA-Induced Mouse Model of Retinal Degeneration." J Cell Biochem **118**(7): 1699-1711.

Retinal ganglion cell layer (RGCs) is one of the important layers of retina, depleted in Glaucoma. Loss of RGC neurons is a major cellular mechanism involved in its pathogenesis resulting in severe vision loss. Stem cell therapy has emerged as a potential strategy to arrest the apoptotic loss of RGCs and also replace the degenerative cells in damaged retina. Here, we have investigated the incorporation and survival of mouse bone marrow derived Lin-ve stem cells in N-methyl-d-aspartate (NMDA)-induced mouse model of retinal degeneration. Two days after intravitreal injection of NMDA (100 mM) showed significant decrease in ganglion cell number and increase in TUNEL positive apoptotic cells in retinal layers. The injury was further characterized by immunohistochemical expression of Brn3b, GFAP, Bcl2, pCREB, CNTF, GDNF, and BDNF in retinal layers. Lin-ve cells (100,000 dose) were intravitreally transplanted after 2 days of injury and evaluated after 7, 14, and 21 days of transplantation. Transplanted cells were found to have migrated from intravitreal space and incorporated into injured retina at 7, 14, and 21 days post-transplantation. At 21 days Brn3b, CNTF, and BDNF expression was found to be upregulated whereas GDNF was downregulated when compared to respective injury time points. Molecular data showed decrease in the expression of Brn3b, BDNF, CNTF, and GDNF post transplantation when compared with injury groups. This study reveals that Lin-ve stem cells may exert neuroprotective effect in damaged retina mediated by participation of neurotrophic factors induced by stem cell transplantation at the site of injury. J. Cell. Biochem. 118: 1699-1711, 2017. (c) 2016 Wiley Periodicals, Inc.

Kachouie, N. N. and P. W. Fieguth (2007). "Extended-Hungarian-JPDA: exact single-frame stem cell tracking." IEEE Trans Biomed Eng **54**(11): 2011-2019.

The fields of bioinformatics and biotechnology rely on the collection, processing and analysis of huge numbers of biocellular images, including cell features such as cell size, shape, and motility. Thus, cell tracking is of crucial importance in the study of cell behaviour and in drug and disease research. Such a multitarget tracking is essentially an assignment problem, NP-hard, with the solution normally found in

practice in a reduced hypothesis space. In this paper we introduce a novel approach to find the exact association solution over time for single-frame scan-back stem cell tracking. Our proposed method employs a class of linear programming optimization methods known as the Hungarian method to find the optimal joint probabilistic data association for nonlinear dynamics and non-Gaussian measurements. The proposed method, an optimal joint probabilistic data association approach, has been successfully applied to track hematopoietic stem cells.

Kamao, H., et al. (2014). "Objective evaluation of the degree of pigmentation in human induced pluripotent stem cell-derived RPE." Invest Ophthalmol Vis Sci **55**(12): 8309-8318.

PURPOSE: For the transplantation of human induced pluripotent stem cell-derived retinal pigment epithelium (hiPSC-RPE), determination of the maturation status of these cells is essential, and the degree of pigmentation (dPG) can serve as a good indicator of this status. The aim of this study was to establish a method of objectively and quantitatively evaluating the dPG of hiPSC-RPE. METHODS: Two observers determined the dPG subjectively by observing recorded images of hiPSC-RPE as follows: the dPG of a single cell was classified into three different pigmentation stages, and the overall dPG was compared between two cell groups to identify the group with the higher dPG. The kappa statistic was applied to assess interobserver reproducibility. Next, the dPG of single cells and cell groups was objectively determined by the lightness of the hue, saturation, and value (HSL) color space, and the correlation between the subjective evaluation and time-dependent change in the objective dPG of hiPSC-RPE was investigated. RESULTS: The kappa statistic was 0.88 and 0.81 in the single-cell and cell-group observations, respectively. The objective dPG of single cells and cell groups was highly correlated with the subjective dPG. However, the observers were occasionally unable to subjectively determine the group with the higher dPG. The objective dPG increased in a time-dependent manner. CONCLUSIONS: The lightness of the HSL color space can be used to objectively and quantitatively evaluate the dPG of hiPSC-RPE in culture. The objective evaluation was consistent and was able to better identify small differences than subjective evaluation.

Kamboj, M., et al. (2016). "Transmission of Clostridium difficile During Hospitalization for Allogeneic Stem Cell Transplant." Infect Control Hosp Epidemiol **37**(1): 8-15.

OBJECTIVE To determine the role of unit-based transmission that accounts for cases of early Clostridium difficile infection (CDI) during hospitalization for allogeneic stem cell transplant. SETTING Stem cell transplant unit at a tertiary care cancer center. METHODS Serially collected stool from patients admitted for transplant was screened for toxigenic C. difficile through the hospital stay and genotyping was performed by multilocus sequence typing. In addition, isolates retrieved from cases of CDI that occurred in other patients hospitalized on the same unit were similarly characterized. Transmission links were established by time-space clustering of cases and carriers of shared toxigenic C. difficile strains. RESULTS During the 27-month period, 1,099 samples from 264 patients were screened, 69 of which had evidence of toxigenic C. difficile; 52 patients developed CDI and 17 were nonsymptomatic carriers. For the 52 cases, 41 had evidence of toxigenic C. difficile on the first study sample obtained within a week of admission, among which 22 were positive within the first 48 hours. A total of 24 sequence types were isolated from this group; 1 patient had infection with the NAP1 strain. A total of 11 patients had microbiologic evidence of acquisition; donor source could be established in half of these cases. CONCLUSIONS Most cases of CDI after stem cell transplant represent delayed onset disease in nonsymptomatic carriers. Transmission on stem cell transplant unit was confirmed in 19% of early CDI cases in our cohort with a probable donor source established in half of the cases.

Kawada, J., et al. (2012). "Spatiotemporally controlled delivery of soluble factors for stem cell differentiation." Lab Chip **12**(21): 4508-4515.

Despite the fact that cells in vivo are largely affected by the spatial heterogeneity in their surroundings, in vitro experimental procedures for stem cell differentiation have been relying on spatially uniform culture environments so far. Here, we present a method to form spatiotemporally non-uniform culture environments for stem cell differentiation using a membrane-based microfluidic device. By adopting a porous membrane with relatively large pores, patterned delivery of soluble factors is maintained stably over a period of time long enough for cell differentiation. We report that spatial patterns of mouse induced pluripotent stem cells (miPSCs) differentiation can be controlled by the present method. Furthermore, it is shown that the cell fate decision of miPSCs is determined by time-dependent switching of the delivery pattern. The present technique could be of relevance to the detailed analyses of the characteristics of stem cell differentiation in time and space, opening up a new insight into regenerative biology.

Lara-Ramirez, R., et al. (2019). "A Notch-regulated proliferative stem cell zone in the developing spinal cord is an ancestral vertebrate trait." Development **146**(1).

Vertebrates have evolved the most sophisticated nervous systems we know. These differ from the nervous systems of invertebrates in several ways, including the evolution of new cell types, and the emergence and elaboration of patterning mechanisms to organise cells in time and space. Vertebrates also generally have many more cells in their central nervous systems than invertebrates, and an increase in neural cell number may have contributed to the sophisticated anatomy of the brain and spinal cord. Here, we study how increased cell number evolved in the vertebrate central nervous system, investigating the regulation of cell proliferation in the lamprey spinal cord. Markers of proliferation show that a ventricular progenitor zone is found throughout the lamprey spinal cord. We show that inhibition of Notch signalling disrupts the maintenance of this zone. When Notch is blocked, progenitor cells differentiate precociously, the proliferative ventricular zone is lost and differentiation markers become expressed throughout the spinal cord. Comparison with other chordates suggests that the emergence of a persistent Notch-regulated proliferative progenitor zone was a crucial step for the evolution of vertebrate spinal cord complexity.

Lau, T., et al. (2009). "Monitoring mouse serotonin transporter internalization in stem cell-derived serotonergic neurons by confocal laser scanning microscopy." Neurochem Int **54**(3-4): 271-276.

In the central nervous system serotonergic neurotransmission is terminated by the rapid removal of serotonin (5-hydroxytryptamine, 5HT) out of the extra-cellular space back into the presynaptic neuron. This task is fulfilled by a specific serotonin transporter (SERT) protein which controls the concentration of extra-cellular 5HT. Consequently, one mechanism to regulate the efficacy of serotonergic neurotransmission is via modulation of the density of SERT molecules on the cell membrane. In this regard it has been shown, that chronic activation of the p38 mitogen-activated protein kinase (p38 MAPK) leads to enhanced SERT surface expression whereas activation of protein kinase C (PKC) reduces SERT surface expression. In addition, it has been reported that exposure to selective serotonin re-uptake inhibitors (SSRIs) leads to a down-regulation of SERT expression in vivo and in vitro in different cellular systems. Here, we have studied interactions between kinase- and SSRI-induced SERT internalization in mouse stem cell-derived serotonergic neurons expressing the native SERT allele in its natural surroundings. Therefore we established a method to quantify the amount of cell surface-expressed SERT molecules on individual cells by antibody detection combined with confocal laser scanning microscopy. Using this methodology we could show that activation of PKC, inhibition of the p38 MAPK as well as exposure to the SSRI citalopram each induced a significant reduction of cell surface-expressed SERT over time. Combinations of PKC activation, p38 MAPK inhibition and SSRI exposure led to a more pronounced down-regulation of SERT surface expression depending on the time of drug exposure.

Lee, Y., et al. (2013). "Hormonal regulation of stem cell maintenance in roots." J Exp Bot **64**(5): 1153-1165.

During plant embryogenesis, the apical-basal axis is established and both the shoot apical meristem (SAM) and the root apical meristem (RAM) are formed. In both meristems, there are slowly dividing cells which control the differentiation of their surrounding cells called the organizing centre (OC) and the quiescent centre (QC) in the shoot and root, respectively. These centres with their surrounding initial cells form a 'stem cell niche'. The initial cells eventually differentiate into various plant tissues, giving rise to plant organs such as lateral shoots, flowers, leaves, and lateral roots. Plant hormones are important factors involved in the balance between cell division and differentiation such that plant growth and development are tightly controlled in space and time. No single hormone acts by itself in regulating the meristematic activity in the root meristem. Division and differentiation are controlled by interactions between several hormones. Intensive research on plant stem cells has focused on how cell division is regulated to form specific plant organs and tissues, how differentiation is controlled, and how stem cell fate is coordinated. In this review, recent knowledge pertaining to the role of plant hormones in maintaining root stem cells including the QC is summarized and discussed. Furthermore, we suggest diverse approaches to answering the main question of how root stem cells are regulated and maintained by plant hormones.

