Induced Pluripotent Stem Cell Research Literatures

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Abstract: Stem cells are derived from embryonic and non-embryonic tissues. Most stem cell studies are for animal stem cells and plants have also stem cell. Stem cells were discovered in 1981 from early mouse embryos. Stem cells have the potential to develop into all different cell types in the living body. Stem cell is a body repair system. When a stem cell divides it can be still a stem cell or become adult cell, such as a brain cell. Stem cells are unspecialized cells and can renew themselves by cell division, and stem cells can also differentiate to adult cells with special functions. Stem cells replace the old cells and repair the damaged tissues. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. This article introduces recent research reports as references in the related studies.

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Introduction

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

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

Aberdam, E., et al. (2017). "Induced pluripotent stem cell-derived limbal epithelial cells (LiPSC) as a cellular alternative for in vitro ocular toxicity testing." PLoS One **12**(6): e0179913.

Induced pluripotent stem cells hold great potential to produce unlimited amount of differentiated cells as cellular source for regenerative medicine but also for in vitro drug screening and cytotoxicity tests. Ocular toxicity testing is mandatory to evaluate the risks of drugs and cosmetic products before their application to human patients by preventing eye irritation or insult. Since the global ban to use animals, many human-derived alternatives have been proposed, from ex-vivo enucleated postmortem cornea, primary corneal cell culture and immortalized corneal epithelial cell lines. All of them share limitations for their routine use. Using an improved protocol, we derived limbal epithelial cells from human induced pluripotent stem cells, named LiPSC, that are able to be passaged and differentiate further into corneal epithelial cells. Comparative RT-qPCR, immunofluorescence staining, flow cytometry analysis and zymography assays demonstrate that LiPSC are morphologically and molecularly similar to the adult stem cells. Moreover, contrary to HCE, LiPSC and primary limbal cells display similarly sensitive to cytotoxicity treatment among passages. Our data strongly suggest that LiPSC could become a powerful alternative cellular model for cosmetic and drug tests.

Abu-Hassan, D. W., et al. (2015). "Induced pluripotent stem cells restore function in a human cell loss model of open-angle glaucoma." Stem Cells **33**(3): 751-761.

Normally, trabecular meshwork (TM) and Schlemm's canal inner wall endothelial cells within the aqueous humor outflow pathway maintain intraocular pressure within a narrow safe range. Elevation in intraocular pressure, because of the loss of homeostatic regulation by these outflow pathway cells, is the primary risk factor for vision loss due to glaucomatous optic neuropathy. A notable feature associated with glaucoma is outflow pathway cell loss. Using controlled cell loss in ex vivo perfused human outflow pathway organ culture, we developed compelling experimental evidence that this level of cell loss compromises intraocular pressure homeostatic function. This function was restored by repopulation of the model with fresh TM cells. We then differentiated induced pluripotent stem cells (iPSCs) and used them to repopulate this cell depletion model. These differentiated cells (TM-like iPSCs) became similar to TM cells in both morphology and expression patterns. When transplanted, they were able to fully restore intraocular pressure homeostatic function. This successful transplantation of TM-like iPSCs establishes the conceptual feasibility of using

autologous stem cells to restore intraocular pressure regulatory function in open-angle glaucoma patients, providing a novel alternative treatment option.

Acharya, M. M., et al. (2015). "Defining the optimal window for cranial transplantation of human induced pluripotent stem cell-derived cells to ameliorate radiation-induced cognitive impairment." Stem Cells Transl Med **4**(1): 74-83.

Past preclinical studies have demonstrated the capability of using human stem cell transplantation in the irradiated brain to ameliorate radiation-induced cognitive dysfunction. Intrahippocampal transplantation of human embryonic stem cells and human neural stem cells (hNSCs) was found to functionally restore cognition in rats 1 and 4 months after cranial irradiation. To optimize the potential therapeutic benefits of human stem cell transplantation, we have further defined optimal transplantation windows for maximizing cognitive benefits after irradiation and used induced pluripotent stem cell-derived hNSCs (iPSC-hNSCs) that may eventually help minimize graft rejection in the host brain. For these studies, animals given an acute head-only dose of 10 Gy were grafted with iPSC-hNSCs at 2 days, 2 weeks, or 4 weeks following irradiation. Animals receiving stem cell grafts showed improved hippocampal spatial memory and contextual fear-conditioning performance compared with irradiated sham-surgery controls when analyzed 1 month after transplantation surgery. Importantly, superior performance was evident when stem cell grafting was delayed by 4 weeks following irradiation compared with animals grafted at earlier times. Analysis of the 4-week cohort showed that the surviving grafted cells migrated throughout the CA1 and CA3 subfields of the host hippocampus and differentiated into neuronal (approximately 39%) and astroglial (approximately 14%) subtypes. Furthermore, radiation-induced inflammation was significantly attenuated across multiple hippocampal subfields in animals receiving iPSC-hNSCs at 4 weeks after irradiation. These studies expand our prior findings to demonstrate that protracted stem cell grafting provides improved cognitive benefits following irradiation that are associated with reduced neuroinflammation.

Alipio, Z. A., et al. (2011). "Epithelial to mesenchymal transition (EMT) induced by bleomycin or TFG (b1)/EGF in murine induced pluripotent stem cell-derived alveolar Type II-like cells." Differentiation **82**(2): 89-98.

Induced pluripotent stem (iPS) cells are derived from reprogrammed somatic cells and are similar to embryonic stem (ES) cells in morphology, gene/protein expression, and pluripotency. In this study, we explored the potential of iPS cells to differentiate into alveolar Type II (ATII)-like epithelial cells. Analysis using quantitative real time polymerase chain reaction and immunofluorescence staining showed that pulmonary surfactant proteins commonly expressed by ATII cells such as surfactant protein A (SPA), surfactant protein B (SPB), and surfactant protein C (SPC) were upregulated in the differentiated cells. Microphilopodia characteristics and lamellar bodies were observed by transmission electron microscopy and lipid deposits were verified by Nile Red and Periodic Acid Schiff staining. C3 complement protein, a specific feature of ATII cells, was present at high levels in culture supernatants demonstrating functionality of these cells in culture. These data show that the differentiated cells generated from iPS cells using a culture method developed previously (Rippon et al., 2006) are ATII-like cells. To further characterize these ATII-like cells, we tested whether they could undergo epithelial to mesenchymal transition (EMT) by exposure to drugs that induce lung fibrosis in mice, such as bleomycin, and the combination of transforming growth factor beta1 (TGF (b1)) and epidermal growth factor (EGF). When the ATII-like cells were exposed to either bleomycin or a TGF (b1)-EGF cocktail, they underwent phenotypic changes including acquisition of a mesenchymal/fibroblastic morphology, upregulation of mesenchymal markers (Col1, Vim, a-Sma, and S100A4), and downregulation of surfactant proteins and E-cadherin. We have shown that ATII-like cells can be derived from skin fibroblasts and that they respond to fibrotic stimuli. These cells provide a valuable tool for screening of agents that can potentially ameliorate or prevent diseases involving lung fibrosis.

Altomare, C., et al. (2016). "Human-induced pluripotent stem cell-derived cardiomyocytes from cardiac progenitor cells: effects of selective ion channel blockade." Europace **18**(suppl 4): iv67-iv76.

AIM: Human-induced pluripotent stem cell (hiPSC)-derived cardiomyocytes are likely to revolutionize electrophysiological approaches to arrhythmias. Recent evidence suggests the somatic cell origin of hiPSCs may influence their differentiation potential. Owing to their cardiomyogenic potential, cardiac-stromal progenitor cells (CPCs) are an interesting cellular source for generation of hiPSC-derived cardiomyocytes. The effect of ionic current blockade in hiPSC-derived cardiomyocytes generated from CPCs has not been characterized yet. METHODS AND RESULTS: Human-induced pluripotent stem cell-derived cardiomyocytes were generated from adult CPCs and skin fibroblasts from the same individuals. The effect of selective ionic

current blockade on spontaneously beating hiPSC-derived cardiomyocytes was assessed using multi-electrode arrays. Cardiac-stromal progenitor cells could be reprogrammed into hiPSCs, then differentiated into hiPSC-derived cardiomyocytes. Human-induced pluripotent stem cell-derived cardiomyocytes of cardiac origin showed higher upregulation of cardiac-specific genes compared with those of fibroblastic origin. Human-induced pluripotent stem cell-derived cardiomyocytes of both somatic cell origins exhibited sensitivity to tetrodotoxin, a blocker of Na (+)current (INa), nifedipine, a blocker of L-type Ca (2+)current (ICaL), and E4031, a blocker of the rapid component of delayed rectifier K (+)current (IKr). Human-induced pluripotent stem cell-derived cardiomyocytes of cardiac origin exhibited sensitivity to JNJ303, a blocker of the slow component of delayed rectifier K (+)current (IKs). CONCLUSION: In hiPSC-derived cardiomyocytes of cardiac origin, INa, ICaL, IKr, and IKs were present as tetrodotoxin-, nifedipine-, E4031-, and JNJ303-sensitive currents, respectively. Although cardiac differentiation efficiency was improved in hiPSCs of cardiac vs. non-cardiac origin, no major functional differences were observed between hiPSC-derived cardiomyocytes of different somatic cell origins. Further studies are warranted to characterize electrophysiological properties of hiPSC-derived cardiomyocytes generated from CPCs.

Alvisi, G., et al. (2018). "Generation of a transgene-free human induced pluripotent stem cell line (UNIPDi001-A) from oral mucosa epithelial stem cells." Stem Cell Res **28**: 177-180.

Human oral mucosa epithelial stem cells (hOMESCs) were obtained from a fresh oral biopsy collected from a healthy subject at the Fondazione Banca degli Occhi del Veneto (FBOV). An integration-free reprogramming protocol was applied exploiting episomal plasmids transfected into cells using a Nucleofector device. Around day 20 post transfection, several human induced pluripotent stem cell (hiPSC) colonies were manually picked and expanded. One of these (UNIPDi001-A-hiPSCs) expressed undifferentiated state marker alkaline phosphatase along with a panel of pluripotency state markers and was able to differentiate into the derivatives of all the three germ layers.

Anand, T., et al. (2016). "Differentiation of Induced Pluripotent Stem Cells to Lentoid Bodies Expressing a Lens Cell-Specific Fluorescent Reporter." PLoS One **11**(6): e0157570.

Curative approaches for eye cataracts and other eye abnormalities, such as myopia and hyperopia currently suffer from a lack of appropriate models. Here, we present a new approach for in vitro growth of lentoid bodies from induced pluripotent stem (iPS) cells as a tool for ophthalmological research. We generated a transgenic mouse line with lens-specific expression of a fluorescent reporter driven by the alphaA crystallin promoter. Fetal fibroblasts were isolated from transgenic fetuses, reprogrammed to iPS cells, and differentiated to lentoid bodies exploiting the specific fluorescence of the lens cell-specific reporter. The employment of cell type-specific reporters for establishing and optimizing differentiation in vitro seems to be an efficient and generally applicable approach for developing differentiation protocols for desired cell populations.

Araki, R., et al. (2017). "The Number of Point Mutations in Induced Pluripotent Stem Cells and Nuclear Transfer Embryonic Stem Cells Depends on the Method and Somatic Cell Type Used for Their Generation." Stem Cells **35**(5): 1189-1196.

Induced pluripotent stem cells hold great promise for regenerative medicine but point mutations have been identified in these cells and have raised serious concerns about their safe use. We generated nuclear transfer embryonic stem cells (ntESCs) from both mouse embryonic fibroblasts (MEFs) and tail-tip fibroblasts (TTFs) and by whole genome sequencing found fewer mutations compared with iPSCs generated by retroviral gene transduction. Furthermore, TTF-derived ntESCs showed only a very small number of point mutations, approximately 80% less than the number observed in iPSCs generated using retrovirus. Base substitution profile analysis confirmed this greatly reduced number of point mutations. The point mutations in iPSCs are therefore not a Yamanaka factor-specific phenomenon but are intrinsic to genome reprogramming. Moreover, the dramatic reduction in point mutations in ntESCs suggests that most are not essential for genome reprogramming. Our results suggest that it is feasible to reduce the point mutation frequency in iPSCs by optimizing various genome reprogramming conditions. We conducted whole genome sequencing of ntES cells derived from MEFs or TTFs.

Armstrong, L., et al. (2010). "Human induced pluripotent stem cell lines show stress defense mechanisms and mitochondrial regulation similar to those of human embryonic stem cells." Stem Cells **28**(4): 661-673.

The generation of induced pluripotent stem cells (iPSC) has enormous potential for the development of patient-specific regenerative medicine. Human embryonic stem cells (hESC) are able to defend their genomic integrity by maintaining low levels of reactive oxygen species (ROS) through a combination

of enhanced removal capacity and limited production of these molecules. Such limited ROS production stems partly from the small number of mitochondria present in hESC; thus, it was important to determine that human iPSC (hiPSC) generation is able to eliminate the extra mitochondria present in the parental fibroblasts (reminiscent of "bottleneck" situation after fertilization) and to show that hiPSC have antioxidant defenses similar to hESC. We were able to generate seven hiPSC lines from adult human dermal fibroblasts and have fully characterized two of those clones. Both hiPSC clones express pluripotency markers and are able to differentiate in vitro into cells belonging to all three germ layers. One of these clones is able to produce fully differentiated teratoma, whereas the other hiPSC clone is unable to silence the viral expression of OCT4 and c-MYC, produce fully differentiated teratoma, and unable to downregulate the expression of some of the pluripotency genes during the differentiation process. In spite of these differences, both clones show ROS stress defense mechanisms and mitochondrial biogenesis similar to hESC. Together our data suggest that, during the reprogramming process, certain cellular mechanisms are in place to ensure that hiPSC are provided with the same defense mechanisms against accumulation of ROS as the hESC.

Asgari, S., et al. (2013). "Differentiation and transplantation of human induced pluripotent stem cell-derived hepatocyte-like cells." Stem Cell Rev **9**(4): 493-504.

The generation of human induced pluripotent stem cells (hiPSCs) with a high differentiation potential provided a new source for hepatocyte generation not only for drug discovery and in vitro disease models, but also for cell replacement therapy. However, the reported hiPSC-derived hepatocyte-like cells (HLCs) were not well characterized and their transplantation, as the most promising clue of cell function was not reported. Here, we performed a growth factor-mediated differentiation of functional HLCs from hiPSCs and evaluated their potential for recovery of a carbon tetrachloride (CCl4)-injured mouse liver following transplantation. The hiPSC-derived hepatic lineage cells expressed hepatocyte-specific markers, showed glycogen and lipid storage activity, secretion of albumin (ALB), alpha-fetoprotein (AFP), urea, and CYP450 metabolic activity in addition to low-density lipoprotein (LDL) and indocyanin green (ICG) uptake. Similar results were observed with human embryonic stem cell (hESC)-derived HLCs. The transplantation of hiPSC-HLCs into a CCl4-injured liver showed incorporation of the hiPSC-HLCs into the mouse liver which resulted in a significant enhancement in total serum ALB after 1 week. A reduction of total serum LDH and bilirubin was seen when compared with the control and sham groups 1 and 5 weeks post-transplantation. Additionally, we detected human serum ALB and ALB-positive transplanted cells in both the host serum and livers, respectively, which showed functional integration of transplanted cells within the mouse livers. Therefore, our results have opened up a proof of concept that functional HLCs can be generated from hiPSCs, thus improving the general condition of a CCl4-injured mouse liver after their transplantation. These results may bring new insights in the clinical applications of hiPSCs once safety issues are overcome.