Lehotzky, D. and G. K. H. Zupanc (2019). "Cellular Automata Modeling of Stem-Cell-Driven Development of Tissue in the Nervous System." Dev Neurobiol **79**(5): 497-517.

Mathematical and computational modeling enables biologists to integrate data from observations and experiments into a theoretical framework. In this review, we describe how developmental processes associated with stem-cell-driven growth of tissue in

both the embryonic and adult nervous system can be modeled using cellular automata (CA). A cellular automaton is defined by its discrete nature in time, space, and state. The discrete space is represented by a uniform grid or lattice containing agents that interact with other agents within their local neighborhood. This possibility of local interactions of agents makes the cellular automata approach particularly well suited for studying through modeling how complex patterns at the tissue level emerge from fundamental developmental processes (such as proliferation, migration, differentiation, and death) at the single-cell level. As part of this review, we provide a primer for how to define biologically inspired rules governing these processes so that they can be implemented into a CA model. We then demonstrate the power of the CA approach by presenting simulations (in the form of figures and movies) based on building models of three developmental systems: the formation of the enteric nervous system through invasion by neural crest cells; the growth of normal and tumorous neurospheres induced by proliferation of adult neural stem/progenitor cells; and the neural fate specification through lateral inhibition of embryonic stem cells in the neurogenic region of Drosophila.

Levengood, S. L. and W. L. Murphy (2010). "Biomaterials for high-throughput stem cell culture." Curr Stem Cell Res Ther **5**(3): 261-267.

A cell's microenvironment plays a primary role in defining cell fate during tissue development, physiological function, and pathological dysfunction. Understanding the key components and interactions within these microenvironments is critical for effective use of stem cells for disease modeling and therapeutic applications. Yet cell microenvironments are difficult to study, as there are tens or hundreds of parameters that can influence cell behavior simultaneously. Additionally, parameters such as cell-cell interactions, cell-ECM interactions, cell shape, soluble signals, and mechanical forces vary dynamically in 3-dimensional space and time. The number of relevant experimental conditions in these cell-based biological systems quickly becomes intractable using standard experimental platforms and techniques. A new set of strategies involving high-throughput experimental formats and 3-dimensional culture is required to achieve significant progress in understanding and exploiting stem cell biology. This mini-review describes bioengineering approaches that are enabling for high-throughput stem cell culture, screening and analysis.

Li, S. C. and M. H. Kabeer (2018). "Spatiotemporal switching signals for cancer stem cell activation in pediatric origins of adulthood cancer: Towards a watch-and-wait lifetime strategy for cancer treatment." World J Stem Cells **10**(2): 15-22.

Pediatric origin of cancer stem cell hypothesis holds great promise and potential in adult cancer treatment, however; the road to innovation is full of obstacles as there are plenty of questions left unanswered. First, the key question is to characterize the nature of such stem cells (concept). Second, the quantitative imaging of pediatric stem cells should be implemented (technology). Conceptually, pediatric stem cell origins of adult cancer are based on the notion that plasticity in early life developmental programming evolves local environments to cancer. Technologically, such imaging in children is lacking as all imaging is designed for adult patients. We postulate that the need for quantitative imaging to measure space-time changes of plasticity in early life developmental programming in children may trigger research and development of the imaging technology. Such quantitative imaging of pediatric origin of adulthood cancer will help develop a spatiotemporal monitoring system to determine cancer initiation and progression. Clinical validation of such speculative hypothesis-that cancer originates in a pediatric environment-will help implement a wait-and-watch strategy for cancer treatment.

Li, Y., et al. (2012). "Long-term safety and efficacy of human-induced pluripotent stem cell (iPS) grafts in a preclinical model of retinitis pigmentosa." Mol Med **18**: 1312-1319.

The U.S. Food and Drug Administration recently approved phase I/II clinical trials for embryonic stem (ES) cell-based retinal pigmented epithelium (RPE) transplantation, but this allograft transplantation requires lifelong immunosuppressive therapy. Autografts from patient-specific induced pluripotent stem (iPS) cells offer an alternative solution to this problem. However, more data are required to establish the safety and efficacy of iPS transplantation in animal models before moving iPS therapy into clinical trials. This study examines the efficacy of iPS transplantation in restoring functional vision in Rpe65(rd12)/Rpe65(rd12) mice, a clinically relevant model of retinitis pigmentosa (RP). Human iPS cells were differentiated into morphologically and functionally RPE-like tissue. Quantitative real-time polymerase chain reaction (RT-PCR) and immunoblots confirmed RPE fate. The iPS-derived RPE cells were injected into the subretinal space of Rpe65(rd12)/Rpe65(rd12) mice at 2 d postnatally. After transplantation, the long-term surviving iPS-derived RPE graft colocalized with the host native RPE cells and assimilated into the host retina without disruption. None of the mice receiving transplants developed tumors over their lifetimes. Furthermore,

electroretinogram, a standard method for measuring efficacy in human trials, demonstrated improved visual function in recipients over the lifetime of this RP mouse model. Our study provides the first direct evidence of functional recovery in a clinically relevant model of retinal degeneration using iPS transplantation and supports the feasibility of autologous iPS cell transplantation for retinal and macular degenerations featuring significant RPE loss.

Li, Z. and H. J. Deeg (2001). "Pros and cons of splenectomy in patients with myelofibrosis undergoing stem cell transplantation." Leukemia **15**(3): 465-467.

During fetal development, the spleen is a major hemopoietic organ. In the adult human, this task is relinquished to the bone marrow. However, under the stress of certain pathologic conditions, extramedullary hemopoiesis may again occur in the spleen. This is especially true for diseases of the marrow, in particular, myeloproliferative disorders such as agnogenic myeloid metaplasia, which is associated with severe fibrosis of the marrow space. At the same time, the spleen sequesters blood cells and contributes to peripheral blood cytopenias, which may improve following splenectomy. However, success is unpredictable, and the operative mortality of splenectomy is on the order of 10%. As a growing number of patients undergo hemopoietic stem cell transplantation as definitive therapy for myelofibrosis, the decision on splenectomy has additional ramifications since the spleen plays an important role in the kinetics of engraftment of donor cells and in immune reconstitution. We conclude from our analysis of available information that the benefit of splenectomy is difficult to predict, although after transplantation splenectomized patients have faster hemopoietic recovery. It appears that the most important indication for splenectomy in these patients is the relief of symptoms from massive spleen enlargement.

Lippmann, E. S., et al. (2013). "Modeling the blood-brain barrier using stem cell sources." Fluids Barriers CNS **10**(1): 2.

The blood-brain barrier (BBB) is a selective endothelial interface that controls trafficking between the bloodstream and brain interstitial space. During development, the BBB arises as a result of complex multicellular interactions between immature endothelial cells and neural progenitors, neurons, radial glia, and pericytes. As the brain develops, astrocytes and pericytes further contribute to BBB induction and maintenance of the BBB phenotype. Because BBB development, maintenance, and disease states are difficult and time-consuming to study in vivo, researchers often utilize in vitro models for simplified analyses and higher throughput. The in vitro format also provides a platform for screening brain-penetrating therapeutics. However, BBB models derived from adult tissue, especially human sources, have been hampered by limited cell availability and model fidelity. Furthermore, BBB endothelium is very difficult if not impossible to isolate from embryonic animal or human brain, restricting capabilities to model BBB development in vitro. In an effort to address some of these shortcomings, advances in stem cell research have recently been leveraged for improving our understanding of BBB development and function. Stem cells, which are defined by their capacity to expand by self-renewal, can be coaxed to form various somatic cell types and could in principle be very attractive for BBB modeling applications. In this review, we will describe how neural progenitor cells (NPCs), the in vitro precursors to neurons, astrocytes, and oligodendrocytes, can be used to study BBB induction. Next, we will detail how these same NPCs can be differentiated to more mature populations of neurons and astrocytes and profile their use in co-culture modeling of the adult BBB. Finally, we will describe our recent efforts in differentiating human pluripotent stem cells (hPSCs) to endothelial cells with robust BBB characteristics and detail how these cells could ultimately be used to study BBB development and maintenance, to model neurological disease, and to screen neuropharmaceuticals.

Liu, M., et al. (2010). "Automated tracking of stem cell lineages of Arabidopsis shoot apex using local graph matching." Plant J **62**(1): 135-147.

Shoot apical meristems (SAMs) of higher plants harbor stem-cell niches. The cells of the stem-cell niche are organized into spatial domains of distinct function and cell behaviors. A coordinated interplay between cell growth dynamics and changes in gene expression is critical to ensure stem-cell homeostasis and organ differentiation. Exploring the causal relationships between cell growth patterns and gene expression dynamics requires quantitative methods to analyze cell behaviors from time-lapse imagery. Although technical breakthroughs in live-imaging methods have revealed spatio-temporal dynamics of SAM-cell growth patterns, robust computational methods for cell segmentation and automated tracking of cells have not been developed. Here we present a local graph matching-based method for automated-tracking of cells and cell divisions of SAMs of Arabidopsis thaliana. The cells of the SAM are tightly clustered in space which poses a unique challenge in computing spatio-temporal correspondences of cells. The local graph-matching principle efficiently exploits the geometric structure and topology of the relative positions of cells in obtaining spatio-temporal

correspondences. The tracker integrates information across multiple slices in which a cell may be properly imaged, thus providing robustness to cell tracking in noisy live-imaging datasets. By relying on the local geometry and topology, the method is able to track cells in areas of high curvature such as regions of primordial outgrowth. The cell tracker not only computes the correspondences of cells across spatio-temporal scale, but it also detects cell division events, and identifies daughter cells upon divisions, thus allowing automated estimation of cell lineages from images captured over a period of 72 h. The method presented here should enable quantitative analysis of cell growth patterns and thus facilitating the development of in silico models for SAM growth.

Maeda, S., et al. (2014). "The conflict between cell proliferation and expansion primarily affects stem organogenesis in Arabidopsis." Plant Cell Physiol **55**(11): 1994-2007.

Plant shoot organs such as stems, leaves and flowers are derived from specialized groups of stem cells organized at the shoot apical meristem (SAM). Organogenesis involves two major processes, namely cell proliferation and differentiation, whereby the former contributes to increasing the cell number and the latter involves substantial increases in cell volume through cell expansion. Co-ordination between the above processes in time and space is essential for proper organogenesis. To identify regulatory factors involved in proper organogenesis, heavy-ion beam-irradiated de-etiolated (det) 3-1 seeds have been used to identify striking phenotypes in the A#26-2; det3-1 mutant. In addition to the stunted plant stature mimicking det3-1, the A#26-2; det3-1 mutant exhibited stem thickening, increased floral organ number and a fruit shape reminiscent of clavata (clv) mutants. DNA sequencing analysis demonstrated that A#26-2; det3-1 harbors a mutation in the CLV3 gene. Importantly, A#26-2; det3-1 displayed cracks that randomly occurred on the main stem with a frequency of approximately 50%. Furthermore, the double mutants clv3-8 det3-1, clv1-4 det3-1 and clv2-1 det3-1 consistently showed stem cracks with frequencies of approximately 97, 38 and 35%, respectively. Cross-sections of stems further revealed an increase in vascular bundle number, cell number and size in the pith of clv3-8 det3-1 compared with det3-1. These findings suggest that the stem inner volume increase due to clv mutations exerts an outward mechanical stress; that in a det3-1 background (defective in cell expansion) resulted in cracking of the outermost layer of epidermal cells.