Ayoubi, S., et al. (2017). "Human induced pluripotent stem cell-derived vascular smooth muscle cells: differentiation and therapeutic potential." Cardiovasc Res **113**(11): 1282-1293.

Cardiovascular diseases remain the leading cause of death worldwide and current treatment strategies have limited effect of disease progression. It would be desirable to have better models to study developmental and pathological processes and model vascular diseases in laboratory settings. To this end, human induced pluripotent stem cells (hiPSCs) have generated great enthusiasm, and have been a driving force for development of novel strategies in drug discovery and regenerative cell-therapy for the last decade.

Azhdari, M., et al. (2013). "Therapeutic potential of human-induced pluripotent stem cell-derived endothelial cells in a bleomycin-induced scleroderma mouse model." Stem Cell Res **10**(3): 288-300.

Vascular injury and destruction of endothelial cells (ECs) are the early events in scleroderma (SSc) patients. This study aims to investigate the therapeutic potential of human-induced pluripotent stem cell-derived ECs (hiPSC-ECs) to treat SSc. We have assessed the functional differentiation of hiPSC-ECs and compared them with human embryonic stem cell-derived ECs (hESC-ECs) by a variety of in vitro experimental approaches. Additionally, we evaluated the therapeutic potential of hiPSC-ECs in a bleomycin-induced SSc mouse model. Our results demonstrated that hiPSC-ECs and hESC-ECs showed similar maximum expressions of FLK1 (early EC marker) at day five during differentiation. After sorting and culturing, the FLK1-positive cells exhibited spindle and subsequent endothelial cobblestone morphology in EGM2 medium. The hESC-ECs and hiPSC-ECs also expressed late EC markers CD31 (68% and 75%), CD144 (50% and 61%), CD146 (46% and 61%), and DiI-labeled acetylated low-density lipoprotein (DiI-ac-LDL) uptake (55% and 63%), respectively. They

additionally formed capillary-like structures on Matrigel. Analyses of the transplantation of sorted CD31-positive hiPSC-ECs into the bleomycin-induced SSc mouse model demonstrated that these cells participate in recovery of the damaged vessels. There was a reduction in collagen content; the number of total and degranulated mast cells returned to their normal state, and bleomycin-induced wounds as well as skin fibrosis improved four weeks after transplantation of hiPSC-ECs. Our findings have shown that the differentiation process from hESCs and hiPSCs to vascular cell components is similar. Additionally, this is the first study to determine the therapeutic potential of vascular cells from hiPSCs in the treatment of an SSc model. In the future, with further validation, these may be used as an appropriate source for the treatment of SSc patients.

Bajpai, V. K., et al. (2012). "Functional vascular smooth muscle cells derived from human induced pluripotent stem cells via mesenchymal stem cell intermediates." Cardiovasc Res **96**(3): 391-400.

AIMS: Smooth muscle cells (SMC) play an important role in vascular homeostasis and disease. Although adult mesenchymal stem cells (MSC) have been used as a source of contractile SMC, they suffer from limited proliferation potential and culture senescence, particularly when originating from older donors. By comparison, human induced pluripotent stem cells (hiPSC) can provide an unlimited source of functional SMC for autologous cell-based therapies and for creating models of vascular disease. Our goal was to develop an efficient strategy to derive functional, contractile SMC from hiPSC. METHODS AND RESULTS: We developed a robust, stage-wise, feeder-free strategy for hiPSC differentiation into functional SMC through an intermediate stage of multipotent MSC, which could be coaxed to differentiate into fat, bone, cartilage, and muscle. At this stage, the cells were highly proliferative and displayed higher clonogenic potential and reduced senescence when compared with parental hair follicle mesenchymal stem cells. In addition, when exposed to differentiation medium, the myogenic proteins such as alpha-smooth muscle actin, calponin, and myosin heavy chain were significantly upregulated and displayed robust fibrillar organization, suggesting the development of a contractile phenotype. Indeed, tissue constructs prepared from these cells exhibited high levels of contractility in response to receptor- and non-receptor-mediated agonists. CONCLUSION: We developed an efficient stage-wise strategy that enabled hiPSC differentiation into contractile SMC through an intermediate population of clonogenic and multipotent MSC. The high yield of MSC and SMC derivation suggests that our strategy may facilitate an acquisition of the large numbers of cells required for regenerative medicine or for studying vascular disease pathophysiology.

Bamba, Y., et al. (2014). "Differentiation, polarization, and migration of human induced pluripotent stem cell-derived neural progenitor cells co-cultured with a human glial cell line with radial glial-like characteristics." Biochem Biophys Res Commun **447**(4): 683-688.

Here we established a unique human glial cell line, GDC90, derived from a human glioma and demonstrated its utility as a glial scaffold for the polarization and differentiation of human induced pluripotent stem cell-derived neural progenitor cells (iPSC-NPCs). When co-cultured with GDC90 cells, iPSC-NPCs underwent rapid polarization and neurite extension along the radially spreading processes of the GDC90 cells, and showed migratory behavior. This method is potentially useful for detailed examination of neurites or for controlling neurites behavior for regenerative medicine.

Barrett, R., et al. (2014). "Reliable generation of induced pluripotent stem cells from human lymphoblastoid cell lines." Stem Cells Transl Med **3**(12): 1429-1434.

Patient-specific induced pluripotent stem cells (iPSCs) hold great promise for many applications, including disease modeling to elucidate mechanisms involved in disease pathogenesis, drug screening, and ultimately regenerative medicine therapies. A frequently used starting source of cells for reprogramming has been dermal fibroblasts isolated from skin biopsies. However, numerous repositories containing lymphoblastoid cell lines (LCLs) generated from a wide array of patients also exist in abundance. To date, this rich bioresource has been severely underused for iPSC generation. We first attempted to create iPSCs from LCLs using two existing methods but were unsuccessful. Here we report a new and more reliable method for LCL reprogramming using episomal plasmids expressing pluripotency factors and p53 shRNA in combination with small molecules. The LCL-derived iPSCs (LCL-iPSCs) exhibited identical characteristics to fibroblast-derived iPSCs (fib-iPSCs), wherein they retained their genotype, exhibited a normal pluripotency profile, and readily differentiated into all three germ-layer cell types. As expected, they also maintained rearrangement of the heavy chain immunoglobulin locus. Importantly, we also show efficient iPSC generation from LCLs of patients with spinal muscular atrophy and inflammatory bowel disease.

Belair, D. G., et al. (2015). "Human vascular tissue models formed from human induced pluripotent stem cell derived endothelial cells." Stem Cell Rev **11**(3): 511-525.

Here we describe a strategy to model blood vessel development using a well-defined induced pluripotent stem cell-derived endothelial cell type (iPSC-EC) cultured within engineered platforms that mimic the 3D microenvironment. The iPSC-ECs used here were first characterized by expression of endothelial markers and functional properties that included VEGF responsiveness, TNF-alpha-induced upregulation of cell adhesion molecules (MCAM/CD146; ICAM1/CD54), thrombin-dependent barrier function, shear stress-induced alignment, and 2D and 3D capillary-like network formation in Matrigel. The iPSC-ECs also formed 3D vascular networks in a variety of engineering contexts, yielded perfusable, interconnected lumen when co-cultured with primary human fibroblasts, and aligned with flow in microfluidics devices. iPSC-EC function during tubule network formation, barrier formation, and sprouting was consistent with that of primary ECs, and the results suggest a VEGF-independent mechanism for sprouting, which is relevant to therapeutic anti-angiogenesis strategies. Our combined results demonstrate the feasibility of using a well-defined, stable source of iPSC-ECs to model blood vessel formation within a variety of contexts using standard in vitro formats.

Ben-Ari, M., et al. (2014). "From beat rate variability in induced pluripotent stem cell-derived pacemaker cells to heart rate variability in human subjects." Heart Rhythm **11**(10): 1808-1818.

BACKGROUND: We previously reported that induced pluripotent stem cell-derived cardiomyocytes manifest beat rate variability (BRV) resembling heart rate variability (HRV) in the human sinoatrial node. We now hypothesized the BRV-HRV continuum originates in pacemaker cells. OBJECTIVE: To investigate whether cellular BRV is a source of HRV dynamics, we hypothesized 3 levels of interaction among different cardiomyocyte entities: (1) single pacemaker cells, (2) networks of electrically coupled pacemaker cells, and (3) the in situ sinoatrial node. METHODS: We measured BRV/HRV properties in single pacemaker cells, induced pluripotent stem cell-derived contracting embryoid bodies (EBs), and electrocardiograms from the same individual. RESULTS: Pronounced BRV/HRV was present at all 3 levels. The coefficient of variance of interbeat intervals and Poincare plot indices SD1 and SD2 for single cells were 20 times greater than those for EBs ($P < .05$) and the in situ heart (the latter two were similar; $P > .05$). We also compared BRV magnitude among single cells, small EBs (~5-10 cells), and larger EBs (>10 cells): BRV indices progressively increased with the decrease in the cell number ($P < .05$). Disrupting intracellular Ca (2+) handling markedly augmented BRV magnitude, revealing a unique bimodal firing pattern, suggesting that intracellular mechanisms contribute to BRV/HRV and the fractal behavior of heart rhythm.

Bershteyn, M., et al. (2014). "Cell-autonomous correction of ring chromosomes in human induced pluripotent stem cells." Nature **507**(7490): 99-103.

Ring chromosomes are structural aberrations commonly associated with birth defects, mental disabilities and growth retardation. Rings form after fusion of the long and short arms of a chromosome, and are sometimes associated with large terminal deletions. Owing to the severity of these large aberrations that can affect multiple contiguous genes, no possible therapeutic strategies for ring chromosome disorders have been proposed. During cell division, ring chromosomes can exhibit unstable behaviour leading to continuous production of aneuploid progeny with low viability and high cellular death rate. The overall consequences of this chromosomal instability have been largely unexplored in experimental model systems. Here we generated human induced pluripotent stem cells (iPSCs) from patient fibroblasts containing ring chromosomes with large deletions and found that reprogrammed cells lost the abnormal chromosome and duplicated the wild-type homologue through the compensatory uniparental disomy (UPD) mechanism. The karyotypically normal iPSCs with isodisomy for the corrected chromosome outgrew co-existing aneuploid populations, enabling rapid and efficient isolation of patient-derived iPSCs devoid of the original chromosomal aberration. Our results suggest a fundamentally different function for cellular reprogramming as a means of 'chromosome therapy' to reverse combined loss-of-function across many genes in cells with large-scale aberrations involving ring structures.

Bobis-Wozowicz, S., et al. (2015). "Human Induced Pluripotent Stem Cell-Derived Microvesicles Transmit RNAs and Proteins to Recipient Mature Heart Cells Modulating Cell Fate and Behavior." Stem Cells **33**(9): 2748-2761.

Microvesicles (MVs) are membrane-enclosed cytoplasmic fragments released by normal and activated cells that have been described as important mediators of cell-to-cell communication. Although the ability of human induced pluripotent stem cells (hiPSCs) to participate in tissue repair is being increasingly recognized, the use of hiPSC-derived MVs (hiPSC-MVs) in this regard remains unknown.

Accordingly, we investigated the ability of hiPSC-MVs to transfer bioactive molecules including mRNA, microRNA (miRNA), and proteins to mature target cells such as cardiac mesenchymal stromal cells (cMSCs), and we next analyzed effects of hiPSC-MVs on fate and behavior of such target cells. The results show that hiPSC-MVs derived from integration-free hiPSCs cultured under serum-free and feeder-free conditions are rich in mRNA, miRNA, and proteins originated from parent cells; however, the levels of expression vary between donor cells and MVs. Importantly, we found that transfer of hiPSC components by hiPSC-MVs impacted on transcriptome and proteomic profiles of target cells as well as exerted proliferative and protective effects on cMSCs, and enhanced their cardiac and endothelial differentiation potential. hiPSC-MVs also transferred exogenous transcripts from genetically modified hiPSCs that opens new perspectives for future strategies to enhance MV content. We conclude that hiPSC-MVs are effective vehicles for transferring iPSC attributes to adult somatic cells, and hiPSC-MV-mediated horizontal transfer of RNAs and proteins to injured tissues may be used for therapeutic tissue repair. In this study, for the first time, we propose a new concept of use of hiPSCs as a source of safe acellular bioactive derivatives for tissue regeneration.

Boonkaew, B., et al. (2018). "Establishment of an integration-free induced pluripotent stem cell line (MUSIi005-A) from exfoliated renal epithelial cells." Stem Cell Res **30**: 34-37.

Human induced pluripotent stem cells (iPSCs) were generated from exfoliated renal epithelial cells isolated from a urine sample of a 31-year-old healthy woman. Epithelial cells were characterized for the expression of E-cadherin and reprogrammed using non-integrating Sendai viral vectors. The urine-derived iPSC line (designated as MUSIi005-A) was karyotypically normal, expressed pluripotent markers, differentiated into cells of three embryonic germ layers, and showed no viral and transgene expressions at passage 29. Our protocol offers a non-invasive and efficient approach for iPSC generation from patients with genetic or acquired disorders.

Borestrom, C., et al. (2014). "Footprint-free human induced pluripotent stem cells from articular cartilage with redifferentiation capacity: a first step toward a clinical-grade cell source." Stem Cells Transl Med **3**(4): 433-447.

Human induced pluripotent stem cells (iPSCs) are potential cell sources for regenerative medicine; however, clinical applications of iPSCs are restricted because of undesired genomic modifications associated with most reprogramming protocols. We show, for the first time, that chondrocytes from autologous chondrocyte implantation (ACI) donors can be efficiently reprogrammed into iPSCs using a nonintegrating method based on mRNA delivery, resulting in footprint-free iPSCs (no genome-sequence modifications), devoid of viral factors or remaining reprogramming molecules. The search for universal allogeneic cell sources for the ACI regenerative treatment has been difficult because making chondrocytes with high matrix-forming capacity from pluripotent human embryonic stem cells has proven challenging and human mesenchymal stem cells have a predisposition to form hypertrophic cartilage and bone. We show that chondrocyte-derived iPSCs can be redifferentiated in vitro into cartilage matrix-producing cells better than fibroblast-derived iPSCs and on par with the donor chondrocytes, suggesting the existence of a differentiation bias toward the somatic cell origin and making chondrocyte-derived iPSCs a promising candidate universal cell source for ACI. Whole-genome single nucleotide polymorphism array and karyotyping were used to verify the genomic integrity and stability of the established iPSC lines. Our results suggest that RNA-based technology eliminates the risk of genomic integrations or aberrations, an important step toward a clinical-grade cell source for regenerative medicine such as treatment of cartilage defects and osteoarthritis.

Brauer, P. M., et al. (2016). "Modeling altered T-cell development with induced pluripotent stem cells from patients with RAG1-dependent immune deficiencies." Blood **128**(6): 783-793.

Primary immunodeficiency diseases comprise a group of heterogeneous genetic defects that affect immune system development and/or function. Here we use in vitro differentiation of human induced pluripotent stem cells (iPSCs) generated from patients with different recombination-activating gene 1 (RAG1) mutations to assess T-cell development and T-cell receptor (TCR) V (D)J recombination. RAG1-mutants from severe combined immunodeficient (SCID) patient cells showed a failure to sustain progression beyond the CD3(--)CD4(-)CD8(-)CD7(+)CD5(+)CD38(-)CD31(-/lo)CD45RA (+) stage of T-cell development to reach the CD3(-/+)CD4(+)CD8(+)CD7(+)CD5(+)CD38(+)CD31(+)CD45RA (-) stage. Despite residual mutant RAG1 recombination activity from an Omenn syndrome (OS) patient, similar impaired T-cell differentiation was observed, due to increased single-strand DNA breaks that likely occur due to heterodimers consisting of both an N-terminal truncated and a catalytically dead RAG1. Furthermore, deep-sequencing analysis of TCR-beta (TRB) and TCR-alpha (TRA) rearrangements of CD3(-)CD4(+)CD8(-) immature

single-positive and CD3(+)CD4(+)CD8(+) double-positive cells showed severe restriction of repertoire diversity with preferential usage of few Variable, Diversity, and Joining genes, and skewed length distribution of the TRB and TRA complementary determining region 3 sequences from SCID and OS iPSC-derived cells, whereas control iPSCs yielded T-cell progenitors with a broadly diversified repertoire. Finally, no TRA/delta excision circles (TRECs), a marker of TRA/delta locus rearrangements, were detected in SCID and OS-derived T-lineage cells, consistent with a pre-TCR block in T-cell development. This study compares human T-cell development of SCID vs OS patients, and elucidates important differences that help to explain the wide range of immunologic phenotypes that result from different mutations within the same gene of various patients.

Bremer, S. and R. Vogel (1999). "Pluripotent stem cells of the mouse as a potential in vitro model for mammalian germ cells. Sister chromatid exchanges induced by MMC and ENU in undifferentiated cell lines compared to differentiated cell lines." Mutat Res **444**(1): 97-102.

We tried to develop an in-vitro test system which could serve as a model for mammalian germ cells in vivo. Two pluripotent cell types were used, because they express some germ cell specific immunological and biochemical markers: (1) Embryonal carcinoma cells (ECC) of the line P19 had been isolated from a teratocarcinoma of murine primordial germ cells (PGC). (2) Embryonal stem cells (ESC) are obtained from the inner cell mass of mouse blastocysts. Sister chromatid exchanges (SCE) induced by mitomycin C and ethylnitrosourea (ENU) were analysed in the two undifferentiated cell lines, ECC and ESC, to detect differences in their sensitivity compared with differentiated cell lines of the mouse. Neither of the model cell lines have shown a greater sensibility after exposure to MMC and ENU. In contrary, the carcinoma cell line was able to tolerate higher concentrations of these genotoxicants. Therefore, SCE analysis in the ECC and ESC lines used does not provide a suitable model for genotoxicity testing on mammalian germ cells.

Briggs, S. F., et al. (2015). "Single-Cell XIST Expression in Human Preimplantation Embryos and Newly Reprogrammed Female Induced Pluripotent Stem Cells." Stem Cells **33**(6): 1771-1781.

The process of X chromosome inactivation (XCI) during reprogramming to produce human induced pluripotent stem cells (iPSCs), as well as during the extensive programming that occurs in human preimplantation development, is not well-understood. Indeed, studies of XCI during reprogramming to iPSCs report cells with two active X chromosomes and/or cells with one inactive X chromosome. Here, we examine expression of the long noncoding RNA, XIST, in single cells of human embryos through the oocyte-to-embryo transition and in new mRNA reprogrammed iPSCs. We show that XIST is first expressed beginning at the 4-cell stage, coincident with the onset of embryonic genome activation in an asynchronous manner. Additionally, we report that mRNA reprogramming produces iPSCs that initially express XIST transcript; however, expression is rapidly lost with culture. Loss of XIST and H3K27me3 enrichment at the inactive X chromosome at late passage results in X chromosome expression changes. Our data may contribute to applications in disease modeling and potential translational applications of female stem cells.

Bylund, J. B., et al. (2017). "Coordinated Proliferation and Differentiation of Human-Induced Pluripotent Stem Cell-Derived Cardiac Progenitor Cells Depend on Bone Morphogenetic Protein Signaling Regulation by GREMLIN 2." Stem Cells Dev **26**(9): 678-693.

Heart development depends on coordinated proliferation and differentiation of cardiac progenitor cells (CPCs), but how the two processes are synchronized is not well understood. Here, we show that the secreted Bone Morphogenetic Protein (BMP) antagonist GREMLIN 2 (GREM2) is induced in CPCs shortly after cardiac mesoderm specification during differentiation of human pluripotent stem cells. GREM2 expression follows cardiac lineage differentiation independently of the differentiation method used, or the origin of the pluripotent stem cells, suggesting that GREM2 is linked to cardiogenesis. Addition of GREM2 protein strongly increases cardiomyocyte output compared to established procardiogenic differentiation methods. Our data show that inhibition of canonical BMP signaling by GREM2 is necessary to promote proliferation of CPCs. However, canonical BMP signaling inhibition alone is not sufficient to induce cardiac differentiation, which depends on subsequent JNK pathway activation specifically by GREM2. These findings may have broader implications in the design of approaches to orchestrate growth and differentiation of pluripotent stem cell-derived lineages that depend on precise regulation of BMP signaling.

Cai, S., et al. (2018). "Human Induced Pluripotent Stem Cell-Derived Sensory Neurons for Fate Commitment of Bone Marrow Stromal Cell-Derived Schwann Cells." Methods Mol Biol **1739**: 149-160.

Here we describe the in vitro derivation of sensory neurons for use in effecting fate commitment of Schwann cell-like cells derived from human bone marrow stromal cells (hBMSCs). We adopt a novel combination of small molecules in an 8-day program that induces the differentiation of human induced pluripotent stem cells into sensory neurons. In co-cultures, the derived sensory neurons present contact-dependent cues to direct hBMSC-derived Schwann cell-like cells toward the Schwann cell fate. These derived human Schwann cells survive passaging and cryopreservation, retain marker expression despite withdrawal of glia-inducing medium and neuronal cues, demonstrate capacity for myelination, and therefore promise application in autologous transplantation and re-myelination therapy.

Carcamo-Orive, I., et al. (2017). "Induced Pluripotent Stem Cell-Derived Endothelial Cells in Insulin Resistance and Metabolic Syndrome." Arterioscler Thromb Vasc Biol **37**(11): 2038-2042.

Insulin resistance leads to a number of metabolic and cellular abnormalities including endothelial dysfunction that increase the risk of vascular disease. Although it has been particularly challenging to study the genetic determinants that predispose to abnormal function of the endothelium in insulin-resistant states, the possibility of deriving endothelial cells from induced pluripotent stem cells generated from individuals with detailed clinical phenotyping, including accurate measurements of insulin resistance accompanied by multilevel omic data (eg, genetic and genomic characterization), has opened new avenues to study this relationship. Unfortunately, several technical barriers have hampered these efforts. In the present review, we summarize the current status of induced pluripotent stem cell-derived endothelial cells for modeling endothelial dysfunction associated with insulin resistance and discuss the challenges to overcoming these limitations.

Carlessi, L., et al. (2013). "Brain and induced pluripotent stem cell-derived neural stem cells as an in vitro model of neurodegeneration in ataxia-telangiectasia." Exp Biol Med (Maywood) **238**(3): 301-307.

The ataxia telangiectasia mutated (ATM) kinase is a key transducer of the cellular response to DNA double strand breaks and its deficiency causes ataxia-telangiectasia (A-T), a pleiotropic genetic disorder primarily characterized by cerebellar neuropathy, immunodeficiency and cancer predisposition. While enormous progress has been achieved in elucidating the biochemical and functional regulation of ATM in DNA damage response, and more recently in redox signalling and antioxidant defence, the factors that make neurons in A-T extremely vulnerable remain unclear. Given also that ATM knockout mice do not recapitulate the central nervous system phenotype, a number of human neural stem cell (hNSC) model systems have been developed to provide insights into the mechanisms of neurodegeneration associated with ATM dysfunction. Here we review the hNSC systems developed by us an others to model A-T.

Carpenter, L., et al. (2011). "Human induced pluripotent stem cells are capable of B-cell lymphopoiesis." Blood **117**(15): 4008-4011.

Induced pluripotent stem (iPS) cells offer a unique potential for understanding the molecular basis of disease and development. Here we have generated several human iPS cell lines, and we describe their pluripotent phenotype and ability to differentiate into erythroid cells, monocytes, and endothelial cells. More significantly, however, when these iPS cells were differentiated under conditions that promote lympho-hematopoiesis from human embryonic stem cells, we observed the formation of pre-B cells. These cells were CD45(+)CD19(+)CD10(+) and were positive for transcripts Pax5, IL7alphaR, lambda-like, and VpreB receptor. Although they were negative for surface IgM and CD5 expression, iPS-derived CD45(+)CD19(+) cells also exhibited multiple genomic D-J (H) rearrangements, which supports a pre-B-cell identity. We therefore have been able to demonstrate, for the first time, that human iPS cells are able to undergo hematopoiesis that contributes to the B-cell lymphoid lineage.

Casaroli-Marano, R. P., et al. (2015). "Potential Role of Induced Pluripotent Stem Cells (IPSCs) for Cell-Based Therapy of the Ocular Surface." J Clin Med **4**(2): 318-342.

The integrity and normal function of the corneal epithelium are crucial for maintaining the cornea's transparency and vision. The existence of a cell population with progenitor characteristics in the limbus maintains a dynamic of constant epithelial repair and renewal. Currently, cell-based therapies for bio replacement-cultured limbal epithelial transplantation (CLET) and cultured oral mucosal epithelial transplantation (COMET)-present very encouraging clinical results for treating limbal stem cell deficiency (LSCD) and restoring vision. Another emerging therapeutic approach consists of obtaining and implementing human progenitor cells of different origins in association with tissue engineering methods. The development of cell-based therapies using stem cells, such as human adult mesenchymal or induced pluripotent stem cells (IPSCs), represent a significant breakthrough in the treatment of certain eye diseases, offering a more rational, less invasive, and better

physiological treatment option in regenerative medicine for the ocular surface. This review will focus on the main concepts of cell-based therapies for the ocular surface and the future use of IPSCs to treat LSCD.

Chan, Y. S., et al. (2013). "A PRC2-dependent repressive role of PRDM14 in human embryonic stem cells and induced pluripotent stem cell reprogramming." Stem Cells **31**(4): 682-692.

PRDM14 is an important determinant of the human embryonic stem cell (ESC) identity and works in concert with the core ESC regulators to activate pluripotency-associated genes. PRDM14 has been previously reported to exhibit repressive activity in mouse ESCs and primordial germ cells; and while PRDM14 has been implicated to suppress differentiation genes in human ESCs, the exact mechanism of this repressive activity remains unknown. In this study, we provide evidence that PRDM14 is a direct repressor of developmental genes in human ESCs. PRDM14 binds to silenced genes in human ESCs and its global binding profile is enriched for the repressive trimethylation of histone H3 lysine 27 (H3K27me3) modification. Further investigation reveals that PRDM14 interacts directly with the chromatin regulator polycomb repressive complex 2 (PRC2) and PRC2 binding is detected at PRDM14-bound loci in human ESCs. Depletion of PRDM14 reduces PRC2 binding at these loci and the concomitant reduction of H3K27me3 modification. Using reporter assays, we demonstrate that gene loci bound by PRDM14 exhibit repressive activity that is dependent on both PRDM14 and PRC2.

Chang, C. W., et al. (2014). "Broad T-cell receptor repertoire in T-lymphocytes derived from human induced pluripotent stem cells." PLoS One **9**(5): e97335.

Human induced pluripotent stem cells (hiPSCs) have enormous potential for the treatment of inherited and acquired disorders. Recently, antigen-specific T lymphocytes derived from hiPSCs have been reported. However, T lymphocyte populations with broad T cell receptor (TCR) diversity have not been generated. We report that hiPSCs derived from skin biopsy are capable of producing T lymphocyte populations with a broad TCR repertoire. In vitro T cell differentiation follows a similar developmental program as observed in vivo, indicated by sequential expression of CD7, intracellular CD3 and surface CD3. The gammadelta TCR locus is rearranged first and is followed by rearrangement of the alphabeta locus. Both gammadelta and alphabeta T cells display a diverse TCR repertoire. Upon activation, the cells express CD25, CD69, cytokines (TNF-alpha, IFN-gamma, IL-2) and cytolytic proteins (Perforin and Granzyme-B). These results suggest that most, if not all, mechanisms required to generate functional T cells with a broad TCR repertoire are intact in our in vitro differentiation protocol. These data provide a foundation for production of patient-specific T cells for the treatment of acquired or inherited immune disorders and for cancer immunotherapy.

Chao, H. M. and E. Chern (2018). "Patient-derived induced pluripotent stem cells for models of cancer and cancer stem cell research." J Formos Med Assoc.

Induced pluripotent stem cells (iPSCs) are embryonic stem cell-like cells reprogrammed from somatic cells by four transcription factors, OCT4, SOX2, KLF4 and c-MYC. iPSCs derived from cancer cells (cancer-iPSCs) could be a novel strategy for studying cancer. During cancer cell reprogramming, the epigenetic status of the cancer cell may be altered, such that it acquires stemness and pluripotency. The cellular behavior of the reprogrammed cells exhibits dynamic changes during the different stages of reprogramming. The cells may acquire the properties of cancer stem cells (CSCs) during the process of reprogramming, and lose their carcinogenic properties during reprogramming into a cancer-iPSCs. Differentiation of cancer-iPSCs by teratoma formation or organoid culturing could mimic the process of tumorigenesis. Some of the molecular mechanisms associated with cancer progression could be elucidated using the cancer-iPSC model. Furthermore, cancer-iPSCs could be expanded in culture system or bioreactors, and serve as cell sources for research, and as personal disease models for therapy and drug screening. This article introduces cancer studies that used the cell reprogramming strategy.

Chatterjee, I., et al. (2016). "Induced Pluripotent Stem (iPS) Cell Culture Methods and Induction of Differentiation into Endothelial Cells." Methods Mol Biol **1357**: 311-327.

The study of stem cell behavior and differentiation in a developmental context is complex, time-consuming, and expensive, and for this reason, cell culture remains a method of choice for developmental and regenerative biology and mechanistic studies. Similar to ES cells, iPS cells have the ability to differentiate into endothelial cells (ECs), and the route for differentiation appears to mimic the developmental process that occurs during the formation of an embryo. Traditional EC induction methods from embryonic stem (ES) cells rely mostly on the formation of embryoid body (EB), which employs feeder or feeder-free conditions in the presence or absence of supporting cells. Similar to ES

cells, iPS cells can be cultured in feeder layer or feeder-free conditions. Here, we describe the iPS cell culture methods and induction differentiation of these cells into ECs. We use anti-mouse Flk1 and anti-mouse VE-cadherin to isolate and characterize mouse ECs, because these antibodies are commercially available and their use has been described in the literature, including by our group. The ECs produced by this method have been used by our laboratory, and we have demonstrated their in vivo potential. We also discuss how iPS cells differ in their ability to differentiate into endothelial cells in culture.