Maidhof, R., et al. (2017). "Timing of mesenchymal stem cell delivery impacts the fate and therapeutic potential in intervertebral disc repair." J Orthop Res **35**(1): 32-40.

Cell-based therapies offer a promising approach to treat intervertebral disc (IVD) degeneration. The impact of the injury microenvironment on treatment efficacy has not been established. This study used a rat disc stab injury model with administration of mesenchymal stromal cells (MSCs) at 3, 14, or 30 days post injury (DPI) to evaluate the impact of interventional timing on IVD biochemistry and biomechanics. We also evaluated cellular localization within the disc with near infrared imaging to track the time and spatial profile of cellular migration after in vivo delivery. Results showed that upon injection into a healthy disc, MSCs began to gradually migrate outwards over the course of 14 days, as indicated by decreased signal intensity from the disc space and increased signal within the adjacent vertebrae. Cells administered 14 or 30 DPI also tended to migrate out 14 days after injection but cells injected 3 DPI were retained at a significantly higher amount versus the other groups ($p < 0.05$). Correspondingly the 3 DPI group, but not 14 or 30 DPI groups, had a higher GAG content in the MSC injected discs ($p = 0.06$). Enrichment of MSCs and increased GAG content in 3 DPI group did not lead to increased compressive biomechanical properties. Findings suggest that cell therapies administered at an early stage of injury/disease progression may have greater chances of mitigating matrix loss, possibly through promotion of MSC activity by the inflammatory microenvironment associated with injury. Future studies will evaluate the mode and driving factors that regulate cellular migration out of the disc. (c) 2016 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 35:32-40, 2017.

Matsushita, T., et al. (2011). "Mesenchymal stem cells transmigrate across brain microvascular endothelial cell monolayers through transiently formed inter-endothelial gaps." Neurosci Lett **502**(1): 41-45.

Mesenchymal stem cells (MSCs) hold much promise for cell therapy for neurological diseases such as cerebral ischemia and Parkinson's disease. Intravenously administered MSCs accumulate in lesions within the brain parenchyma, but little is known of the details of MSC transmigration across the blood-brain barrier (BBB). To study MSC transmigration across the BBB, we developed an in vitro culture system consisting of rat brain microvascular endothelial cells (BMECs) and bone marrow-derived MSCs using Transwell or Millicell culture inserts. Using this system, we first investigated the influence of the number of MSCs added to the upper chamber on BMEC barrier integrity. The addition of MSCs at a density of 1.5 x 10(5) cells/cm

(2) led to disruption of the BMEC monolayer structure and decreased barrier function as measured by the transendothelial electrical resistance (TEER). When applied at a density of 1.5 x 10(4) cells/cm (2), neither remarkable disruption of the BMEC monolayers nor a significant decrease in TEER was observed until at least 12 h. After cultivation for 24 h under this condition, MSCs were found in the subendothelial space or beneath the insert membrane, suggesting that MSCs transmigrate across BMEC monolayers. Time-lapse imaging revealed that MSCs transmigrated across the BMEC monolayers through transiently formed intercellular gaps between the BMECs. These results show that our in vitro culture system consisting of BMECs and MSCs is useful for investigating the molecular and cellular mechanisms underlying MSC transmigration across the BBB.

McBride, J. D., et al. (2017). "Bone Marrow Mesenchymal Stem Cell-Derived CD63(+) Exosomes Transport Wnt3a Exteriorly and Enhance Dermal Fibroblast Proliferation, Migration, and Angiogenesis In Vitro." Stem Cells Dev **26**(19): 1384-1398.

Wnts are secreted glycoproteins that regulate stem cell self-renewal, differentiation, and cell-to-cell communication during embryonic development and in adult tissues. Bone marrow mesenchymal stem cells (BM-MSCs) have been shown to stimulate dermis repair and regeneration; however, it is unclear how BM-MSCs may modulate downstream Wnt signaling. While recent reports implicate that Wnt ligands and Wnt messenger RNAs (such as Wnt4) exist within the interior compartment of exosomes, it has been debated whether or not Wnts exist on the exterior surface of exosomes to travel in the extracellular space. To help answer this question, we utilized flow cytometry of magnetic beads coated with anti-CD63 antibodies and found, for the first time, that Wnt3a protein is detectable exteriorly on CD63(+) exosomes derived from BM-MSCs over-secreting Wnt3a into serum-free conditioned media (Wnt3a CM). Our data suggest that CD63(+) exosomes significantly help transport exterior Wnt3a signal to recipient cells to promote fibroblast and endothelial functions. During purification of exosomes, we unexpectedly found that use of ultracentrifugation alone significantly decreased the ability to detect exteriorly bound Wnt3a on CD63(+) exosomes, however, polyethylene glycol (PEG)-mediated exosome-enrichment before exosome-purification (with ultracentrifugation into a sucrose cushion) resulted in exosomes more likely to retain exterior Wnt3a detectability and downstream Wnt/beta-catenin activity. Our findings indicate the important role that purification methods may have on stem cell-derived Wnt-exosome activity in downstream assays. The ability for BM-MSC Wnt3a CM and exosomes to stimulate dermal fibroblast proliferation and migration, and endothelial angiogenesis in vitro, was significantly decreased after CD63(+)-exosome depletion or knockdown of Wnt coreceptor LRP6 in recipient cells, suggesting both are required for optimal Wnt-exosome activity in our system. Thus, BM-MSC-derived CD63(+) exosomes are a significant carrier of exterior Wnt3a within high Wnt environments, resulting in downstream fibroblast proliferation, migration, and angiogenesis in vitro.

Molina-Pena, R. and M. M. Alvarez (2012). "A simple mathematical model based on the cancer stem cell hypothesis suggests kinetic commonalities in solid tumor growth." PLoS One **7**(2): e26233.

BACKGROUND: The Cancer Stem Cell (CSC) hypothesis has gained credibility within the cancer research community. According to this hypothesis, a small subpopulation of cells within cancerous tissues exhibits stem-cell-like characteristics and is responsible for the maintenance and proliferation of cancer. METHODOLOGIES/PRINCIPAL FINDINGS: We present a simple compartmental pseudo-chemical mathematical model for tumor growth, based on the CSC hypothesis, and derived using a "chemical reaction" approach. We defined three cell subpopulations: CSCs, transit progenitor cells, and differentiated cells. Each event related to cell division, differentiation, or death is then modeled as a chemical reaction. The resulting set of ordinary differential equations was numerically integrated to describe the time evolution of each cell subpopulation and the overall tumor growth. The parameter space was explored to identify combinations of parameter values that produce biologically feasible and consistent scenarios. CONCLUSIONS/SIGNIFICANCE: Certain kinetic relationships apparently must be satisfied to sustain solid tumor growth and to maintain an approximate constant fraction of CSCs in the tumor lower than 0.01 (as experimentally observed): (a) the rate of symmetrical and asymmetrical CSC renewal must be in the same order of magnitude; (b) the intrinsic rate of renewal and differentiation of progenitor cells must be half an order of magnitude higher than the corresponding intrinsic rates for cancer stem cells; (c) the rates of apoptosis of the CSC, transit amplifying progenitor (P) cells, and terminally differentiated (D) cells must be progressively higher by approximately one order of magnitude. Simulation results were consistent with reports that have suggested that encouraging CSC differentiation could be an effective therapeutic strategy for fighting cancer in addition to selective killing or inhibition of symmetric division of CSCs.

Morrison, M., et al. (2015). "StemBANCC: Governing Access to Material and Data in a Large Stem Cell Research Consortium." Stem Cell Rev **11**(5): 681-687.

This paper makes the case for implementing an internal governance framework for sharing materials and data in stem cell research consortia. A governance framework can facilitate a transparent and accountable system while building trust among partner institutions. However, avoiding excessive bureaucracy is essential. The development and implementation of a governance framework for materials and data access in the Stem cells for Biological Assays of Novel drugs and prediCtive toxiCology (StemBANCC) consortium is presented as a practical example. The StemBANCC project is a multi-partner European research consortium, which aims to build a resource of 1,500 well characterised induced pluripotent stem cell (iPSC) lines for in vitro disease modelling and toxicology studies. The project governance framework was developed in two stages. A small working group identified key components of a framework and translated the project legal agreements into a draft policy document. The second phase allowed input from all consortium partners to shape the iterative development of a final policy document that could be agreed by all parties. Careful time management strategies were needed to manage the duration of this component. This part of the process also served as an exploratory space where different options could be proposed, potential gaps in planning identified, and project co-ordination activities specified.

Movahednia, M. M., et al. (2015). "Differential effects of the extracellular microenvironment on human embryonic stem cell differentiation into keratinocytes and their subsequent replicative life span." Tissue Eng Part A **21**(7-8): 1432-1443.

Culture microenvironment plays a critical role in the propagation and differentiation of human embryonic stem cells (hESCs) and their differentiated progenies. Although high efficiency of hESC differentiation to keratinocytes (hESC-Kert) has been achieved, little is known regarding the effects of early culture microenvironment and pertinent extracellular matrix (ECM) interactions during epidermal commitment on subsequent proliferative capacity of hESC-Kert. The aim of this study is to evaluate the effects of the different ECM microenvironments during hESC differentiation on subsequent replicative life span of hESC-Kert. In doing so, H1-hESCs were differentiated to keratinocytes (H1-Kert) in two differentiation systems. The first system employed autologous fibroblast feeder support, in which keratinocytes (H1-Kert (ACC)) were derived by coculture of hESCs with hESC-derived fibroblasts (H1-ebFs). The second system employed a novel decellularized matrix from H1-ebFs to create a dermoepidermal junction-like (DEJ) matrix. H1-Kert (AFF) were derived by differentiation of hESCs on the feeder-free system employing the DEJ matrix. Our study indicated that the feeder-free system with the use of DEJ matrix was more efficient in differentiation of hESCs toward epidermal progenitors. However, the feeder-free system was not sufficient to support the subsequent replicative capacity of differentiated keratinocytes. Of note, H1-Kert (AFF) showed limited replicative capacity with reduced telomere length and early cellular senescence. We further showed that the lack of cell-cell interactions during epidermal commitment led to heightened production of TGF-beta1 by hESC-Kert during extended culture, which in turn was responsible for resulting in the limited replicative life span with cellular senescence of hESC-Kert derived under the feeder-free culture system. This study highlights for the first time the importance of the culture microenvironment and cell-ECM interactions during differentiation of hESCs on subsequent replicative life span and cellular senescence of the differentiated keratinocytes, with implications for use of these cells for applications in tissue engineering and regenerative medicine.