Chen, C., et al. (2013). "Inhibition of neuronal nitric oxide synthase activity promotes migration of human-induced pluripotent stem cell-derived neural stem cells toward cancer cells." J Neurochem **126**(3): 318-330.

The breakthrough in derivation of human-induced pluripotent stem cells (hiPSCs) provides an approach that may help overcome ethical and allergenic challenges posed in numerous medical applications involving human cells, including neural stem/progenitor cells (NSCs). Considering the great potential of NSCs in targeted cancer gene therapy, we investigated in this study the tumor tropism of hiPSC-derived NSCs and attempted to enhance the tropism by manipulation of biological activities of proteins that are involved in regulating the migration of NSCs toward cancer cells. We first demonstrated that hiPSC-NSCs displayed tropism for both glioblastoma cells and breast cancer cells in vitro and in vivo. We then compared gene expression profiles between migratory and non-migratory hiPSC-NSCs toward these cancer cells and observed that the gene encoding neuronal nitric oxide synthase (nNOS) was down-regulated in migratory hiPSC-NSCs. Using nNOS inhibitors and nNOS siRNAs, we demonstrated that this protein is a relevant regulator in controlling migration of hiPSC-NSCs toward cancer cells, and that inhibition of its activity or down-regulation of its expression can sensitize poorly migratory NSCs and be used to improve their tumor tropism. These findings suggest a novel application of nNOS inhibitors in neural stem cell-mediated cancer therapy.

Chen, H. F., et al. (2011). "Surface marker epithelial cell adhesion molecule and E-cadherin facilitate the identification and selection of induced pluripotent stem cells." Stem Cell Rev **7**(3): 722-735.

The derivation of induced pluripotent stem cells (iPSCs) requires not only efficient reprogramming methods, but also reliable markers for identification and purification of iPSCs. Here, we demonstrate that surface markers, epithelial cells adhesion molecule (EpCAM) and epithelial cadherin (E-cadherin) can be used for efficient identification and/or isolation of reprogrammed mouse iPSCs. By viral transduction of Oct4, Sox2, Klf4 and n- or c-Myc into mouse embryonic fibroblasts, we observed that the conventional mouse embryonic stem cell (mESC) markers, alkaline phosphatase (AP) and stage-specific embryonic antigen 1 (SSEA1), were expressed in incompletely reprogrammed cells that did not express all the exogenous reprogramming factors or failed to acquire pluripotent status even though exogenous reprogramming factors were expressed. EpCAM and E-cadherin, however, remained inactivated in these cells. Expression of EpCAM and E-cadherin correlated with the activation of Nanog and endogenous Oct4, and was only seen in the successfully reprogrammed iPSCs. Furthermore, purification of EpCAM-expressing cells at late reprogramming stage by FACS enriched the Nanog-expressing cell population suggesting the feasibility of selecting successful reprogrammed mouse iPSCs by EpCAM expression. We have thus identified new surface markers that can efficiently identify successfully reprogrammed iPSCs and provide an effective means for iPSC isolation.

Chen, L., et al. (2014). "Mouse induced pluripotent stem cell microenvironment generates epithelial-mesenchymal transition in mouse Lewis lung cancer cells." Am J Cancer Res **4**(1): 80-88.

Induced pluripotent stem (iPS) cells may be a powerful tool in regenerative medicine, but their potential tumorigenicity is a significant challenge for the clinical use of iPS cells. Previously, we succeeded in converting miPS cells into cancer stem cells (CSCs) under the conditions of tumor microenvironment. Both stem cells and tumor cells are profoundly influenced by bi-directional communication with their respective microenvironment, which dictates cell fate determination and behavior. The microenvironment derived from iPS cells has not been well studied. In this paper, we have investigated the effects of secreted factors from Nanog-mouse iPS (miPS) cells on mouse Lewis lung cancer (LLC) cells that are found in the conditioned media. The results demonstrated that miPS cells secrete factors that can convert the epithelia phenotype of LLC cells to a mesenchymal phenotype, and that can promote tumorigenisity, migration and invasion. Furthermore, LLC cells that have been exposed to miPS conditioned medium became resistant to apoptosis. These various biological effects suggest that the miPS microenvironment contain factors that can promote an epithelial-mesenchymal transition (EMT) through an active Snail-MMP axis or by suppressing differentiation in LLC cells.

Chen, L. W., et al. (2011). "Potential application of induced pluripotent stem cells in cell replacement

therapy for Parkinson's disease." CNS Neurol Disord Drug Targets **10**(4): 449-458.

Parkinson's disease (PD), a common degenerative disease in humans, is known to result from loss of dopamine neurons in the substantia nigra and is characterized by severe motor symptoms of tremor, rigidity, bradykinsia and postural instability. Although levodopa administration, surgical neural lesion, and deep brain stimulation have been shown to be effective in improving parkinsonian symptoms, cell replacement therapy such as transplantation of dopamine neurons or neural stem cells has shed new light on an alternative treatment strategy for PD. While the difficulty in securing donor dopamine neurons and the immuno-rejection of neural transplants largely hinder application of neural transplants in clinical treatment, induced pluripotent stem cells (iPS cells) derived from somatic cells may represent a powerful tool for studying the pathogenesis of PD and provide a source for replacement therapies in this neurodegenerative disease. Yamanaka et al. [2006, 2007] first succeeded in generating iPS cells by reprogramming fibroblasts with four transcription factors, Oct4, Sox2, Klf4, and c-Myc in both mouse and human. Animal studies have further shown that iPS cells from fibroblasts could be induced into dopamine neurons and transplantation of these cells within the central nervous system improved motor symptoms in the 6-OHDA model of PD. More interestingly, neural stem cells or fibroblasts from patients can be efficiently reprogrammed and subsequently differentiated into dopamine neurons. Derivation of patient-specific iPS cells and subsequent differentiation into dopamine neurons would provide a disease-specific in vitro model for disease pathology, drug screening and personalized stem cell therapy for PD. This review summarizes current methods and modifications in producing iPS cells from somatic cells as well as safety concerns of reprogramming procedures. Novel reprogramming strategies that deter abnormal permanent genetic and epigenetic alterations are essential for propagating clinically-qualified iPS cells.

Chen, W., et al. (2018). "Angiogenic and osteogenic regeneration in rats via calcium phosphate scaffold and endothelial cell co-culture with human bone marrow mesenchymal stem cells (MSCs), human umbilical cord MSCs, human induced pluripotent stem cell-derived MSCs and human embryonic stem cell-derived MSCs." J Tissue Eng Regen Med **12**(1): 191-203.

Angiogenesis is a limiting factor in regenerating large bone defects. The objective of this study was to investigate angiogenic and osteogenic effects of co-culture on calcium phosphate cement (CPC) scaffold using human umbilical vein endothelial cells (hUVECs) and mesenchymal stem cells (MSCs) from different origins for the first time. hUVECs were co-cultured with four types of cell: human umbilical cord MSCs (hUCMSCs), human bone marrow MSCs (hBMSCs) and MSCs from induced pluripotent stem cells (hiPSC-MSCs) and embryonic stem cells (hESC-MSCs). Constructs were implanted in 8 mm cranial defects of rats for 12 weeks. CPC without cells served as control 1. CPC with hBMSCs served as control 2. Microcapillary-like structures were successfully formed on CPC in vitro in all four co-cultured groups. Microcapillary lengths increased with time ($p < 0.05$). Osteogenic and angiogenic gene expressions were highly elevated and mineralization by co-cultured cells increased with time ($p < 0.05$). New bone amount and blood vessel density of co-cultured groups were much greater than controls ($p < 0.05$) in an animal study. hUVECs co-cultured with hUCMSCs, hiPSC-MSCs and hESC-MSCs achieved new bone and vessel density similar to hUVECs co-cultured with hBMSCs ($p > 0.1$). Therefore, hUCMSCs, hiPSC-MSCs and hESC-MSCs could serve as alternative cell sources to hBMSCs, which require an invasive procedure to harvest. In conclusion, this study showed for the first time that co-cultures of hUVECs with hUCMSCs, hiPSC-MSCs, hESC-MSCs and hBMSCs delivered via CPC scaffold achieved excellent osteogenic and angiogenic capabilities in vivo. The novel co-culture constructs are promising for bone reconstruction with improved angiogenesis for craniofacial/orthopaedic applications. Copyright (c) 2017 John Wiley & Sons, Ltd.

Chen, W., et al. (2013). "Nonviral cell labeling and differentiation agent for induced pluripotent stem cells based on mesoporous silica nanoparticles." ACS Nano **7**(10): 8423-8440.

The generation of induced pluripotent stem cells (iPSCs) is an innovative personalized-regenerative technology, which can transform own-self somatic cells into embryonic stem (ES)-like cells, which have the potential to differentiate into all cell types of three dermal lineages. However, how to quickly, efficiently, and safely produce specific-lineage differentiation from pluripotent-state cells and iPSCs is still an open question. The objective of the present study was to develop a platform of a nonviral gene delivery system of mesoporous silica nanoparticles (MSNs) to rapidly generate iPSC-derived definitive-lineage cells, including endodermal-differentiated cells. We also evaluated the feasibility and efficiency of FITC-conjugated MSNs (FMSNs) for labeling of iPSCs and utilized the multifunctional properties of FMSNs for a suitable carrier for biomolecule delivery. We showed that FMSNs of various surface charges could be efficiently internalized by iPSCs without causing

cytotoxicity. The levels of reactive oxygen species and pluripotent status, including in vitro stemness signatures and in vivo teratoma formation, remained unaltered. Notably, positive-charged FMSN enhanced cellular uptake efficiency and retention time. Moreover, when using positive-charged FMSN to deliver hepatocyte nuclear factor 3beta (HNF3beta) plasmid DNA (pDNA), the treated iPSCs exhibited significantly improved definitive endoderm formation and further quickly differentiated into hepatocyte-like cells with mature functions (low-density lipoprotein uptake and glycogen storage) within 2 weeks in vitro. Double delivery of pHNF3beta further improved mRNA expression levels of liver-specific genes. These findings reveal the multiple advantages of FMSNs to serve as ideal vectors not only for stem cell labeling but also for safe gene delivery to promote the production of hepatocyte-like cells from iPSCs.

Chen, Y. C., et al. (2018). "ATOH1/RFX1/RFX3 transcription factors facilitate the differentiation and characterisation of inner ear hair cell-like cells from patient-specific induced pluripotent stem cells harbouring A8344G mutation of mitochondrial DNA." Cell Death Dis **9**(4): 437.

Degeneration or loss of inner ear hair cells (HCs) is irreversible and results in sensorineural hearing loss (SHL). Human-induced pluripotent stem cells (hiPSCs) have been employed in disease modelling and cell therapy. Here, we propose a transcription factor (TF)-driven approach using ATOH1 and regulatory factor of x-box (RFX) genes to generate HC-like cells from hiPSCs. Our results suggest that ATOH1/RFX1/RFX3 could significantly increase the differentiation capacity of iPSCs into MYO7A (mCherry)-positive cells, upregulate the mRNA expression levels of HC-related genes and promote the differentiation of HCs with more mature stereociliary bundles. To model the molecular and stereociliary structural changes involved in HC dysfunction in SHL, we further used ATOH1/RFX1/RFX3 to differentiate HC-like cells from the iPSCs from patients with myoclonus epilepsy associated with ragged-red fibres (MERRF) syndrome, which is caused by A8344G mutation of mitochondrial DNA (mtDNA), and characterised by myoclonus epilepsy, ataxia and SHL. Compared with isogenic iPSCs, MERRF-iPSCs possessed ~42-44% mtDNA with A8344G mutation and exhibited significantly elevated reactive oxygen species (ROS) production and CAT gene expression. Furthermore, MERRF-iPSC-differentiated HC-like cells exhibited significantly elevated ROS levels and MnSOD and CAT gene expression. These MERRF-HCs that had more single cilia with a shorter length could be observed only by using a non-TF method, but those with fewer stereociliary bundle-like protrusions than isogenic iPSCs-differentiated-HC-like cells could be further observed using ATOH1/RFX1/RFX3 TFs. We further analysed and compared the whole transcriptome of M1(ctrl)-HCs and M1-HCs after treatment with ATOH1 or ATOH1/RFX1/RFX3. We revealed that the HC-related gene transcripts in M1(ctrl)-iPSCs had a significantly higher tendency to be activated by ATOH1/RFX1/RFX3 than M1-iPSCs. The ATOH1/RFX1/RFX3 TF-driven approach for the differentiation of HC-like cells from iPSCs is an efficient and promising strategy for the disease modelling of SHL and can be employed in future therapeutic strategies to treat SHL patients.

Chichagova, V., et al. (2016). "Generation of Human Induced Pluripotent Stem Cells Using RNA-Based Sendai Virus System and Pluripotency Validation of the Resulting Cell Population." Methods Mol Biol **1353**: 285-307.

Human induced pluripotent stem cells (hiPSCs) provide a platform for studying human disease in vitro, increase our understanding of human embryonic development, and provide clinically relevant cell types for transplantation, drug testing, and toxicology studies. Since their discovery, numerous advances have been made in order to eliminate issues such as vector integration into the host genome, low reprogramming efficiency, incomplete reprogramming and acquisition of genomic instabilities. One of the ways to achieve integration-free reprogramming is by using RNA-based Sendai virus. Here we describe a method to generate hiPSCs with Sendai virus in both feeder-free and feeder-dependent culture systems. Additionally, we illustrate methods by which to validate pluripotency of the resulting stem cell population.

Chiou, S. S., et al. (2013). "Control of Oxidative Stress and Generation of Induced Pluripotent Stem Cell-like Cells by Jun Dimerization Protein 2." Cancers (Basel) **5**(3): 959-984.

We report here that the Jun dimerization protein 2 (JDP2) plays a critical role as a cofactor for the transcription factors nuclear factor-erythroid 2-related factor 2 (Nrf2) and MafK in the regulation of the antioxidants and production of reactive oxygen species (ROS). JDP2 associates with Nrf2 and MafK (Nrf2-MafK) to increase the transcription of antioxidant response element-dependent genes. Oxidative-stress-inducing reagent led to an increase in the intracellular accumulation of ROS and cell proliferation in Jdp2 knock-out mouse embryonic fibroblasts. In Jdp2-Cre mice mated with reporter mice, the expression of JDP2 was restricted to granule cells in the brain cerebellum. The induced pluripotent stem cells (iPSC)-like cells were generated from DAOY medulloblastoma cell by

introduction of JDP2, and the defined factor OCT4. iPSC-like cells expressed stem cell-like characteristics including alkaline phosphatase activity and some stem cell markers. However, such iPSC-like cells also proliferated rapidly, became neoplastic, and potentiated cell malignancy at a later stage in SCID mice. This study suggests that medulloblastoma cells can be reprogrammed successfully by JDP2 and OCT4 to become iPSC-like cells. These cells will be helpful for studying the generation of cancer stem cells and ROS homeostasis.