Mukaiyama, Y., et al. (2009). "[Pyogenic spondylitis following unrelated hematopoietic stem cell transplantation]." Rinsho Ketsueki **50**(12): 1706-1710.

Pyogenic spondylitis is regarded as a rare infectious disease. The incidence of this disease has been increasing recently due to an increase in the ratio of elderly people in the population as well as an increase in immunocompromised hosts complicated by cancer, diabetes mellitus and liver cirrhosis. Allogeneic hematopoietic stem cell transplantation (HSCT) is now performed widely as a curative treatment for various malignant hematological diseases. However, allogeneic HSCT causes chronic immunocompromise. There is no case report describing infectious spondylitis after HSCT. Here we describe a case of infectious spondylitis after HSCT and discuss risk factors and treatment. The patient was a 56-year-old female with AML-M1 who underwent allogeneic HSCT in our hospital. She developed back pain and fever about 150 days after HSCT and became unable to walk due to the severity of back pain. MRI T1 images showed a low intensity area, T2 images showed a high intensity area and Gd-DTPA-enhanced images showed a high intensity area at the S1-2 disk space. Clinical findings and MRI findings suggested pyogenic spondylitis. Back pain improved gradually after conservative treatment with meropenem (MEPM) for two weeks. After 4 weeks of MEPM administration,

she had fully recovered and there has not been any recurrence of back pain to date. In conclusion, pyogenic spondylitis should be considered in the differential diagnoses for HSCT recipients with severe back pain.

Na, S., et al. (2016). "Regeneration of dental pulp/dentine complex with a three-dimensional and scaffold-free stem-cell sheet-derived pellet." J Tissue Eng Regen Med **10**(3): 261-270.

Dental pulp/dentine complex regeneration is indispensable to the construction of biotissue-engineered tooth roots and represents a promising approach to therapy for irreversible pulpitis. We used a tissue-engineering method based on odontogenic stem cells to design a three-dimensional (3D) and scaffold-free stem-cell sheet-derived pellet (CSDP) with the necessary physical and biological properties. Stem cells were isolated and identified and stem cells from root apical papilla (SCAPs)-based CSDPs were then fabricated and examined. Compact cell aggregates containing a high proportion of extracellular matrix (ECM) components were observed, and the CSDP culture time was prolonged. The expression of alkaline phosphatase (ALP), dentine sialoprotein (DSPP), bone sialoprotein (BSP) and runt-related gene 2 (RUNX2) mRNA was higher in CSDPs than in cell sheets (CSs), indicating that CSDPs have greater odonto/osteogenic potential. To further investigate this hypothesis, CSDPs and CSs were inserted into human treated dentine matrix fragments (hTDMFs) and transplanted into the subcutaneous space in the backs of immunodeficient mice, where they were cultured in vivo for 6 weeks. The root space with CSDPs was filled entirely with a dental pulp-like tissue with well-established vascularity, and a continuous layer of dentine-like tissue was deposited onto the existing dentine. A layer of odontoblast-like cells was found to express DSPP, ALP and BSP, and human mitochondria lined the surface of the newly formed dentine-like tissue. These results clearly indicate that SCAP-CSDPs with a mount of endogenous ECM have a strong capacity to form a heterotopic dental pulp/dentine complex in empty root canals; this method can be used in the fabrication of bioengineered dental roots and also provides an alternative treatment approach for pulp disease.

O'Grady, B., et al. (2019). "Spatiotemporal control and modeling of morphogen delivery to induce gradient patterning of stem cell differentiation using fluidic channels." Biomater Sci **7**(4): 1358-1371.

The process of cell differentiation in a developing embryo is influenced by numerous factors, including various biological molecules whose presentation varies dramatically over space and time. These morphogens regulate cell fate based on concentration profiles, thus creating discrete populations of cells and ultimately generating large, complex tissues and organs. Recently, several in vitro platforms have attempted to recapitulate the complex presentation of extrinsic signals found in nature. However, it has been a challenge to design versatile platforms that can dynamically control morphogen gradients over extended periods of time. To address some of these issues, we introduce a platform using channels patterned in hydrogels to deliver multiple morphogens to cells in a 3D scaffold, thus creating a spectrum of cell phenotypes based on the resultant morphogen gradients. The diffusion coefficient of a common small molecule morphogen, retinoic acid (RA), was measured within our hydrogel platform using Raman spectroscopy and its diffusion in our platform's geometry was modeled using finite element analysis. The predictive model of spatial gradients was validated in a cell-free hydrogel, and temporal control of morphogen gradients was then demonstrated using a reporter cell line that expresses green fluorescent protein in the presence of RA. Finally, the utility of this approach for regulating cell phenotype was demonstrated by generating opposing morphogen gradients to create a spectrum of mesenchymal stem cell differentiation states.

Orciani, M., et al. (2013). "Alterations of ROS pathways in scleroderma begin at stem cell level." J Biol Regul Homeost Agents **27**(1): 211-224.

Scleroderma is a chronic systemic autoimmune disease (primarily of the skin) characterized by fibrosis (or hardening), vascular alterations and autoantibodies production.There are currently no effective therapies against this devastating and often lethal disorder. Despite the interest for the immunomodulatory effects of mesenchymal stem cells (MSCs) in autoimmune diseases, the role of MSCs in scleroderma is still unknown. A pivotal role in scleroderma onset is played by oxidative stress associated with the accumulation of great amounts of reactive oxygen species (ROS). This study depicts some phenotypic and functional features of MSCs isolated from the skin of healthy and scleroderma patients; the ROS production and accumulation, the expression of ERK1/2 and the effects of the stimulation with PDGF, were analyzed in MSCs; results were compared to those observed in primary fibroblasts (Fbs) isolated from the same subjects. We found that the pro-oxidant environment exerted by scleroderma affects MSCs, which are still able to counteract the ROS accumulation by improving the antioxidant defenses. On the contrary, scleroderma fibroblasts show a disruption of these mechanisms, with consequent ROS

increase and the activation of the cascade triggered by scleroderma auto-antibodies against PDGFR.

Paci, M., et al. (2012). "Mathematical modelling of the action potential of human embryonic stem cell derived cardiomyocytes." Biomed Eng Online **11**: 61.

BACKGROUND: Human embryonic stem cell derived cardiomyocytes (hESC-CMs) hold high potential for basic and applied cardiovascular research. The development of a reliable simulation platform able to mimic the functional properties of hESC-CMs would be of considerable value to perform preliminary test complementing in vitro experimentations. METHODS: We developed the first computational model of hESC-CM action potential by integrating our original electrophysiological recordings of transient-outward, funny, and sodium-calcium exchanger currents and data derived from literature on sodium, calcium and potassium currents in hESC-CMs. RESULTS: The model is able to reproduce basal electrophysiological properties of hESC-CMs at 15 40 days of differentiation (Early stage). Moreover, the model reproduces the modifications occurring through the transition from Early to Late developmental stage (50-110, days of differentiation). After simulated blockade of ionic channels and pumps of the sarcoplasmic reticulum, Ca2+ transient amplitude was decreased by 12% and 33% in Early and Late stage, respectively, suggesting a growing contribution of a functional reticulum during maturation. Finally, as a proof of concept, we tested the effects induced by prototypical channel blockers, namely E4031 and nickel, and their qualitative reproduction by the model. CONCLUSIONS: This study provides a novel modelling tool that may serve useful to investigate physiological properties of hESC-CMs.

Perez Mdel, C., et al. (2013). "Space-time dynamics of stem cell niches: a unified approach for plants." J Integr Bioinform **10**(2): 219.

Many complex systems cannot be analyzed using traditional mathematical tools, due to their irreducible nature. This makes it necessary to develop models that can be implemented computationally to simulate their evolution. Examples of these models are cellular automata, evolutionary algorithms, complex networks, agent-based models, symbolic dynamics and dynamical systems techniques. We review some representative approaches to model the stem cell niche in Arabidopsis thaliana and the basic biological mechanisms that underlie its formation and maintenance. We propose a mathematical model based on cellular automata for describing the space-time dynamics of the stem cell niche in the root. By making minimal assumptions on the cell communication process documented in experiments, we classify the basic developmental features of the stem-cell niche, including the basic structural architecture, and suggest that they could be understood as the result of generic mechanisms given by short and long range signals. This could be a first step in understanding why different stem cell niches share similar topologies, not only in plants. Also the fact that this organization is a robust consequence of the way information is being processed by the cells and to some extent independent of the detailed features of the signaling mechanism.

Petrus-Reurer, S., et al. (2017). "Integration of Subretinal Suspension Transplants of Human Embryonic Stem Cell-Derived Retinal Pigment Epithelial Cells in a Large-Eyed Model of Geographic Atrophy." Invest Ophthalmol Vis Sci **58**(2): 1314-1322.

Purpose: Subretinal suspension transplants of human embryonic stem cell-derived retinal pigment epithelial cells (hESC-RPE) have the capacity to form functional monolayers in naive eyes. We explore hESC-RPE integration when transplanted in suspension to a large-eyed model of geographic atrophy (GA). Methods: Derivation of hESC-RPE was performed in a xeno-free and defined manner. Subretinal bleb injection of PBS or sodium iodate (NaIO3) was used to induce a GA-like phenotype. Suspensions of hESC-RPE were transplanted to the subretinal space of naive or PBS-/NaIO3-treated rabbits using a transvitreal pars plana technique. Integration of hESC-RPE was monitored by multimodal real-time imaging and by immunohistochemistry. Results: Subretinal blebs of PBS or NaIO3 caused different degrees of outer neuroretinal degeneration, RPE hyperautofluorescence, focal RPE loss, and choroidal atrophy; that is, hallmark characteristics of GA. In nonpretreated naive eyes, hESC-RPE integrated as subretinal monolayers with preserved overlying photoreceptors, yet not in areas with outer neuroretinal degeneration and native RPE loss. When transplanted to eyes with PBS-/NaIO3-induced degeneration, hESC-RPE failed to integrate. Conclusions: In a large-eyed preclinical model, subretinal suspension transplants of hESC-RPE did not integrate in areas with GA-like degeneration.

Pittet, M. J., et al. (2014). "The journey from stem cell to macrophage." Ann N Y Acad Sci **1319**: 1-18.