Choi, H. Y., et al. (2018). "Generation of a human induced pluripotent stem cell line, KSCBi003-A, from human adipose tissue-derived mesenchymal stem cells using a chromosomal integration-free system." Stem Cell Res **31**: 1-4.

We generated a human induced pluripotent stem cell (hiPSC) line, KSCBi003-A, from adipose tissue-derived mesenchymal stem cells (Ad-MSCs) using a Sendai virus-based gene delivery system. We confirmed that the KSCBi003-A has a normal karyotype and short tandem repeat (STR)-based identities that match the parent cells. We also confirmed that the cell line expresses pluripotent stem cell markers such as Nanog, OCT4, SSEA-4, TRA-1-60, and TRA-1-81. We also analyzed that the KSCBi003-A has an ability to differentiate three germ layers (ectoderm, mesoderm, endoderm). This cell line is registered and available at the National Stem Cell Bank, Korea National Institute of Health.

Choi, S. M., et al. (2011). "Reprogramming of EBV-immortalized B-lymphocyte cell lines into induced pluripotent stem cells." Blood **118**(7): 1801-1805.

EBV-immortalized B lymphocyte cell lines have been widely banked for studying a variety of diseases, including rare genetic disorders. These cell lines represent an important resource for disease modeling with the induced pluripotent stem cell (iPSC) technology. Here we report the generation of iPSCs from EBV-immortalized B-cell lines derived from multiple inherited disease patients via a nonviral method. The reprogramming method for the EBV cell lines involves a distinct protocol compared with that of patient fibroblasts. The B-cell line-derived iPSCs expressed pluripotency markers, retained the inherited mutation and the parental V (D)J rearrangement profile, and differentiated into all 3 germ layer cell types. There was no integration of the reprogramming-related transgenes or the EBV-associated genes in these iPSCs. The ability to reprogram the widely banked patient B-cell lines will offer an unprecedented opportunity to generate human disease models and provide novel drug therapies.

Chow, L., et al. (2017). "Safety and immune regulatory properties of canine induced pluripotent stem cell-derived mesenchymal stem cells." Stem Cell Res **25**: 221-232.

Mesenchymal stem cells (MSCs) exhibit broad immune modulatory activity in vivo and can suppress T cell proliferation and dendritic cell activation in vitro. Currently, most MSC for clinical usage are derived from younger donors, due to ease of procurement and to the superior immune modulatory activity. However, the use of MSC from multiple unrelated donors makes it difficult to standardize study results and compare outcomes between different clinical trials. One solution is the use of MSC derived from induced pluripotent stem cells (iPSC); as iPSC-derived MSC have nearly unlimited proliferative potential and exhibit in vitro phenotypic stability. Given the value of dogs as a spontaneous disease model for pre-clinical evaluation of stem cell therapeutics, we investigated the functional properties of canine iPSC-derived MSC (iMSC), including immune modulatory properties and potential for teratoma formation. We found that canine iMSC downregulated expression of pluripotency genes and appeared morphologically similar to conventional MSC. Importantly, iMSC retained a stable phenotype after multiple passages, did not form teratomas in immune deficient mice, and did not induce tumor formation in dogs following systemic injection. We concluded therefore that iMSC were phenotypically stable, immunologically potent, safe with respect to tumor formation, and represented an important new source of cells for therapeutic modulation of inflammatory disorders.

Chuang, C. Y., et al. (2015). "Granulosa cell-derived induced pluripotent stem cells exhibit pro-trophoblastic differentiation potential." Stem Cell Res Ther **6**: 14.

INTRODUCTION: Human induced pluripotent stem cells (hiPSCs) have been derived from various somatic cell types. Granulosa cells, a group of cells which surround oocytes and are obtained from the (normally discarded) retrieved egg follicles of women undergoing infertility treatment, are a possible cell source for induced pluripotent stem cell (iPSC) generation. Here, we explored the possibility of using human granulosa cells as a donor cell type for iPSC reprogramming, and compared granulosa cell-derived iPSCs (iGRAs) with those derived from other cell sources, to determine the potential ability of iGRA differentiation. METHODS: Granulosa cells were collected from egg follicles retrieved from women undergoing infertility treatment. After short-term culture, the granulosa cells derived from different

patients were mixed in culture, and infected with retroviruses encoding reprogramming factors. The resulting iPSC clones were selected and subjected to microsatellite DNA analysis to determine their parental origin. IGRAs were subjected to RT-PCR, immunofluorescence staining, and in vitro and in vivo differentiation assays to further establish their pluripotent characteristics. RESULTS: Microsatellite DNA analysis was used to demonstrate that hiPSCs with different parental origins can be simultaneously reprogrammed by retroviral transfection of a mixed human granulosa cell population obtained from multiple individuals. The iGRAs resemble human embryonic stem cells (hESCs) in many respects, including morphological traits, growth requirements, gene and marker expression profiles, and in vitro and in vivo developmental propensities. We also demonstrate that the iGRAs express low levels of NLRP2, and differentiating iGRAs possess a biased differentiation potential toward the trophoblastic lineage. Although NLRP2 knockdown in hESCs promotes trophoblastic differentiation of differentiating hESCs, it does not result in exit from pluripotency. These results imply that NLRP2 may play a role in regulating the trophoblastic differentiation of human pluripotent stem cells. CONCLUSIONS: These findings provide a means of generating iPSCs from multiple granulosa cell populations with different parental origins. The ability to generate iPSCs from granulosa cells not only enables modeling of infertility-associated disease, but also provides a means of identifying potential clinical interventions through iPSC-based drug screening.

Chung, H. C., et al. (2012). "Human induced pluripotent stem cells derived under feeder-free conditions display unique cell cycle and DNA replication gene profiles." Stem Cells Dev **21**(2): 206-216.

Use of animal feeder layers and serum containing media in the derivation and propagation of induced pluripotent stem cells (iPSCs) can hinder clinical translation, because of the presence of xeno-material/pathogens. A defined and standardized system would be ideal for generating a homogenous population of iPSCs, which closely resembles human embryonic stem cells (hESCs). This article presents a novel and extensive comparison between in-house produced iPSCs and hESCs under "feeder" and "feeder-free" conditions, using transcriptomic genome-wide microarray analysis. We generated a list of pluripotency-associated and bivalent domain-containing genes by meta-analysis to measure qualitatively the degree of reprogramming in feeder-free derived iPSCs, in which both profiles displayed similar levels of gene expression as in hESCs. Gene ontology analysis showed that feeder-free iPSCs have enriched terms belonging to DNA repair/replication and cell cycle, which are signature to pluripotent cells. Transcriptomic data combined with directed differentiation assays, indicated that variability among iPSC lines is minimized when using a feeder-free cultural system, which may serve as a platform for further developing regenerative medicine compliant human iPSCs.

Cieslar-Pobuda, A., et al. (2016). "Human induced pluripotent stem cell differentiation and direct transdifferentiation into corneal epithelial-like cells." Oncotarget **7**(27): 42314-42329.

The corneal epithelium is maintained by a small pool of tissue stem cells located at the limbus. Through certain injuries or diseases this pool of stem cells may get depleted. This leads to visual impairment. Standard treatment options include autologous or allogeneic limbal stem cell (LSC) transplantation, however graft rejection and chronic inflammation lowers the success rate over long time. Induced pluripotent stem (iPS) cells have opened new possibilities for treating various diseases with patient specific cells, eliminating the risk of immune rejection. In recent years, several protocols have been developed, aimed at the differentiation of iPS cells into the corneal epithelial lineage by mimicking the environmental niche of limbal stem cells. However, the risk of teratoma formation associated with the use of iPS cells hinders most applications from lab into clinics. Here we show that the differentiation of iPS cells into corneal epithelial cells results in the expression of corneal epithelial markers showing a successful differentiation, but the process is long and the level of gene expression for the pluripotency markers does not vanish completely. Therefore we set out to determine a direct transdifferentiation approach to circumvent the intermediate state of pluripotency (iPS-stage). The resulting cells, obtained by direct transdifferentiation of fibroblasts into limbal cells, exhibited corneal epithelial cell morphology and expressed corneal epithelial markers. Hence we shows for the first time a direct transdifferentiation of human dermal fibroblasts into the corneal epithelial lineage that may serve as source for corneal epithelial cells for transplantation approaches.

Citro, L., et al. (2014). "Comparison of human induced pluripotent stem-cell derived cardiomyocytes with human mesenchymal stem cells following acute myocardial infarction." PLoS One **9**(12): e116281.

INTRODUCTION: Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have recently been shown to express key cardiac proteins and improve in vivo cardiac function when

administered following myocardial infarction. However, the efficacy of hiPSC-derived cell therapies, in direct comparison to current, well-established stem cell-based therapies, is yet to be elucidated. The goal of the current study was to compare the therapeutic efficacy of human mesenchymal stem cells (hMSCs) with hiPSC-CMs in mitigating myocardial infarction (MI). METHODS: Male athymic nude hyrats were subjected to permanent ligation of the left-anterior-descending (LAD) coronary artery to induce acute MI. Four experimental groups were studied: 1) control (non-MI), 2) MI, 3) hMSCs (MI+MSC), and 4) hiPSC-CMs (MI+hiPSC-derived cardiomyocytes). The hiPSC-CMs and hMSCs were labeled with superparamagnetic iron oxide (SPIO) in vitro to track the transplanted cells in the ischemic heart by high-field cardiac MRI. These cells were injected into the ischemic heart 30-min after LAD ligation. Four-weeks after MI, cardiac MRI was performed to track the transplanted cells in the infarct heart. Additionally, echocardiography (M-mode) was performed to evaluate the cardiac function. Immunohistological and western blot studies were performed to assess the cell tracking, engraftment and cardiac fibrosis in the infarct heart tissues. RESULTS: Echocardiography data showed a significantly improved cardiac function in the hiPSC-CMs and hMSCs groups, when compared to MI. Immunohistological studies showed expression of connexin-43, alpha-actinin and myosin heavy chain in engrafted hiPSC-CMs. Cardiac fibrosis was significantly decreased in hiPSC-CMs group when compared to hMSCs or MI groups. Overall, this study demonstrated improved cardiac function with decreased fibrosis with both hiPSC-CMs and hMSCs groups when compared with MI group.

Clayton, Z. E., et al. (2015). "Generating induced pluripotent stem cell derived endothelial cells and induced endothelial cells for cardiovascular disease modelling and therapeutic angiogenesis." Int J Cardiol **197**: 116-122.

Standard therapy for atherosclerotic coronary and peripheral arterial disease is insufficient in a significant number of patients because extensive disease often precludes effective revascularization. Stem cell therapy holds promise as a supplementary treatment for these patients, as pre-clinical and clinical research has shown transplanted cells can promote angiogenesis via direct and paracrine mechanisms. Induced pluripotent stem cells (iPSCs) are a novel cell type obtained by reprogramming somatic cells using exogenous transcription factor cocktails, which have been introduced to somatic cells via viral or plasmid constructs, modified mRNA or small molecules. IPSCs are now being used in disease modelling and drug testing and are undergoing their first clinical trial, but despite recent advances, the inefficiency of the reprogramming process remains a major limitation, as does the lack of consensus regarding the optimum transcription factor combination and delivery method and the uncertainty surrounding the genetic and epigenetic stability of iPSCs. IPSCs have been successfully differentiated into vascular endothelial cells (iPSC-ECs) and, more recently, induced endothelial cells (iECs) have also been generated by direct differentiation, which bypasses the pluripotent intermediate. IPSC-ECs and iECs demonstrate endothelial functionality in vitro and have been shown to promote neovessel growth and enhance blood flow recovery in animal models of myocardial infarction and peripheral arterial disease. Challenges remain in optimising the efficiency, safety and fidelity of the reprogramming and endothelial differentiation processes and establishing protocols for large-scale production of clinical-grade, patient-derived cells.

Clayton, Z. E., et al. (2018). "Induced pluripotent stem cell-derived endothelial cells promote angiogenesis and accelerate wound closure in a murine excisional wound healing model." Biosci Rep **38**(4).

Chronic wounds are a major complication in patients with cardiovascular diseases. Cell therapies have shown potential to stimulate wound healing, but clinical trials using adult stem cells have been tempered by limited numbers of cells and invasive procurement procedures. Induced pluripotent stem cells (iPSCs) have several advantages of other cell types, for example they can be generated in abundance from patients' somatic cells (autologous) or those from a matched donor. iPSCs can be efficiently differentiated to functional endothelial cells (iPSC-ECs). Here, we used a murine excisional wound model to test the pro-angiogenic properties of iPSC-ECs in wound healing. Two full-thickness wounds were made on the dorsum of NOD-SCID mice and splinted. iPSC-ECs (5 x 10(5)) were topically applied to one wound, with the other serving as a control. Treatment with iPSC-ECs significantly increased wound perfusion and accelerated wound closure. Expression of endothelial cell (EC) surface marker, platelet endothelial cell adhesion molecule (PECAM-1) (CD31), and pro-angiogenic EC receptor, Tie1, mRNA was up-regulated in iPSC-EC treated wounds at 7 days post-wounding. Histological analysis of wound sections showed increased capillary density in iPSC-EC wounds at days 7 and 14 post-wounding, and increased collagen content at day 14. Anti-GFP fluorescence confirmed presence of iPSC-ECs in the wounds. Bioluminescent imaging (BLI) showed progressive decline of iPSC-ECs over time, suggesting that iPSC-ECs are acting primarily through short-term paracrine effects. These results highlight the pro-regenerative

effects of iPSC-ECs and demonstrate that they are a promising potential therapy for intractable wounds.

Clayton, Z. E., et al. (2017). "A comparison of the pro-angiogenic potential of human induced pluripotent stem cell derived endothelial cells and induced endothelial cells in a murine model of peripheral arterial disease." Int J Cardiol **234**: 81-89.

BACKGROUND: Endothelial cells derived from human induced pluripotent stem cells (iPSC-ECs) promote angiogenesis, and more recently induced endothelial cells (iECs) have been generated via fibroblast trans-differentiation. These cell types have potential as treatments for peripheral arterial disease (PAD). However, it is unknown whether different reprogramming methods produce cells that are equivalent in terms of their pro-angiogenic capabilities. OBJECTIVES: We aimed to directly compare iPSC-ECs and iECs in an animal model of PAD, in order to identify which cell type, if any, displays superior therapeutic potential. METHODS: IPSC-ECs and iECs were generated from human fibroblasts, and transduced with a reporter construct encoding GFP and firefly luciferase for bioluminescence imaging (BLI). Endothelial phenotype was confirmed using in vitro assays. NOD-SCID mice underwent hindlimb ischaemia surgery and received an intramuscular injection of either 1x10(6) iPSC-ECs, 1x10(6) iECs or control vehicle only. Perfusion recovery was measured by laser Doppler. Hindlimb muscle samples were taken for histological analyses. RESULTS: Perfusion recovery was enhanced in iPSC-EC treated mice on day 14 (Control vs. iPSC-EC; 0.35+/-0.04 vs. 0.54+/-0.08, $p<0.05$) and in iEC treated mice on days 7 (Control vs. iEC; 0.23+/-0.02 vs. 0.44+/-0.06, $p<0.05$), 10 (0.31+/-0.04 vs. 0.64+/-0.07, $p<0.001$) and 14 (0.35+/-0.04 vs. 0.68+/-0.07, $p<0.001$) post-treatment. IEC-treated mice also had greater capillary density in the ischaemic gastrocnemius muscle (Control vs. iEC; 125+/-10 vs. 179+/-11 capillaries/image; $p<0.05$). BLI detected iPSC-EC and iEC presence in vivo for two weeks post-treatment. CONCLUSIONS: IPSC-ECs and iECs exhibit similar, but not identical, endothelial functionality and both cell types enhance perfusion recovery after hindlimb ischaemia.

Collado, M. S., et al. (2017). "Exposure of Induced Pluripotent Stem Cell-Derived Vascular Endothelial and Smooth Muscle Cells in Coculture to Hemodynamics Induces Primary Vascular Cell-Like Phenotypes." Stem Cells Transl Med **6**(8): 1673-1683.

Human induced pluripotent stem cells (iPSCs) can be differentiated into vascular endothelial (iEC) and smooth muscle (iSMC) cells. However, because iECs and iSMCs are not derived from an intact blood vessel, they represent an immature phenotype. Hemodynamics and heterotypic cell:cell communication play important roles in vascular cell phenotypic modulation. Here we tested the hypothesis that hemodynamic exposure of iECs in coculture with iSMCs induces an in vivo-like phenotype. iECs and iSMCs were cocultured under vascular region-specific blood flow hemodynamics, and compared to hemodynamic cocultures of blood vessel-derived endothelial (pEC) and smooth muscle (pSMC) cells. Hemodynamic flow-induced gene expression positively correlated between pECs and iECs as well as pSMCs and iSMCs. While endothelial nitric oxide synthase 3 protein was lower in iECs than pECs, iECs were functionally mature as seen by acetylated-low-density lipoprotein (LDL) uptake. SMC contractile protein markers were also positively correlated between pSMCs and iSMCs. Exposure of iECs and pECs to atheroprone hemodynamics with oxidized-LDL induced an inflammatory response in both. Dysfunction of the transforming growth factor beta (TGFbeta) pathway is seen in several vascular diseases, and iECs and iSMCs exhibited a transcriptomic prolife similar to pECs and pSMCs, respectively, in their responses to LY2109761-mediated transforming growth factor beta receptor I/II (TGFbetaRI/II) inhibition. Although there are differences between ECs and SMCs derived from iPSCs versus blood vessels, hemodynamic coculture restores a high degree of similarity in their responses to pathological stimuli associated with vascular diseases. Thus, iPSC-derived vascular cells exposed to hemodynamics may provide a viable system for modeling rare vascular diseases and testing new therapeutic approaches. Stem Cells Translational Medicine 2017;6:1673-1683.

Critchley, C. R., et al. (2013). "The impact of commercialisation on public perceptions of stem cell research: exploring differences across the use of induced pluripotent cells, human and animal embryos." Stem Cell Rev **9**(5): 541-554.

The development of pluripotent cells that enable stem cell research (SCR) without destroying human embryos is now a leading priority for science. Public and political controversies associated with human embryonic SCR experienced in the recent past should be alleviated if scientists no longer need to harvest cells from human embryos. This research suggests however additional issues needing attention in order to gain the public's trust and support: the use of mouse embryos and the commercialisation of research. Using a representative sample of 2,800 Australians, and an experimental telephone survey design, this research compared levels and predictors of public support for stem cell research across three cell source conditions: human embryo (HE), mouse embryo (ME) and induced pluripotent cells (iPSCs). The results revealed

that the public were significantly more likely to support research using iPSCs than HE and ME cells and public compared to private research (regardless of the cell source). There was no significant difference in support for HE compared to ME research, but the former was viewed as more likely to lead to accessible health care benefits and to be associated with more trustworthy scientists. The results of a multimediation structural equation model showed that the primary reason support for SCR significantly dropped in a private compared to public context (i.e., the commercialisation effect) was because public scientists were trusted more than private scientists. This effect was consistent across all three SCR materials, suggesting that the use of mouse embryos or even iPSCs will not reduce the publics' concern with commercialised science. The implications these results have for public acceptance of stem cell and animal research are discussed in relation to possible solutions such as increasing public awareness of the regulation of animal research and benefit sharing.

Csobonyeiova, M., et al. (2017). "Induced pluripotent stem cells in modeling and cell-based therapy of amyotrophic lateral sclerosis." J Physiol Pharmacol **68**(5): 649-657.

Amyotrophic lateral sclerosis (ALS) is a fatal neurological disease characterized by neuromuscular degeneration and the progressive loss of upper and lower motor neurons (MNs), causing weakness and paralysis. However, the underlying mechanisms of this disease are still unknown and there is no cure, or even treatment to stop or reverse its pathology. Consequently, most ALS patients die within 3 - 5 years after disease onset. While considerable progress has been made in studying animal models of ALS, they lack clinical suitability due to genetic differences. However, the recent development of induced pluripotent stem cells (iPSCs) has made it possible to study human disease-specific neuronal and glial cells to identify disease mechanisms and develop phenotypic screens for drug discovery. iPSCs provide researchers with a model of naturally occurring pathology under the human genetic background and MNs differentiated from human iPSCs bearing ALS-associated mutations offer a powerful model to study disease pathology. This paper reviews recent methods of differentiating iPSCs into neuronal cells and suggests further applications of these iPSCs-derived cells for ALS disease modeling, drug screening, and possible cell-based therapy.

Czaplewski, S. K., et al. (2014). "Tenogenic differentiation of human induced pluripotent stem cell-derived mesenchymal stem cells dictated by properties of braided submicron fibrous scaffolds." Biomaterials **35**(25): 6907-6917.

Tendon and ligament (T/L) engineering is a growing area of research with potential to address the inadequacies of current T/L defect treatments. Our group previously developed braided submicron fibrous scaffolds (BSMFSs) and demonstrated the viability of BSMFSs for T/L tissue engineering. The objective of this study was to investigate the effect of fiber chemistry and braiding angle on BSMFS mechanical properties and in turn, tenogenic differentiation of human induced pluripotent stem cell-derived mesenchymal stem cells (hiPSC-MSCs) seeded on BSMFSs subjected to cyclic tensile stimulation in the absence of tenogenic medium. By varying fiber chemistry and/or braiding angle, BSMFSs with a range of mechanical properties were produced. We found that fiber chemistry dictated cell adhesion while braiding angle dictated the tissue-specific lineage commitment of hiPSC-MSCs. Scaffolds braided with large angles better supported hiPSC-MSC tenogenic differentiation as evidenced by the production of T/L-associated markers, downregulation of osteogenic markers, and expression of fibroblast-like, spindle cell morphology compared to scaffolds braided with small angles. Our results demonstrate the importance of substrate properties and mechanical stimulation on tenogenic differentiation. These results also demonstrate the versatility of BSMFSs and the potential of hiPSC-MSCs for T/L tissue engineering.

D'Aiuto, L., et al. (2012). "Human induced pluripotent stem cell-derived models to investigate human cytomegalovirus infection in neural cells." PLoS One **7**(11): e49700.

Human cytomegalovirus (HCMV) infection is one of the leading prenatal causes of congenital mental retardation and deformities world-wide. Access to cultured human neuronal lineages, necessary to understand the species specific pathogenic effects of HCMV, has been limited by difficulties in sustaining primary human neuronal cultures. Human induced pluripotent stem (iPS) cells now provide an opportunity for such research. We derived iPS cells from human adult fibroblasts and induced neural lineages to investigate their susceptibility to infection with HCMV strain Ad169. Analysis of iPS cells, iPS-derived neural stem cells (NSCs), neural progenitor cells (NPCs) and neurons suggests that (i) iPS cells are not permissive to HCMV infection, i.e., they do not permit a full viral replication cycle; (ii) Neural stem cells have impaired differentiation when infected by HCMV; (iii) NPCs are fully permissive for HCMV infection; altered expression of genes related to neural metabolism or neuronal differentiation is also observed; (iv) most iPS-derived neurons are not

permissive to HCMV infection; and (v) infected neurons have impaired calcium influx in response to glutamate.

Dannenmann, B., et al. (2015). "High glutathione and glutathione peroxidase-2 levels mediate cell-type-specific DNA damage protection in human induced pluripotent stem cells." Stem Cell Reports **4**(5): 886-898.

Pluripotent stem cells must strictly maintain genomic integrity to prevent transmission of mutations. In human induced pluripotent stem cells (iPSCs), we found that genome surveillance is achieved via two ways, namely, a hypersensitivity to apoptosis and a very low accumulation of DNA lesions. The low apoptosis threshold was mediated by constitutive p53 expression and a marked upregulation of proapoptotic p53 target genes of the BCL-2 family, ensuring the efficient iPSC removal upon genotoxic insults. Intriguingly, despite the elevated apoptosis sensitivity, both mitochondrial and nuclear DNA lesions induced by genotoxins were less frequent in iPSCs compared to fibroblasts. Gene profiling identified that mRNA expression of several antioxidant proteins was considerably upregulated in iPSCs. Knockdown of glutathione peroxidase-2 and depletion of glutathione impaired protection against DNA lesions. Thus, iPSCs ensure genomic integrity through enhanced apoptosis induction and increased antioxidant defense, contributing to protection against DNA damage.

Das, A. K. and R. Pal (2010). "Induced pluripotent stem cells (iPSCs): the emergence of a new champion in stem cell technology-driven biomedical applications." J Tissue Eng Regen Med **4**(6): 413-421.

Pluripotent stem cells possess the unique property of differentiating into all other cell types of the human body. Further, the discovery of induced pluripotent stem cells (iPSCs) in 2006 has opened up new avenues in clinical medicine. In simple language, iPSCs are nothing but somatic cells reprogrammed genetically to exhibit pluripotent characteristics. This process utilizes retroviruses/lentiviruses/adenovirus/plasmids to incorporate candidate genes into somatic cells isolated from any part of the human body. It is also possible to develop disease-specific iPSCs which are most likely to revolutionize research in respect to the pathophysiology of most debilitating diseases, as these can be mimicked ex vivo in the laboratory. These models can also be used to study the safety and efficacy of known drugs or potential drug candidates for a particular diseased condition, limiting the need for animal studies and considerably reducing the time and money required to develop new drugs. Recently, functional neurons, cardiomyocytes, pancreatic islet cells, hepatocytes and retinal cells have been derived from human iPSCs, thus re-confirming the pluripotency and differentiation capacity of these cells. These findings further open up the possibility of using iPSCs in cell replacement therapy for various degenerative disorders. In this review we highlight the development of iPSCs by different methods, their biological characteristics and their prospective applications in regenerative medicine and drug screening. We further discuss some practical limitations pertaining to this technology and how they can be averted for the betterment of human life.

Dash, B. C., et al. (2015). "Induced pluripotent stem cell-derived vascular smooth muscle cells: methods and application." Biochem J **465**(2): 185-194.

Vascular smooth muscle cells (VSMCs) play a major role in the pathophysiology of cardiovascular diseases. The advent of induced pluripotent stem cell (iPSC) technology and the capability of differentiating into virtually every cell type in the human body make this field a ray of hope for vascular regenerative therapy and understanding of the disease mechanism. In the present review, we first discuss the recent iPSC technology and vascular smooth muscle development from an embryo and then examine different methodologies to derive VSMCs from iPSCs, and their applications in regenerative therapy and disease modelling.

de Lazaro, I., et al. (2014). "Induced pluripotent stem (iPS) cells: a new source for cell-based therapeutics?" J Control Release **185**: 37-44.

The generation of induced pluripotent stem (iPS) cells from somatic cells by the ectopic expression of defined transcription factors has provided the regenerative medicine field with a new tool for cell replacement strategies. The advantages that these pluripotent cells can offer in comparison to other sources of stem cells include the generation of patient-derived cells and the lack of embryonic tissue while maintaining a versatile differentiation potential. The promise of iPS cell derivatives for therapeutic applications is encouraging albeit very early in development, with the first clinical study currently ongoing in Japan. Many challenges are yet to be circumvented before this technology can be clinically translated widely though. The delivery and expression of the reprogramming factors, the genomic instability, epigenetic memory and impact of cell propagation in culture are only some of the concerns. This article aims to critically discuss the potential of iPS cells as a new source of cell therapeutics.

de Peppo, G. M. and D. Marolt (2012). "State of the art in stem cell research: human embryonic stem

cells, induced pluripotent stem cells, and transdifferentiation." J Blood Transfus **2012**: 317632.

Stem cells divide by asymmetric division and display different degrees of potency, or ability to differentiate into various specialized cell types. Owing to their unique regenerative capacity, stem cells have generated great enthusiasm worldwide and represent an invaluable tool with unprecedented potential for biomedical research and therapeutic applications. Stem cells play a central role in the understanding of molecular mechanisms regulating tissue development and regeneration in normal and pathological conditions and open large possibilities for the discovery of innovative pharmaceuticals to treat the most devastating diseases of our time. Not least, their intrinsic characteristics allow the engineering of functional tissues for replacement therapies that promise to revolutionize the medical practice in the near future. In this paper, the authors present the characteristics of pluripotent stem cells and new developments of transdifferentiation technologies and explore some of the biomedical applications that this emerging technology is expected to empower.

Deng, J., et al. (2018). "Cell Transplantation for Spinal Cord Injury: Tumorigenicity of Induced Pluripotent Stem Cell-Derived Neural Stem/Progenitor Cells." Stem Cells Int **2018**: 5653787.

Spinal cord injury (SCI) is an intractable and worldwide difficult medical challenge with limited treatments. Neural stem/progenitor cell (NS/PC) transplantation derived from fetal tissues or embryonic stem cells (ESCs) has demonstrated therapeutic effects via replacement of lost neurons and severed axons and creation of permissive microenvironment to promote repair of spinal cord and axon regeneration but causes ethnical concerns and immunological rejections as well. Thus, the implementation of induced pluripotent stem cells (iPSCs), which can be generated from adult somatic cells and differentiated into NS/PCs, provides an effective alternation in the treatment of SCI. However, as researches further deepen, there is accumulating evidence that the use of iPSC-derived NS/PCs shows mounting concerns of safety, especially the tumorigenicity. This review discusses the tumorigenicity of iPSC-derived NS/PCs focusing on the two different routes of tumorigenicity (teratomas and true tumors) and underlying mechanisms behind them, as well as possible solutions to circumvent them.

Deshmukh, R. S., et al. (2012). "Drug discovery models and toxicity testing using embryonic and induced pluripotent stem-cell-derived cardiac and neuronal cells." Stem Cells Int **2012**: 379569.

Development of induced pluripotent stem cells (iPSCs) using forced expression of specific sets of transcription factors has changed the field of stem cell research extensively. Two important limitations for research application of embryonic stem cells (ESCs), namely, ethical and immunological issues, can be circumvented using iPSCs. Since the development of first iPSCs, tremendous effort has been directed to the development of methods to increase the efficiency of the process and to reduce the extent of genomic modifications associated with the reprogramming procedure. The established lineage-specific differentiation protocols developed for ESCs are being applied to iPSCs, as they have great potential in regenerative medicine for cell therapy, disease modeling either for drug development or for fundamental science, and, last but not least, toxicity testing. This paper reviews efforts aimed at practical development of iPSC differentiation to neural/cardiac lineages and further the use of these iPSCs-derived cells for drug development and toxicity testing.