Essential protectors against infection and injury, macrophages can also contribute to many common and fatal diseases. Here, we discuss the mechanisms that control different types of macrophage activities in mice. We follow the cells' maturational pathways over time and space and elaborate on events that influence the type of macrophage eventually settling a particular

destination. The nature of the precursor cells, developmental niches, tissues, environmental cues, and other connecting processes appear to contribute to the identity of macrophage type. Together, the spatial and developmental relationships of macrophages compose a topo-ontogenic map that can guide our understanding of their biology.

Porter, J. R., et al. (2009). "Biocompatibility and mesenchymal stem cell response to poly (epsilon-caprolactone) nanowire surfaces for orthopedic tissue engineering." Tissue Eng Part A **15**(9): 2547-2559.

Concerns over utilizing autogenous cancellous bone grafts (such as donor-site morbidity, increased surgical time/complication rate, and restricted availability) as the gold-standard treatment for critical-sized defects in bone have motivated the development of a wide variety of sophisticated synthetic bone scaffolds in recent years. In this work, a novel solvent-free template synthesis technique was utilized to fabricate poly (epsilon-caprolactone) (PCL) nanowire surfaces as a building block for the development of three-dimensional bone scaffolds. Bone marrow-derived mesenchymal stem cells (MSCs) were used to characterize the short- and long-term in vitro biocompatibility and cellular response to these surfaces. A 4-week study in rats was conducted to assess in vivo biocompatibility as well. Short-term in vitro studies revealed that PCL nanowire surfaces enhanced MSC response in terms of survivability, viability, cytoskeleton changes, and morphology as compared with control surfaces (smooth PCL and polystyrene). In long-term in vitro studies, nanowire surfaces induced a rapid production of bone extracellular matrix by differentiated MSCs as indicated by accelerated calcium phosphate mineralization, and osteocalcin and osteopontin production. In vivo studies and histological analysis confirmed that nanowire surfaces are biocompatible. Preliminary biodegradation studies were conducted and indicated that rate of PCL biodegradation can, to some extent, be controlled through the inclusion of nanowires and ester-degrading enzymes. In addition to demonstrating enhanced short- and long-term MSC response to PCL nanowire surfaces, this work presents a simple technique for solvent-free fabrication and bioactive molecule encapsulation of biocompatible, biodegradable three-dimensional bone scaffold components and warrants further investigation.

Rasi Ghaemi, S., et al. (2018). "High-Throughput Assessment and Modeling of a Polymer Library Regulating Human Dental Pulp-Derived Stem Cell Behavior." ACS Appl Mater Interfaces **10**(45): 38739-38748.

The identification of biomaterials that modulate cell responses is a crucial task for tissue engineering and cell therapy. The identification of novel materials is complicated by the immense number of synthesizable polymers and the time required for testing each material experimentally. In the current study, polymeric biomaterial-cell interactions were assessed rapidly using a microarray format. The attachment, proliferation, and differentiation of human dental pulp stem cells (hDPSCs) were investigated on 141 homopolymers and 400 diverse copolymers. The copolymer of isooctyl acrylate and 2-(methacryloyloxy)ethyl acetoacetate achieved the highest attachment and proliferation of hDPSC, whereas high cell attachment and differentiation of hDPSC were observed on the copolymer of isooctyl acrylate and trimethylolpropane ethoxylate triacrylate. Computational models were generated, relating polymer properties to cellular responses. These models could accurately predict cell behavior for up to 95% of materials within a test set. The models identified several functional groups as being important for supporting specific cell responses. In particular, oxygen-containing chemical moieties, including fragments from the acrylate/acrylamide backbone of the polymers, promoted cell attachment. Small hydrocarbon fragments originating from polymer pendant groups promoted cell proliferation and differentiation. These computational models constitute a key tool to direct the discovery of novel materials within the enormous chemical space available to researchers.

Ray, A., et al. (2018). "Cancer Stem Cell Migration in Three-Dimensional Aligned Collagen Matrices." Curr Protoc Stem Cell Biol **46**(1): e57.

Cell migration is strongly influenced by the organization of the surrounding 3-D extracellular matrix. In particular, within fibrous solid tumors, carcinoma cell invasion may be directed by patterns of aligned collagen in the extra-epithelial space. Thus, studying the interactions of heterogeneous populations of cancer cells that include the stem/progenitor-like cancer stem cell subpopulation and aligned collagen networks is critical to our understanding of carcinoma dissemination. Here, we describe a robust method to generate aligned collagen matrices in vitro that mimic in vivo fiber organization. Subsequently, a protocol is presented for seeding aligned matrices with distinct carcinoma cell subpopulations and performing live cell imaging and quantitative analysis of cell migration. Together, the engineered constructs and the imaging techniques laid out here provide a platform to study cancer stem cell migration in 3-D anisotropic collagen with real-time visualization of cellular interactions

with the fibrous matrix. (c) 2018 by John Wiley & Sons, Inc.

Riccitelli, E., et al. (2013). "Extracellular sphingosine-1-phosphate: a novel actor in human glioblastoma stem cell survival." PLoS One **8**(6): e68229.

Glioblastomas are the most frequent and aggressive intracranial neoplasms in humans, and despite advances and the introduction of the alkylating agent temozolomide in therapy have improved patient survival, resistance mechanisms limit benefits. Recent studies support that glioblastoma stem-like cells (GSCs), a cell subpopulation within the tumour, are involved in the aberrant expansion and therapy resistance properties of glioblastomas, through still unclear mechanisms. Emerging evidence suggests that sphingosine-1-phosphate (S1P) a potent onco-promoter able to act as extracellular signal, favours malignant and chemoresistance properties in GSCs. Notwithstanding, the origin of S1P in the GSC environment remains unknown. We investigated S1P metabolism, release, and role in cell survival properties of GSCs isolated from either U87-MG cell line or a primary culture of human glioblastoma. We show that both GSC models, grown as neurospheres and expressing GSC markers, are resistant to temozolomide, despite not expressing the DNA repair protein MGMT, a major contributor to temozolomide-resistance. Pulse experiments with labelled sphingosine revealed that both GSC types are able to rapidly phosphorylate the long-chain base, and that the newly produced S1P is efficiently degraded. Of relevance, we found that S1P was present in GSC extracellular medium, its level being significantly higher than in U87-MG cells, and that the extracellular/intracellular ratio of S1P was about ten-fold higher in GSCs. The activity of sphingosine kinases was undetectable in GSC media, suggesting that mechanisms of S1P transport to the extracellular environment are constitutive in GSCs. In addition we found that an inhibitor of S1P biosynthesis made GSCs sensitive to temozolomide (TMZ), and that exogenous S1P reverted this effect, thus involving extracellular S1P as a GSC survival signal in TMZ resistance. Altogether our data implicate for the first time GSCs as a pivotal source of extracellular S1P, which might act as an autocrine/paracrine signal contributing to their malignant properties.

Rissone, A., et al. (2015). "Reticular dysgenesis-associated AK2 protects hematopoietic stem and progenitor cell development from oxidative stress." J Exp Med **212**(8): 1185-1202.

Adenylate kinases (AKs) are phosphotransferases that regulate the cellular adenine nucleotide composition and play a critical role in the energy homeostasis of all tissues. The AK2 isoenzyme is expressed in the mitochondrial intermembrane space and is mutated in reticular dysgenesis (RD), a rare form of severe combined immunodeficiency (SCID) in humans. RD is characterized by a maturation arrest in the myeloid and lymphoid lineages, leading to early onset, recurrent, and overwhelming infections. To gain insight into the pathophysiology of RD, we studied the effects of AK2 deficiency using the zebrafish model and induced pluripotent stem cells (iPSCs) derived from fibroblasts of an RD patient. In zebrafish, Ak2 deficiency affected hematopoietic stem and progenitor cell (HSPC) development with increased oxidative stress and apoptosis. AK2-deficient iPSCs recapitulated the characteristic myeloid maturation arrest at the promyelocyte stage and demonstrated an increased AMP/ADP ratio, indicative of an energy-depleted adenine nucleotide profile. Antioxidant treatment rescued the hematopoietic phenotypes in vivo in ak2 mutant zebrafish and restored differentiation of AK2-deficient iPSCs into mature granulocytes. Our results link hematopoietic cell fate in AK2 deficiency to cellular energy depletion and increased oxidative stress. This points to the potential use of antioxidants as a supportive therapeutic modality for patients with RD.

Rosu, S. and O. Cohen-Fix (2017). "Live-imaging analysis of germ cell proliferation in the C. elegans adult supports a stochastic model for stem cell proliferation." Dev Biol **423**(2): 93-100.

The C. elegans adult hermaphrodite contains a renewable pool of mitotically dividing germ cells that are contained within the progenitor zone (PZ), at the distal region of the germline. From the PZ, cells enter meiosis and differentiate, ensuring the continued production of oocytes. In this study, we investigated the proliferation strategy used to maintain the PZ pool by using a photoconvertible marker to follow the fate of selected germ cells and their descendants in live worms. We found that the most distal pool of 6-8 rows of cells in the PZ (the distal third) behave similarly, with a fold expansion corresponding to one cell division every 6h on average. Proximal to this region, proliferation decreases, and by the proximal third of the PZ, most cells have stopped dividing. In addition, we show that all the descendants of cells in rows 3 and above move proximally and leave the PZ over time. Combining our data with previous studies, we propose a stochastic model for the C. elegans PZ proliferation, where a pool of proliferating stem cells divide symmetrically within the distal most 6-8 rows of the germline and exit from this stem cell niche occurs by displacement due to competition for limited space.

Ruangsawasdi, N., et al. (2017). "Effects of Stem Cell Factor on Cell Homing During Functional Pulp Regeneration in Human Immature Teeth." Tissue Eng Part A **23**(3-4): 115-123.

Conventional root canal treatment in immature permanent teeth can lead to early tooth loss in children because root formation is discontinued. We investigated whether the stem cell factor (SCF) could facilitate cell homing in the pulpless immature root canal and promote regeneration of a functional pulp. In vitro, human mesenchymal stem cells (hMSCs) were exposed to SCF at various concentrations for assessing cell migration, proliferation, and differentiation toward odonto/osteoblasts by 3D-chemotaxis slides, WST-1 assay, and alkaline phosphatase activity, respectively. Fibrin gels were used to deliver 15 mug/mL SCF for in vivo experiments. The release kinetic of SCF was assessed in vitro. Two corresponding human immature premolars, with or without SCF, were placed at rat calvariae for 6 and 12 weeks. All tooth specimens were either analyzed histologically and the percentage of tissue ingrowth determined or the cells were extracted from the pulp space, and the mRNA level of DMP1, DSPP, Col1, NGF, and VEGF were assessed by quantitative polymerase chain reaction. In the presence of SCF, we saw an increase in hMSCs directional migration, proliferation, and odonto/osteogenic differentiation. SCF also increased the extent of tissue ingrowth at 6 weeks but not at 12 weeks. However, at this time point, the formed tissue appeared more mature in samples with SCF. In terms of gene transcription, DMP1, Col1, and VEGF were the significantly upregulated genes, while DSPP and NGF were not affected. Our results suggest that SCF can accelerate cell homing and the maturation of the pulp-dentin complex in human immature teeth.

Rushing, G. and R. A. Ihrie (2016). "Neural stem cell heterogeneity through time and space in the ventricular-subventricular zone." Front Biol (Beijing) **11**(4): 261-284.

BACKGROUND: The origin and classification of neural stem cells (NSCs) has been a subject of intense investigation for the past two decades. Efforts to categorize NSCs based on their location, function and expression have established that these cells are a heterogeneous pool in both the embryonic and adult brain. The discovery and additional characterization of adult NSCs has introduced the possibility of using these cells as a source for neuronal and glial replacement following injury or disease. To understand how one could manipulate NSC developmental programs for therapeutic use, additional work is needed to elucidate how NSCs are programmed and how signals during development are interpreted to determine cell fate. OBJECTIVE: This review describes the identification, classification and characterization of NSCs within the large neurogenic niche of the ventricular-subventricular zone (V-SVZ). METHODS: A literature search was conducted using Pubmed including the keywords "ventricular-subventricular zone," "neural stem cell," "heterogeneity," "identity" and/or "single cell" to find relevant manuscripts to include within the review. A special focus was placed on more recent findings using single-cell level analyses on neural stem cells within their niche (s). RESULTS: This review discusses over 20 research articles detailing findings on V-SVZ NSC heterogeneity, over 25 articles describing fate determinants of NSCs, and focuses on 8 recent publications using distinct single-cell analyses of neural stem cells including flow cytometry and RNA-seq. Additionally, over 60 manuscripts highlighting the markers expressed on cells within the NSC lineage are included in a chart divided by cell type. CONCLUSIONS: Investigation of NSC heterogeneity and fate decisions is ongoing. Thus far, much research has been conducted in mice however, findings in human and other mammalian species are also discussed here. Implications of NSC heterogeneity established in the embryo for the properties of NSCs in the adult brain are explored, including how these cells may be redirected after injury or genetic manipulation.

Sabetkish, S., et al. (2018). "The role of nonautologous and autologous adipose-derived mesenchymal stem cell in acute pyelonephritis." Cell Tissue Bank **19**(3): 301-309.

We compared the therapeutic effects of autologous and nonautologous adipose-derived mesenchymal stem cell (ADMSC), in ameliorating the renal function in a rabbit model of acute pyelonephritis. The difference of perirenal and neck subcutaneous ADMSCs were also evaluated. Twenty female rabbits were apportioned to 5 groups. In group I (n = 4), the rabbits were injected direct inoculation of Escherichia coli (E. coli) into the right kidney. In group II (n = 4), autologous ADMSCs obtained from nape adipose tissue were injected into the subcapsular space 1 week after E. coli injection, while nonautologous ADMSCs of the same origin (from male rabbits) were applied in group III (n = 4). In group IV (n = 4), autologous perirenal ADMSCs were applied with the same method, while perirenal nonautologous ADMSCs from male rabbits were used in group V (n = 4). Technetium-99m-DMSA renal scan was performed 1, 2 and 4 months post-injection in all groups. Kidneys were excised for the evaluation of histopathological changes in the same time points. PCR examination for detection of Y-chromosome (in group III and V) and fluorescent evaluation (in group II and IV) were also performed to determine the fate of injected cells.

Injection of autologous ADMSCs resulted in more satisfactory outcomes in reduction of interstitial fibrosis, tubular, and glomerular atrophy as compared to nonautologous groups. However, histopathological ameliorations were significantly better in group IV in which autologous perirenal ADMSC was applied. Remarkably, two months after the injection, Technetium-99m-DMSA renal scan showed that right kidney reached to near normal cortical function (48 and 45%) in group IV and V, respectively as compared to groups II (41%) and III (37%). Autologous ADMSCs may have better results in cell therapy as compared to nonautologous cells. However, more satisfactory outcomes may be obtained when the cell source is selected from the surrounding adipose tissue.

Santi, P. A. and S. B. Johnson (2013). "Decellularized ear tissues as scaffolds for stem cell differentiation." J Assoc Res Otolaryngol **14**(1): 3-15.

Permanent sensorineural hearing loss is a major medical problem and is due to the loss of hair cells and subsequently spiral ganglion neurons in the cochlea. Since these cells lack the capacity of renewal in mammals, their regeneration would be an optimal solution to reverse hearing loss. In other tissues, decellularized extracellular matrix (ECM) has been used as a mechanical and biochemical scaffold for the induction of stem and other cells toward a target tissue phenotype. Such induced cells have been used for tissue and organ transplants in preclinical animal and human clinical applications. This paper reports for the first time the decellularization of the cochlea and identification of remaining laminin and collagen type IV as a first step in preparing an ECM scaffold for directing stem cells toward an auditory lineage. Fresh ear tissues were removed from euthanized mice, a rat and a human and processed for decellularization using two different detergent extraction methods. Cochleas were imaged with scanning thin-sheet laser imaging microscopy (sTSLIM) and brightfield microscopy. Detergent treatment of fresh tissue removed all cells as evidenced by lack of H & E and DNA staining of the membranous labyrinth while preserving components of the ECM. The organ of Corti was completely removed, as were spiral ganglion neurons, which appeared as hollow sheaths and tubes of basal lamina (BL) material. Cells of the stria vascularis were removed and its only vestige left was its laterally linking network of capillary BL that appeared to "float" in the endolymphatic space. Laminin and type IV collagen were detected in the ECM after decellularization and were localized in vascular, neural and epithelial BL. Further work is necessary to attempt to seed neural and other stem cells into the decellularized ECM to hopefully induce differentiation and subsequent in vivo engraftment into damaged cochleas.

Silva, T. P., et al. (2019). "Design Principles for Pluripotent Stem Cell-Derived Organoid Engineering." Stem Cells Int **2019**: 4508470.

Human morphogenesis is a complex process involving distinct microenvironmental and physical signals that are manipulated in space and time to give rise to complex tissues and organs. Advances in pluripotent stem cell (PSC) technology have promoted the in vitro recreation of processes involved in human morphogenesis. The development of organoids from human PSCs represents one reliable source for modeling a large spectrum of human disorders, as well as a promising approach for drug screening and toxicological tests. Based on the "self-organization" capacity of stem cells, different PSC-derived organoids have been created; however, considerable differences between in vitro-generated PSC-derived organoids and their in vivo counterparts have been reported. Advances in the bioengineering field have allowed the manipulation of different components, including cellular and noncellular factors, to better mimic the in vivo microenvironment. In this review, we focus on different examples of bioengineering approaches used to promote the self-organization of stem cells, including assembly, patterning, and morphogenesis in vitro, contributing to tissue-like structure formation.

Singhal, S., et al. (2010). "Triamcinolone attenuates macrophage/microglia accumulation associated with NMDA-induced RGC death and facilitates survival of Muller stem cell grafts." Exp Eye Res **90**(2): 308-315.

Retinal ganglion cell (RGC) death in glaucoma models is associated with accumulation of activated microglia, a sign of neural degeneration which has been shown to constitute a barrier for transplant cell survival and migration. This study investigated the use of triamcinolone (TA) to control macrophage/microglia accumulation in a model of RGC depletion to create a permissive environment for stem cell grafting. Intravitreal NMDA alone or in combination with TA was used to induce rapid onset of RGC death in 3-4 week old Lister hooded (LH) rat eyes prior to Muller stem cell transplantation into the vitreoretinal space. The effect of NMDA on RGC death and microglial accumulation was assessed immuno-histochemically, whilst electroretinography (ERG) was used to assess RGC function. Post transplantation, survival of grafted cells and their association with microglia were also examined by immunohistochemical methods. Intravitreal injection of NMDA alone resulted in severe

macrophage/microglia accumulation associated with extensive RGC death 4-7 days post-treatment. Combination of NMDA with TA significantly reduced microglial numbers in the RGC when compared to NMDA only treated eyes while still depleting the retina of RGC. At the same time, NMDA/TA treatment also caused functional RGC loss as demonstrated by reduction of the scotopic threshold response. Upon transplantation with Muller stem cells, NMDA/TA treatment caused significant reduction in the number of transplant associated macrophage/microglia compared to eyes treated with NMDA alone. On this basis it is proposed that intravitreal injection of TA may be useful as an effective anti-inflammatory agent to control macrophage/microglia accumulation induced by RGC death, thereby creating a retinal environment permissive to cell transplantation studies.

Sobiesiak, M., et al. (2010). "The mesenchymal stem cell antigen MSCA-1 is identical to tissue non-specific alkaline phosphatase." Stem Cells Dev **19**(5): 669-677.

We have recently identified 2 distinct CD271(bright)MSCA-1(dim)CD56(+) and CD271(bright)MSCA-1(bright)CD56(-) MSC subsets in primary femur-derived bone marrow (BM), which differ in their expression pattern and morphology as well as in their clonogenic and differentiation capacity. Here, we show that MSCA-1 is identical to tissue non-specific alkaline phosphatase (TNAP), an ectoenzyme known to be expressed at high levels in liver, bone, and kidney as well as in embryonic stem (ES) cells. SDS-PAGE of WERI-RB-1 cell lysate and supernatant from phosphatidylinositol-specific phospholipase C (PI-PLC)-treated WERI-RB-1 cells resulted in the appearance of a prominent 68-kDa band. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDITOF MS) sequence analysis revealed TNAP-specific peptides. Screening of the MSCA-1-specific antibody W8B2 on HEK-293 cells transfected with the full-length coding sequence of TNAP showed specific reactivity with transfected but not with parent cell line. In addition, TNAP-specific mRNA expression was selectively detected in the transfectant line. In agreement with these findings, enzymatic activity of TNAP was exclusively detected in sorted MSCA-1(+) BM cells but not in the MSCA-1(-) negative fraction. Surface marker analysis revealed coexpression of the embryonic marker SSEA-3 but not SSEA-4, TRA-1-60, and TRA-1-81. In endometrium, TNAP is expressed at intermediate levels on CD146(+) cells and at high levels in the luminal space of glandular epithelia. Our results demonstrate that TNAP is a selective marker for the prospective isolation of BM-derived MSC and MSC-like cells in endometrium.

Sui, L., et al. (2013). "Transplantation of human embryonic stem cell-derived pancreatic endoderm reveals a site-specific survival, growth, and differentiation." Cell Transplant **22**(5): 821-830.