Devolder, K. (2010). "Complicity in stem cell research: the case of induced pluripotent stem cells." Hum Reprod **25**(9): 2175-2180.

Many who object to human embryonic stem cell (hESC) research because they believe it involves complicity in embryo destruction have welcomed induced pluripotent stem cell (iPSC) research as an ethical alternative. This opinion article aims to show that complicity arguments against hESC research are prima facie inconsistent with accepting iPSC research as it is currently done. Those who oppose hESC research on grounds of complicity should either (i) oppose iPSC research as well, (ii) advocate a radical change in the way iPSC research is done, (iii) demonstrate that complicity arguments against iPSC research are weaker than those against hESC research or (iv) reject complicity arguments against both hESC and iPSC research, either by adopting a more limited conception of complicity that allows acceptance of some hESC research, or by accepting that destroying embryos for important scientific research is not wrong.

Diederichs, S. and R. S. Tuan (2014). "Functional comparison of human-induced pluripotent stem cell-derived mesenchymal cells and bone marrow-derived mesenchymal stromal cells from the same donor." Stem Cells Dev **23**(14): 1594-1610.

Mesenchymal stem cells (MSCs) have a high potential for therapeutic efficacy in treating diverse musculoskeletal injuries and cardiovascular diseases, and for ameliorating the severity of graft-versus-host and autoimmune diseases. While most of these clinical applications require substantial cell quantities, the number of MSCs that can be obtained initially from a single donor is limited. Reports on the derivation of MSC-like cells from pluripotent stem cells (PSCs) are,

thus, of interest, as the infinite proliferative capacity of PSCs opens the possibility to generate large amounts of uniform batches of MSCs. However, characterization of such MSC-like cells is currently inadequate, especially with regard to the question of whether these cells are equivalent or identical to MSCs. In this study, we have derived MSC-like cells [induced PSC-derived MSC-like progenitor cells (iMPCs)] using four different methodologies from a newly established induced PSC line reprogrammed from human bone marrow stromal cells (BMSCs), and compared the iMPCs directly with the originating parental BMSCs. The iMPCs exhibited typical MSC/fibroblastic morphology and MSC-typical surface marker profile, and they were capable of differentiation in vitro along the osteogenic, chondrogenic, and adipogenic lineages. However, compared with the parental BMSCs, iMPCs displayed a unique expression pattern of mesenchymal and pluripotency genes and were less responsive to traditional BMSC differentiation protocols. We, therefore, conclude that iMPCs generated from PSCs via spontaneous differentiation represent a distinct population of cells which exhibit MSC-like characteristics.

Diekman, B. O., et al. (2015). "Knockdown of the cell cycle inhibitor p21 enhances cartilage formation by induced pluripotent stem cells." Tissue Eng Part A **21**(7-8): 1261-1274.

The limited regenerative capacity of articular cartilage contributes to progressive joint dysfunction associated with cartilage injury or osteoarthritis. Cartilage tissue engineering seeks to provide a biological substitute for repairing damaged or diseased cartilage, but requires a cell source with the capacity for extensive expansion without loss of chondrogenic potential. In this study, we hypothesized that decreased expression of the cell cycle inhibitor p21 would enhance the proliferative and chondrogenic potential of differentiated induced pluripotent stem cells (iPSCs). Murine iPSCs were directed to differentiate toward the chondrogenic lineage with an established protocol and then engineered to express a short hairpin RNA (shRNA) to reduce the expression of p21. Cells expressing the p21 shRNA demonstrated higher proliferative potential during monolayer expansion and increased synthesis of glycosaminoglycans (GAGs) in pellet cultures. Furthermore, these cells could be expanded approximately 150-fold over three additional passages without a reduction in the subsequent production of GAGs, while control cells showed reduced potential for GAG synthesis with three additional passages. In pellets from extensively passaged cells, knockdown of p21 attenuated the sharp decrease in cell number that occurred in control cells, and immunohistochemical analysis showed that p21 knockdown limited the production of type I and type X collagen while maintaining synthesis of cartilage-specific type II collagen. These findings suggest that manipulating the cell cycle can augment the monolayer expansion and preserve the chondrogenic capacity of differentiated iPSCs, providing a strategy for enhancing iPSC-based cartilage tissue engineering.

Ding, Q., et al. (2018). "Protective effects of human induced pluripotent stem cell-derived exosomes on high glucose-induced injury in human endothelial cells." Exp Ther Med **15**(6): 4791-4797.

Exosomes are a family of extracellular vesicles that are secreted from almost all types of cells and are associated with cell-to-cell communication. The present study was performed to investigate the effects of human induced pluripotent stem cell-derived exosomes (hiPSC-exo) on cell viability, capillary-like structure formation and senescence in endothelial cells exposed to high glucose. Exosomes were isolated from the conditional medium of hiPSCs and confirmed by transmission electron microscopy, nanoparticle tracking analysis and western blot analysis using Alix and cluster of differentiation-63 as markers. hiPSC-exo were labeled with PKH26 for tracking, and it was determined that spherical exosomes, with a typical cup-shape, were absorbed by human umbilical vascular endothelial cells (HUVECs). Cultured HUVECs were treated with high glucose (33 mM) with or without hiPSC-exo (20 microg/ml) for 48 h, and cell viability, capillary tube formation and senescence were assessed. When exposed to high glucose, viability and tube formation in HUVECs was significantly reduced ($P<0.0001$), whereas the proportion of senescent cells was higher compared with that in control HUVECs ($P<0.0001$). Furthermore, hiPSC-exo restored cell viability and capillary-like structure formation, and reduced senescence in HUVECs exposed to high glucose ($P<0.0001$). However, hiPSC-exo had minimal effects on normal HUVECs. These findings suggest that stem cell-derived exosomes are able to promote cell proliferation, enhance capillary-like structure formation and reduce senescence in endothelial cells exposed to high glucose.

Dirja, B. T., et al. (2016). "Potential of laryngeal muscle regeneration using induced pluripotent stem cell-derived skeletal muscle cells." Acta Otolaryngol **136**(4): 391-396.

Conclusion Induced pluripotent stem (iPS) cells may be a new potential cell source for laryngeal muscle regeneration in the treatment of vocal fold atrophy after recurrent laryngeal nerve paralysis. Objectives Unilateral vocal fold paralysis can lead to

degeneration, atrophy, and loss of force of the thyroarytenoid muscle. At present, there are some treatments such as thyroplasty, arytenoid adduction, and vocal fold injection. However, such treatments cannot restore reduced mass of the thyroarytenoid muscle. iPS cells have been recognized as supplying a potential resource for cell transplantation. The aim of this study was to assess the effectiveness of the use of iPS cells for the regeneration of laryngeal muscle through the evaluation of both in vitro and in vivo experiments. Methods Skeletal muscle cells were generated from tdTomato-labeled iPS cells using embryoid body formation. Differentiation into skeletal muscle cells was analyzed by gene expression and immunocytochemistry. The tdTomato-labeled iPS cell-derived skeletal muscle cells were transplanted into the left atrophied thyroarytenoid muscle. To evaluate the engraftment of these cells after transplantation, immunohistochemistry was performed. Results The tdTomato-labeled iPS cells were successfully differentiated into skeletal muscle cells through an in vitro experiment. These cells survived in the atrophied thyroarytenoid muscle after transplantation.

Dominguez, A. A., et al. (2014). "Human germ cell formation in xenotransplants of induced pluripotent stem cells carrying X chromosome aneuploidies." Sci Rep **4**: 6432.

Turner syndrome is caused by complete or partial loss of the second sex chromosome and is characterized by spontaneous fetal loss in >90% of conceptions. Survivors possess an array of somatic and germline clinical characteristics. Induced pluripotent stem cells (iPSCs) offer an opportunity for insight into genetic requirements of the X chromosome linked to Turner syndrome. We derived iPSCs from Turner syndrome and control individuals and examined germ cell development as a function of X chromosome composition. We demonstrate that two X chromosomes are not necessary for reprogramming or maintenance of pluripotency and that there are minimal differences in gene expression, at the single cell level, linked to X chromosome aneuploidies. Formation of germ cells, as assessed in vivo through a murine xenotransplantation model, indicated that undifferentiated iPSCs, independent of X chromosome composition, are capable of forming germ-cell-like cells (GCLCs) in vivo. In combination with clinical data regarding infertility in women with X chromosome aneuploidies, results suggest that two intact X chromosomes are not required for human germ cell formation, qualitatively or quantitatively, but rather are likely to be required for maintenance of human germ cells to adulthood.

Dorn, I., et al. (2015). "Erythroid differentiation of human induced pluripotent stem cells is independent of donor cell type of origin." Haematologica **100**(1): 32-41.

Epigenetic memory in induced pluripotent stem cells, which is related to the somatic cell type of origin of the stem cells, might lead to variations in the differentiation capacities of the pluripotent stem cells. In this context, induced pluripotent stem cells from human CD34(+) hematopoietic stem cells might be more suitable for hematopoietic differentiation than the commonly used fibroblast-derived induced pluripotent stem cells. To investigate the influence of an epigenetic memory on the ex vivo expansion of induced pluripotent stem cells into erythroid cells, we compared induced pluripotent stem cells from human neural stem cells and human cord blood-derived CD34(+) hematopoietic stem cells and evaluated their potential for differentiation into hematopoietic progenitor and mature red blood cells. Although genome-wide DNA methylation profiling at all promoter regions demonstrates that the epigenetic memory of induced pluripotent stem cells is influenced by the somatic cell type of origin of the stem cells, we found a similar hematopoietic induction potential and erythroid differentiation pattern of induced pluripotent stem cells of different somatic cell origin. All human induced pluripotent stem cell lines showed terminal maturation into normoblasts and enucleated reticulocytes, producing predominantly fetal hemoglobin. Differences were only observed in the growth rate of erythroid cells, which was slightly higher in the induced pluripotent stem cells derived from CD34(+) hematopoietic stem cells. More detailed methylation analysis of the hematopoietic and erythroid promoters identified similar CpG methylation levels in the induced pluripotent stem cell lines derived from CD34(+) cells and those derived from neural stem cells, which confirms their comparable erythroid differentiation potential.

Drowley, L., et al. (2016). "Human Induced Pluripotent Stem Cell-Derived Cardiac Progenitor Cells in Phenotypic Screening: A Transforming Growth Factor-beta Type 1 Receptor Kinase Inhibitor Induces Efficient Cardiac Differentiation." Stem Cells Transl Med **5**(2): 164-174.

Several progenitor cell populations have been reported to exist in hearts that play a role in cardiac turnover and/or repair. Despite the presence of cardiac stem and progenitor cells within the myocardium, functional repair of the heart after injury is inadequate. Identification of the signaling pathways involved in the expansion and differentiation of cardiac progenitor cells (CPCs) will broaden insight into the fundamental mechanisms playing a role in cardiac homeostasis and

disease and might provide strategies for in vivo regenerative therapies. To understand and exploit cardiac ontogeny for drug discovery efforts, we developed an in vitro human induced pluripotent stem cell-derived CPC model system using a highly enriched population of KDR (pos)/CKIT (neg)/NKX2.5(pos) CPCs. Using this model system, these CPCs were capable of generating highly enriched cultures of cardiomyocytes under directed differentiation conditions. In order to facilitate the identification of pathways and targets involved in proliferation and differentiation of resident CPCs, we developed phenotypic screening assays. Screening paradigms for therapeutic applications require a robust, scalable, and consistent methodology. In the present study, we have demonstrated the suitability of these cells for medium to high-throughput screens to assess both proliferation and multilineage differentiation. Using this CPC model system and a small directed compound set, we identified activin-like kinase 5 (transforming growth factor-beta type 1 receptor kinase) inhibitors as novel and potent inducers of human CPC differentiation to cardiomyocytes. Significance: Cardiac disease is a leading cause of morbidity and mortality, with no treatment available that can result in functional repair. This study demonstrates how differentiation of induced pluripotent stem cells can be used to identify and isolate cell populations of interest that can translate to the adult human heart. Two separate examples of phenotypic screens are discussed, demonstrating the value of this biologically relevant and reproducible technology. In addition, this assay system was able to identify novel and potent inducers of differentiation and proliferation of induced pluripotent stem cell-derived cardiac progenitor cells.

Du, C., et al. (2014). "Induced pluripotent stem cell-derived hepatocytes and endothelial cells in multi-component hydrogel fibers for liver tissue engineering." Biomaterials **35**(23): 6006-6014.

Liver tissue engineering requires a suitable cell source, methodologies to assemble the cells within their niche microenvironments in a spatially defined manner, and vascularization of the construct in vivo for maintenance of hepatocyte viability and function. Recently, we have developed methods of encapsulating cells within separate domains in multi-component hydrogel fibers and methods of assembling fibers to form 3D-patterned tissue constructs. In the present work, we have combined these approaches to encapsulate hepatocytes and endothelial cells within their specific niches, and to assemble them into endothelialized liver tissue constructs. The hepatocytes and endothelial cells were obtained in parallel by differentiating human recombinant protein-induced human pluripotent stem cells, resulting in a construct which contained genetically identical endothelial and parenchymal elements. We were able to demonstrate that the presence of endothelial cells in the scaffold significantly improved hepatocyte function in vitro and facilitated vascularization of the scaffold when implanted in a mouse partial hepatectomy model. The in vivo studies further asserted that integration of the scaffold with host vasculature had occurred, as demonstrated by the presence of human albumin in the mouse serum.

Du, Y., et al. (2017). "Exosomes from Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stromal Cells (hiPSC-MSCs) Protect Liver against Hepatic Ischemia/ Reperfusion Injury via Activating Sphingosine Kinase and Sphingosine-1-Phosphate Signaling Pathway." Cell Physiol Biochem **43**(2): 611-625.

BACKGROUND/AIMS: This study aimed to evaluate the effects of exosomes produced by human-induced pluripotent stem cell-derived mesenchymal stromal cells (hiPSC-MSCs-Exo) on hepatic ischemia-reperfusion (I/R) injury, as well as the underlying mechanisms. METHODS: Exosomes derived from hiPSC-MSCs were isolated and characterized both biochemically and biophysically. hiPSC-MSCs-Exo were injected systemically into a murine ischemia/reperfusion injury model via the inferior vena cava, and then the therapeutic effects were evaluated. The serum levels of transaminases (aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as histological changes were examined. Primary hepatocytes and human hepatocyte cell line HL7702 were used to test whether exosomes could induce hepatocytes proliferation in vitro. In addition, the expression levels of proliferation markers (proliferation cell nuclear antigen, PCNA; Phosphohistone-H3, PHH3) were measured by immunohistochemistry and Western blot. Moreover, SK inhibitor (SKI-II) and S1P1 receptor antagonist (VPC23019) were used to investigate the role of sphingosine kinase and sphingosine-1-phosphate-dependent pathway in the effects of hiPSC-MSCs-Exo on hepatocytes. RESULTS: hiPSCs were efficiently induced into hiPSC-MSCs that had typical MSC characteristics. hiPSC-MSCs-Exo had diameters ranging from 100 to 200 nm and expressed exosome markers (Alix, CD63 and CD81). After hiPSC-MSCs-Exo administration, hepatocyte necrosis and sinusoidal congestion were markedly suppressed in the ischemia/reperfusion injury model, with lower histopathological scores. The levels of hepatocyte injury markers AST and ALT were significantly lower in the treatment group compared to control, and the expression levels of proliferation markers (PCNA and

PHH3) were greatly induced after hiPSC-MSCs-Exo administration. Moreover, hiPSC-MSCs-Exo also induced primary hepatocytes and HL7702 cells proliferation in vitro in a dose-dependent manner. We found that hiPSC-MSCs-Exo could directly fuse with target hepatocytes or HL7702 cells and increase the activity of sphingosine kinase and synthesis of sphingosine-1-phosphate (S1P). Furthermore, the inhibition of SK1 or S1P1 receptor completely abolished the protective and proliferative effects of hiPSC-MSCs-Exo on hepatocytes, both in vitro and in vivo. CONCLUSIONS: Our results demonstrated that hiPSC-MSCs-Exo could alleviate hepatic I/R injury via activating sphingosine kinase and sphingosine-1-phosphate pathway in hepatocytes and promote cell proliferation. These findings represent a novel mechanism that potentially contributes to liver regeneration and have important implications for new therapeutic approaches to acute liver disease.