Development of beta-cells from human embryonic stem cells (hESCs) could compensate for the shortage of islet donors required for diabetes therapy. Although pancreatic progenitors have been derived from hESCs using various protocols, no fully functional b-cells could be generated in vitro. We evaluated the in vivo growth and differentiation of PDX1+ pancreatic endoderm cells obtained from hESCs. Here we show site-specific survival and differentiation when comparing cells grafted in the epididymal fat pad or the subcutaneous space of NOD/SCID mice after 12 weeks follow-up. Subcutaneous grafts persisted and expressed PDX1 at all time points analyzed, showed PDX1 and NKX6.1 coexpression after 6 weeks, and contained NGN3+ cells after 12 weeks.These findings suggest that further specification along the pancreatic lineage occured at the subcutaneous site.In sharp contrast, in the fat pad grafts only a minority of PDX1+ cells remained after 2 weeks, and no further pancreatic differentiation was observed later on. In addition, contaminating mesenchymal cells present in the implants further developed into cartilage tissue after 6 weeks implantation in the fat pad, but not in the subcutaneous space. These findings indicate that the in vivo microenvironment plays a critical role in the further differentiation of transplanted pancreatic endoderm cells.

Tian, C., et al. (2013). "Autologous bone marrow mesenchymal stem cell therapy in the subacute stage of traumatic brain injury by lumbar puncture." Exp Clin Transplant **11**(2): 176-181.

OBJECTIVES: To explore the clinical therapeutic effects and safety of autologous bone marrow mesenchymal stem cell therapy for traumatic brain injury by lumbar puncture. MATERIALS AND METHODS: A total of 97 patients (24 with persistent vegetative state and 73 with disturbance motor activity) who developed a complex cerebral lesion after traumatic brain injury received autologous bone marrow mesenchymal stem cell therapy voluntarily. The stem cells were isolated from the bone marrow of the patients and transplanted into the subarachnoid space by lumbar puncture. RESULTS: Fourteen days after cell therapy, no serious complications or adverse events were reported. To a certain extent, 38 of 97 patients (39.2%) improved in the function of brain after transplant (P =.007). Eleven of 24 patients

(45.8%) with persistent vegetative state showed posttherapeutic improvements in consciousness (P =.024). Twenty-seven of 73 patients (37.0%) with a motor disorder began to show improvements in motor functions (P =.025). The age of patients and the time elapsed between injury and therapy had effects on the outcomes of the cellular therapy (P <.05). No correlation was found between the number of cell injections and improvements (P >.05). CONCLUSIONS: This study suggests that the bone marrow stem cell therapy is safe and effective on patients with traumatic brain injury complications, such as persistent vegetative state and motor disorder, through lumbar puncture. Young patients improve more easily than older ones. The earlier the cellular therapy begins in the subacute stage of traumatic brain injury, the better the results.

Tian, C., et al. (2011). "Increased Muller cell de-differentiation after grafting of retinal stem cell in the sub-retinal space of Royal College of Surgeons rats." Tissue Eng Part A **17**(19-20): 2523-2532.

In several vertebrate classes, the Muller glia are capable of de-differentiating, proliferating, and acquiring a progenitor-like state in response to acute retinal injury or in response to exogenous growth factors. Our previous study has shown that Muller cells can be activated and de-differentiated into retinal progenitors during Royal College of Surgeons (RCS) rats' degeneration, although the limited proliferation cannot maintain retinal function. We now report that rat retinal stem cells (rSCs) transplanted into RCS rats slowed the progression of retinal morphological degeneration and prevented the functional disruption. Further, we found that retinal progenitor cells labeled with Chx10 were increased significantly after rSCs transplantation, and most of them are mainly from activated Muller cells. rSCs transplantation also enhances neurogenic potential by producing more recoverin-positive photoreceptors, which was proved coming from Muller glia-derived cells. These results provide evidence that stem cell-based therapy may offer a novel therapeutic approach for the treatment of retinal degeneration, and that Muller glia in mammalian retina can be activated and de-differentiated by rSC transplantation and may have therapeutic effects.

Touraine, J. L., et al. (2004). "Reappraisal of in utero stem cell transplantation based on long-term results." Fetal Diagn Ther **19**(4): 305-312.

The therapeutic field of in utero transplantation of stem cells, into human fetuses, has developed since 1988 with the hope of improved probability of engraftment and tolerance, due to immune immaturity of the host. Fifteen years later, it is possible to evaluate the results that we and others have obtained in the treatment of several fetal diseases. Seven fetal patients have been treated in Lyon: In 2 cases, pregnancy termination was induced by the in utero injection; in the 5 other cases, engraftment was obtained and repeatedly documented with presence of donor HLA antigens and/or Y chromosome in recipients. In the 2 patients with combined immunodeficiency disease, a sustained reconstitution of immunity was obtained as a result of the transplant but other complications occurred thereafter. In patients with thalassemia major, Niemann-Pick disease or hemophilia, a very partial and very transitory benefit was only obtained. Approximately 33 other patients with immunodeficiencies, hemoglobinopathies or inborn errors of metabolism have been treated worldwide, over the last 13 years, with a comparable method, using parental or fetal stem cells transplanted in utero. Successful treatment has usually been recorded in immunodeficiencies, and insufficient results have been obtained in the other cases. This form of treatment can therefore be recommended after prenatal diagnosis of combined immunodeficiency but additional research is required to improve the degree of engraftment, the lack of resistance of the host and the 'space' available for hematopoiesis in the other conditions.

Walther, V. and T. A. Graham (2014). "Location, location, location! The reality of life for an intestinal stem cell in the crypt." J Pathol **234**(1): 1-4.

The intestinal crypt has become the archetypal system to understand stem cell behaviour in vivo. Advances in lineage-tracing technology have identified rapidly cycling stem cells at the crypt base with prominent expression of 'marker' genes such as Lgr5. Elegant quantitative analysis of lineage-tracing data has shown that each stem cell within the crypt is in continual neutral competition with the others in order to retain its place in the niche and so prevent differentiation into a specialized lineage. Accordingly, it appears that the regulation of the stem cell pool occurs primarily at the level of the stem cell population, as a simple consequence of competition for the limited space within the niche. However, contradictory data showing that cells located away from the crypt base niche can also sometimes function as stem cells has challenged the notion that stemness is fundamentally cell-extrinsic. Writing in Nature, Ritsma and colleagues have resolved this debate by performing in vivo live-imaging of the crypt base. By tracking individual stem cells over time, they showed that the relative positioning of the cell within the niche stochastically regulates its fate. Stem cells located in close proximity to the crypt base were more likely to persist long-term, but peripheral cells could sometimes move into privileged crypt-base positions. Thus, while

many cells within the crypt have stem cell potential, only cells lucky enough to reside in the 'Goldilocks zone' behave as functional stem cells in the long term. The hunt for intestinal stem cells is over: the stem cells are simply found in their niche.

Wen, S., et al. (2009). "Dynamic signaling for neural stem cell fate determination." Cell Adh Migr **3**(1): 107-117.

Central nervous system (CNS) development starts from neural stem cells (NSCs) which ultimately give rise to the three major cell types (neurons, oligodendrocytes and astrocytes) of the CNS. NSCs are specified in space- and time-related fashions, becoming spatially heterogeneous and generating a progressively restricted repertoire of cell types. Mammalian NSCs produce different cell types at different time points during development under the influence of multiple signaling pathways. These pathways act in a dynamic web mode to determine the fate of NSCs via modulating the expression and activity of distinct set of transcription factors which in turn trigger the transcription of neural fate-associated genes. This review thus introduces the major signal pathways, transcription factors and their cross-talks and coordinative interactions in NSC fate determination.

Woo, J. H., et al. (2016). "MERS versus Standard Surgical Approaches for Porcine Vocal Fold Scarring with Adipose Stem Cell Constructs." Otolaryngol Head Neck Surg **155**(4): 612-623.

OBJECTIVE: Cells, scaffold, and surgical approaches are important for regeneration of the lamina propria of the scarred vocal fold (VF). Microendoscopy of Reinke's space (MERS) is a surgical approach used to access the lamina propria. The present study evaluated MERS in the treatment of VF scarring as compared with standardized approaches for the treatment of VF scarring with adipose stem cell constructs. STUDY DESIGN: Animal study. SETTING: Academic center. SUBJECTS AND METHODS: VF injury was performed bilaterally to induce scarring in 20 pigs. Eight weeks after injury, pigs were classified into no treatment, minithyrotomy, VF injection, VF incision/dissection, and MERS. All groups (except control) were implanted with adipose stem cell and hyaluronan. Four weeks after treatment, histology for collagen, hyaluronan, and fibronectin; mRNA expression for alpha-smooth muscle actin, tumor growth factor beta1, collagen 1alpha1, collagen 3alpha1, matrix metalloproteinase 2, basic fibroblast growth factor, and hepatocyte growth factor; and tissue rheology were evaluated. RESULTS: Differences were measured among surgical approaches for protein levels of collagen, hyaluronan, and fibronectin (P =.0133, P <.0001, and P =.0025, respectively). Fibroblast growth factor, collagen 1alpha1, and matrix metalloproteinase 2 transcript levels were different among treatment groups (P =.003, P =.0086, and P =.014, respectively), while no differences were measured for alpha-smooth muscle actin, tumor growth factor beta1, hepatocyte growth factor, and collagen 3alpha1. Rheologically, significant differences were not measured between groups. CONCLUSION: MERS is a promising surgical approach for the treatment of VF scarring, optimizing the placement of implanted biomaterials.

Wu, J., et al. (2007). "[Effect of transplanting marrow mesenchymal stem cells via subarachnoid space on spinal cord injury and T cell subpopulation in rats]." Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi **21**(5): 492-496.

OBJECTIVE: To investigate the therapeutic effects of transplanting allogeneic marrow mesenchymal stem cells (MSCs) via subarachnoid space on spinal cord injury (SCI) and the T cell subpopulation. METHODS: Density gradient centrifugation was used to isolate and expand MSCs from bone marrow of 10 six-week-old SD rats. The SCI model was produced by weight-beating from 60 eight-week-old female SD rats. Forty survival SCI rats, which BBB scores were zero, were divided randomly into 2 groups:experimental group (group A) and control group (group B). In addition, 20 normal eight-week-old SI) rats were used as blank group (group C). In group A, 1 ml cells suspention containing MSCs (the 6th generation, 2 x 10(6)/ml) was injected via subarachnoid space. In group B, equal volume of L-DMEM was injected in the same way. The BBB score was obtained after 1st, 2nd and 3rd weeks of injection. At the same time,T cell subpopulation was detected by flow cytometry. RESULTS: The BBB score in group A was better than that in group B, but fewer than that in group C in the 3rd week. CD4+ T cells in group A were less than those in groups B and C in the 1st, 2nd, and 3rd weeks. CD8+ T cells in group A were less than those in groups B and C in the 2nd and 3rd weeks. The ratio of CD4+/CD8+ T cells in group A was less than those in groups B and C in the 1st week. Above differences showed statistically significant difference (P < 0.05). However, there were no statistically significant differences in the ratio of CD4+/CD8+T cells between group A and groups B, C in the 2nd and 3rd weeks (P>0.05). CONCLUSION: The above results suggest that allogeneic MSCs transplantation via subarachnoid space is beneficial to SCI to some extend, do not result in rejection in vivo. Furthermore, it can lead to immunosuppression in short time. So, it

provides clues to apply MSCs to treat SCI and other diseases.

Wu, W., et al. (2016). "Features specific to retinal pigment epithelium cells derived from three-dimensional human embryonic stem cell cultures - a new donor for cell therapy." Oncotarget **7**(16): 22819-22833.

Retinal pigment epithelium (RPE) transplantation is a particularly promising treatment of retinal degenerative diseases affecting RPE-photoreceptor complex. Embryonic stem cells (ESCs) provide an abundant donor source for RPE transplantation. Herein, we studied the time-course characteristics of RPE cells derived from three-dimensional human ESCs cultures (3D-RPE). We showed that 3D-RPE cells possessed morphology, ultrastructure, gene expression profile, and functions of authentic RPE. As differentiation proceeded, 3D-RPE cells could mature gradually with decreasing proliferation but increasing functions. Besides, 3D-RPE cells could form polarized monolayer with functional tight junction and gap junction. When grafted into the subretinal space of Royal College of Surgeons rats, 3D-RPE cells were safe and efficient to rescue retinal degeneration. This study showed that 3D-RPE cells were a new donor for cell therapy of retinal degenerative diseases.

Wu, Y., et al. (2016). "Fabrication and evaluation of electrohydrodynamic jet 3D printed polycaprolactone/chitosan cell carriers using human embryonic stem cell-derived fibroblasts." J Biomater Appl **31**(2): 181-192.

Biological function of adherent cells depends on the cell-cell and cell-matrix interactions in three-dimensional space. To understand the behavior of cells in 3D environment and their interactions with neighboring cells and matrix requires 3D culture systems. Here, we present a novel 3D cell carrier scaffold that provides an environment for routine 3D cell growth in vitro We have developed thin, mechanically stable electrohydrodynamic jet (E-jet) 3D printed polycaprolactone and polycaprolactone/Chitosan macroporous scaffolds with precise fiber orientation for basic 3D cell culture application. We have evaluated the application of this technology by growing human embryonic stem cell-derived fibroblasts within these 3D scaffolds. Assessment of cell viability and proliferation of cells seeded on polycaprolactone and polycaprolactone/Chitosan 3D-scaffolds show that the human embryonic stem cell-derived fibroblasts could adhere and proliferate on the scaffolds over time. Further, using confocal microscopy we demonstrate the ability to use fluorescence-labelled cells that could be microscopically monitored in real-time. Hence, these 3D printed polycaprolactone and polycaprolactone/Chitosan scaffolds could be used as a cell carrier for in vitro 3D cell culture-, bioreactor- and tissue engineering-related applications in the future.

Xie, D., et al. (2010). "Optimization of magnetosonoporation for stem cell labeling." NMR Biomed **23**(5): 480-484.

Recent advances in magnetic cell labeling have taken place with the development of a magnetosonoporation (MSP) technique. The aim of this study was to optimize the MSP protocol in order to achieve high cell viability and intracellular uptake of MR contrast agents. First, we determined the sub-optimal MSP parameters by evaluating the viabilities of C17.2 neural stem cells without Feridex using various MSP intensities ranging from 0.1 to 1 w/cm (2), duty cycles at 20%, 50% or 100%, and exposure times from 1-15 min. The sub-optimized MSP parameters with cell viabilities greater than 90% were further optimized by evaluating both cell viability and intracellular iron uptake when Feridex was used. We then used the optimized MSP parameters to determinate the optimal concentration of Feridex for magnetic cell labeling. Subsequently, we validated the feasibility of using MRI to track the migration of neural stem cells from the transplanted sites to glioma masses in four mouse brains when the cells had been labeled with Feridex using the optimized MSP protocol. The MRI findings were confirmed by histological correlations. In vitro experiments demonstrated that the optimal MSP protocol was achieved at 20% duty cycle, 0.3 w/cm (2) ultrasound intensity, 5-min exposure time and 1 mg/mL Feridex. This study demonstrated that the optimized MSP cell labeling technique can achieve both high cell viability and intracellular uptake of MR contrast agents, and has the potential to be a useful cell labeling technique to facilitate future clinical translation of MRI-integrated cell therapy.

Yang, Y., et al. (2011). "Monitoring bone marrow-originated mesenchymal stem cell traffic to myocardial infarction sites using magnetic resonance imaging." Magn Reson Med **65**(5): 1430-1436.

How stem cells promote myocardial repair in myocardial infarction (MI) is not well understood. The purpose of this study was to noninvasively monitor and quantify mesenchymal stem cells (MSC) from bone marrow to MI sites using magnetic resonance imaging (MRI). MSC were dual-labeled with an enhanced green fluorescent protein and micrometer-sized iron oxide particles prior to intra-bone marrow transplantation into the tibial medullary space of C57Bl/6 mice. Micrometer-sized iron oxide particles

labeling caused signal attenuation in T (2)*-weighted MRI and thus allowed noninvasive cell tracking. Longitudinal MRI demonstrated MSC infiltration into MI sites over time. Fluorescence from both micrometer-sized iron oxide particles and enhanced green fluorescent protein in histology validated the presence of dual-labeled cells at MI sites. This study demonstrated that MSC traffic to MI sites can be noninvasively monitored in MRI by labeling cells with micrometer-sized iron oxide particles. The dual-labeled MSC at MI sites maintained their capability of proliferation and differentiation. The dual-labeling, intra-bone marrow transplantation, and MRI cell tracking provided a unique approach for investigating stem cells' roles in the post-MI healing process. This technique can potentially be applied to monitor possible effects on stem cell mobilization caused by given treatment strategies.

Zhang, B. Y., et al. (2018). "Evaluation of the Curative Effect of Umbilical Cord Mesenchymal Stem Cell Therapy for Knee Arthritis in Dogs Using Imaging Technology." Stem Cells Int **2018**: 1983025.

Objective: The aim of this study was to assess the efficacy of canine umbilical cord mesenchymal stem cells (UC-MSCs) on the treatment of knee osteoarthritis in dogs. Methods: Eight dogs were evenly assigned to two groups. The canine model of knee osteoarthritis was established by surgical manipulation of knee articular cartilage on these eight dogs. UC-MSCs were isolated from umbilical cord Wharton's jelly by 0.1% type collagenase I and identified by immunofluorescence staining and adipogenic and osteogenic differentiation in vitro. A suspension of allogeneic UC-MSCs (1 x 10(6)) and an equal amount of physiological saline was injected into the cavitas articularis in the treated and untreated control groups, respectively, on days 1 and 3 posttreatment. The structure of the canine knee joint was observed by magnetic resonance imaging (MRI), B-mode ultrasonography, and X-ray imaging at the 3rd, 7th, 14th, and 28th days after treatment. Concurrently, the levels of IL-6, IL-7, and TNF-alpha in the blood of the examined dogs were measured. Moreover, the recovery of cartilage and patella surface in the treated group and untreated group was compared using a scanning electron microscope (SEM) after a 35-day treatment. Results: Results revealed that the isolated cells were UC-MSCs, because they were positive for CD44 and negative for CD34 surface markers, and the cells were differentiated into adipocytes and osteoblasts. Imaging technology showed that as treatment time increased, the high signal in the MRI T2-weighted images decreased, the echo-free space in B ultrasonography images disappeared basically, and the continuous linear hypoechoic region at the trochlear sulcus thickened. On X-ray images, the serrate defect at the ventral cortex of the patella improved, and the low-density gap of the ventral patella and trochlear crest gradually increased in the treated group. On the contrary, the high signal in the MRI T2-weighted images and the echo-free space in B ultrasonography images still increased after a 14-day treatment in the untreated control group, and the linear hypoechoic region was discontinuous. On the X-ray images, there was no improvement in the serrate defect of the ventral cortex of the patella. Results for inflammatory factors showed that the blood levels of IL-6, IL-7, and TNF-alpha of the untreated control group were significantly higher than those of the treated group ($P < 0.05$) 7-14 days posttreatment. The result of SEM showed that the cartilage neogenesis in the treated group had visible neonatal tissue and more irregular arrangement of new tissue fibers than that of the untreated control group. Furthermore, more vacuoles but without collagen fibers were observed in the cartilage of the untreated control group, and the thickness of the neogenetic cartilage in the treated group (65.13 +/- 5.29, 65.30 +/- 5.83) and the untreated control group (34.27 +/- 5.42) showed a significant difference ($P < 0.01$). Conclusion: Significantly higher improvement in cartilage neogenesis and recovery was observed in the treated group compared to the untreated control group. The joint fluid and the inflammatory response in the treated group decreased. Moreover, improved recovery in the neogenetic cartilage, damaged skin fascia, and muscle tissue around the joints was more significant in the treated group than in the untreated control group. In conclusion, canine UC-MSCs promote the repair of cartilage and patella injury in osteoarthritis, improve the healing of the surrounding tissues, and reduce the inflammatory response.

Zhou, J., et al. (2016). "Characterization of Induced Pluripotent Stem Cell Microvesicle Genesis, Morphology and Pluripotent Content." Sci Rep **6**: 19743.

Microvesicles (MVs) are lipid bilayer-covered cell fragments that range in diameter from 30 nm-1 uM and are released from all cell types. An increasing number of studies reveal that MVs contain microRNA, mRNA and protein that can be detected in the extracellular space. In this study, we characterized induced pluripotent stem cell (iPSC) MV genesis, content and fusion to retinal progenitor cells (RPCs) in vitro. Nanoparticle tracking revealed that iPSCs released approximately 2200 MVs cell/hour in the first 12 hrs with an average diameter of 122 nm. Electron and light microscopic analysis of iPSCs showed MV release via lipid bilayer budding. The mRNA content of iPSC MVs was characterized and revealed the

presence of the transcription factors Oct-3/4, Nanog, Klf4, and C-Myc. The protein content of iPSCs MVs, detected by immunogold electron microscopy, revealed the presence of the Oct-3/4 and Nanog. Isolated iPSC MVs were shown to fuse with RPCs in vitro at multiple points along the plasma membrane. These findings demonstrate that the mRNA and protein cargo in iPSC MVs have established roles in maintenance of pluripotency. Building on this work, iPSC derived MVs may be shown to be involved in maintaining cellular pluripotency and may have application in regenerative strategies for neural tissue.

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