Easley, C. A., et al. (2014). "Gamete derivation from embryonic stem cells, induced pluripotent stem cells or somatic cell nuclear transfer-derived embryonic stem cells: state of the art." Reprod Fertil Dev **27**(1): 89-92.

Generating gametes from pluripotent stem cells (PSCs) has many scientific justifications and several biomedical rationales. Here, we consider several strategies for deriving gametes from PSCs from mice and primates (human and non-human) and their anticipated strengths, challenges and limitations. Although the 'Weismann barrier', which separates the mortal somatic cell lineages from the potentially immortal germline, has long existed, breakthroughs first in mice and now in humans are artificially creating germ cells from somatic cells. Spermatozoa with full reproductive viability establishing multiple generations of seemingly normal offspring have been reported in mice and, in humans, haploid spermatids with correct parent-of-origin imprints have been obtained. Similar progress with making oocytes has been published using mouse PSCs differentiated in vitro into primordial germ cells, which are then cultured after xenografting reconstructed artificial ovaries. Progress in making human oocytes artificially is proving challenging. The usefulness of these artificial gametes, from assessing environmental exposure toxicity to optimising medical treatments to prevent negative off-target effects on fertility, may prove invaluable, as may basic discoveries on the fundamental mechanisms of gametogenesis.

Eckert, A., et al. (2015). "Bystander Effect Fuels Human Induced Pluripotent Stem Cell-Derived Neural Stem Cells to Quickly Attenuate Early Stage Neurological Deficits After Stroke." Stem Cells Transl Med **4**(7): 841-851.

UNLABELLED:: Present therapies for stroke rest with tissue plasminogen activator (tPA), the sole licensed antithrombotic on the market; however, tPA's effectiveness is limited in that the drug not only must be administered less than 3-5 hours after stroke but often exacerbates blood-brain barrier (BBB) leakage and increases hemorrhagic incidence. A potentially promising therapy for stroke is transplantation of human induced pluripotent stem cell-derived neural stem cells (hiPSC-NSCs). To date, the effects of iPSCs on injuries that take place during early stage ischemic stroke have not been well studied. Consequently, we engrafted iPSC-NSCs into the ipsilesional hippocampus, a natural niche of NSCs, at 24 hours after stroke (prior to secondary BBB opening and when inflammatory signature is abundant). At 48 hours after stroke (24 hours after transplant), hiPSC-NSCs had migrated to the stroke lesion and quickly improved neurological function. Transplanted mice showed reduced expression of proinflammatory factors (tumor necrosis factor-alpha, interleukin 6 [IL-6], IL-1beta, monocyte chemotactic protein 1, macrophage inflammatory protein 1alpha), microglial activation, and adhesion molecules (intercellular adhesion molecule 1, vascular cell adhesion molecule 1) and attenuated BBB damage. We are the first to report that engrafted hiPSC-NSCs rapidly improved neurological function (less than 24 hours after transplant). Rapid hiPSC-NSC therapeutic activity is mainly due to a bystander effect that elicits reduced inflammation and BBB damage. SIGNIFICANCE: Clinically, cerebral vessel occlusion is rarely permanent because of spontaneous or thrombolytic therapy-mediated reperfusion. These results have clinical implications indicating a much extended therapeutic window for transplantation of human induced pluripotent stem cell-derived neural stem cells (hiPSC-NSCs; 24 hours after stroke as opposed to the 5-hour window with tissue plasminogen activator [tPA]). In addition, there is potential for a synergistic effect by combining hiPSC-NSC transplantation with tPA to attenuate stroke's adverse effects.

Efthymiou, A. G., et al. (2014). "Self-renewal and cell lineage differentiation strategies in human embryonic stem cells and induced pluripotent stem cells." Expert Opin Biol Ther **14**(9): 1333-1344.

INTRODUCTION: Since the initial discoveries of human embryonic and induced pluripotent stem cells, many strategies have been developed to utilize the potential of these cells for translational research and disease modeling. The success of these aims and the development of future applications in this area will depend on the ability to generate high-quality and

large numbers of differentiated cell types that genetically, epigenetically, and functionally mimic the cells found in the body. AREAS COVERED: In this review, we highlight the current strategies used to maintain stem cell pluripotency (a measure of stem cell quality), as well as provide an overview of the various differentiation strategies being used to generate cells from all three germ lineages. We also discuss the particular considerations that must be addressed when utilizing these cells for translational therapy, and provide an example of a cell type currently used in clinical trials. EXPERT OPINION: The major challenge in regenerative medicine and disease modeling will be in generating functional cells of sufficient quality that are physiologically and epigenetically similar to the diverse cells that they are modeled after. By meeting these criteria, these differentiated products can be successfully used in disease modeling, drug/toxicology screens, and cellular replacement therapy.

Emborg, M. E., et al. (2013). "Induced pluripotent stem cell-derived neural cells survive and mature in the nonhuman primate brain." Cell Rep **3**(3): 646-650.

The generation of induced pluripotent stem cells (iPSCs) opens up the possibility for personalized cell therapy. Here, we show that transplanted autologous rhesus monkey iPSC-derived neural progenitors survive for up to 6 months and differentiate into neurons, astrocytes, and myelinating oligodendrocytes in the brains of MPTP-induced hemiparkinsonian rhesus monkeys with a minimal presence of inflammatory cells and reactive glia. This finding represents a significant step toward personalized regenerative therapies.

Fan, X. L., et al. (2018). "Induced pluripotent stem cell-derived mesenchymal stem cells activate quiescent T cells and elevate regulatory T cell response via NF-kappaB in allergic rhinitis patients." Stem Cell Res Ther **9**(1): 170.

BACKGROUND: It has been demonstrated previously that induced pluripotent stem cell (iPSC)-derived mesenchymal stem cells (MSCs) have immunosuppressive effects on activated T cells. However, the effects of iPSC-MSCs on quiescent T cells are still unknown. The aim of this study was to identify the immunomodulatory role of iPSC-MSCs on resting peripheral blood mononuclear cells (PBMCs) from allergic rhinitis (AR) patients. METHODS: PBMCs were cocultured with iPSC-MSCs without any stimulation, following which lymphocyte proliferation, activation of T cells, TH1/TH2 and regulatory T (Treg) cell differentiation, and Treg cell function were analyzed. The roles of soluble factors and cell-cell contact were examined to investigate the mechanisms involved. RESULTS: iPSC-MSCs promoted the proliferation of resting lymphocytes, activated CD4(+) and CD8(+) T cells, and upregulated and activated Treg cells without any additional stimulation. In addition, iPSC-MSCs balanced biased TH1/TH2 cytokine levels. Cell-cell contact was confirmed to be a possible mechanism involved. NF-kappaB was identified to play an important role in the immunomodulatory effects of iPSC-MSCs on quiescent T cells. CONCLUSIONS: iPSC-MSCs activate quiescent T cells and elevate regulatory T-cell response in AR patients, suggesting different immunomodulatory functions of iPSC-MSCs according to the phases of diseases. Therefore, iPSC-MSCs are a potential therapeutic candidate for treating allergic airway inflammation.

Fan, Y., et al. (2012). "Generation of human beta-thalassemia induced pluripotent stem cells from amniotic fluid cells using a single excisable lentiviral stem cell cassette." J Reprod Dev **58**(4): 404-409.

Induced pluripotent stem cells (iPSCs) derived from somatic cells of patients represent a powerful tool for biomedical research and may have a wide range of applications in cell and gene therapy. However, the safety issues and the low efficiency associated with generating human iPSCs have limited their usage in clinical settings. The cell type used to create iPSCs can significantly influence the reprogramming efficiency and kinetics. Here, we show that amniotic fluid cells from the prenatal diagnosis of a beta-thalassemia patient can be efficiently reprogrammed using a doxycycline (DOX)-inducible humanized version of the single lentiviral "stem cell cassette" vector flanked by loxP sites, which can be excised with Cre recombinase. We also demonstrated that the patient-derived iPSCs can be characterized based on the expression of pluripotency markers, and they can be differentiated into various somatic cell types in vitro and in vivo. Moreover, microarray analysis demonstrates a high correlation coefficient between human beta-thalassemia iPS cells and human embryonic stem (hES) cells but a low correlation coefficient between human beta-thalassemia amniotic fluid cells and human beta-thalassemia iPS cells. Our data suggest that amniotic fluid cells may be an ideal human somatic cell resource for rapid and efficient generation of patient-specific iPS cells.

Ferreira, A. F., et al. (2018). "Hematopoietic stem cells from induced pluripotent stem cells - considering the role of microRNA as a cell differentiation regulator." J Cell Sci **131**(4).

Although hematopoietic stem cell (HSC) therapy for hematological diseases can lead to a good outcome

from the clinical point of view, the limited number of ideal donors, the comorbidity of patients and the increasing number of elderly patients may limit the application of this therapy. HSCs can be generated from induced pluripotent stem cells (iPSCs), which requires the understanding of the bone marrow and liver niches components and function in vivo iPSCs have been extensively applied in several studies involving disease models, drug screening and cellular replacement therapies. However, the somatic reprogramming by transcription factors is a low-efficiency process. Moreover, the reprogramming process is also regulated by microRNAs (miRNAs), which modulate the expression of the transcription factors OCT-4 (also known as POU5F1), SOX-2, KLF-4 and MYC, leading somatic cells to a pluripotent state. In this Review, we present an overview of the challenges of cell reprogramming protocols with regard to HSC generation from iPSCs, and highlight the potential role of miRNAs in cell reprogramming and in the differentiation of induced pluripotent stem cells.

Focosi, D. and G. Amabile (2017). "Induced Pluripotent Stem Cell-Derived Red Blood Cells and Platelet Concentrates: From Bench to Bedside." Cells **7**(1).

Red blood cells and platelets are anucleate blood components indispensable for oxygen delivery and hemostasis, respectively. Derivation of these blood elements from induced pluripotent stem (iPS) cells has the potential to develop blood donor-independent and genetic manipulation-prone products to complement or replace current transfusion banking, also minimizing the risk of alloimmunization. While the production of erythrocytes from iPS cells has challenges to overcome, such as differentiation into adult-type phenotype that functions properly after transfusion, platelet products are qualitatively and quantitatively approaching a clinically-applicable level owing to advances in expandable megakaryocyte (MK) lines, platelet-producing bioreactors, and novel reagents. Guidelines that assure the quality of iPS cells-derived blood products for clinical application represent a novel challenge for regulatory agencies. Considering the minimal risk of tumorigenicity and the expected significant demand of such products, ex vivo production of iPS-derived blood components can pave the way for iPS translation into the clinic.

Foster, A. A., et al. (2018). "Protein-engineered hydrogels enhance the survival of induced pluripotent stem cell-derived endothelial cells for treatment of peripheral arterial disease." Biomater Sci **6**(3): 614-622.

A key feature of peripheral arterial disease (PAD) is damage to endothelial cells (ECs), resulting in lower limb pain and restricted blood flow. Recent preclinical studies demonstrate that the transplantation of ECs via direct injection into the affected limb can result in significantly improved blood circulation. Unfortunately, the clinical application of this therapy has been limited by low cell viability and poor cell function. To address these limitations we have developed an injectable, recombinant hydrogel, termed SHIELD (Shear-thinning Hydrogel for Injectable Encapsulation and Long-term Delivery) for cell transplantation. SHIELD provides mechanical protection from cell membrane damage during syringe flow. Additionally, secondary in situ crosslinking provides a reinforcing network to improve cell retention, thereby augmenting the therapeutic benefit of cell therapy. In this study, we demonstrate the improved acute viability of human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) following syringe injection delivery in SHIELD, compared to saline. Using a murine hind limb ischemia model of PAD, we demonstrate enhanced iPSC-EC retention in vivo and improved neovascularization of the ischemic limb based on arteriogenesis following transplantation of iPSC-ECs delivered in SHIELD.

Frederich, B. J., et al. (2017). "Electrotaxis of cardiac progenitor cells, cardiac fibroblasts, and induced pluripotent stem cell-derived cardiac progenitor cells requires serum and is directed via PI3'K pathways." Heart Rhythm **14**(11): 1685-1692.

BACKGROUND: The limited regenerative capacity of cardiac tissue has long been an obstacle to treating damaged myocardium. Cell-based therapy offers an enormous potential to the current treatment paradigms. However, the efficacy of regenerative therapies remains limited by inefficient delivery and engraftment. Electrotaxis (electrically guided cell movement) has been clinically used to improve recovery in a number of tissues but has not been investigated for treating myocardial damage. OBJECTIVE: The purpose of this study was to test the electrotactic behaviors of several types of cardiac cells. METHODS: Cardiac progenitor cells (CPCs), cardiac fibroblasts (CFs), and human induced pluripotent stem cell-derived cardiac progenitor cells (hiPSC-CPCs) were used. RESULTS: CPCs and CFs electrotax toward the anode of a direct current electric field, whereas hiPSC-CPCs electrotax toward the cathode. The voltage-dependent electrotaxis of CPCs and CFs requires the presence of serum in the media. Addition of soluble vascular cell adhesion molecule to serum-free media restores directed migration. We provide evidence that CPC and CF electrotaxis is mediated

through phosphatidylinositide 3-kinase signaling. In addition, very late antigen-4, an integrin and growth factor receptor, is required for electrotaxis and localizes to the anodal edge of CPCs in response to direct current electric field. The hiPSC-derived CPCs do not express very late antigen-4, migrate toward the cathode in a voltage-dependent manner, and, similar to CPCs and CFs, require media serum and phosphatidylinositide 3-kinase activity for electrotaxis. CONCLUSION: The electrotactic behaviors of these therapeutic cardiac cells may be used to improve cell-based therapy for recovering function in damaged myocardium.

Freund, C., et al. (2010). "The first reported generation of human induced pluripotent stem cells (iPS cells) and iPS cell-derived cardiomyocytes in the Netherlands." Neth Heart J **18**(1): 51-54.

One of the recent breakthroughs in stem cell research has been the reprogramming of human somatic cells to an embryonic stem cell (ESC)-like state (induced pluripotent stem cells, iPS cells). Similar to ESCs, iPS cells can differentiate into derivatives of the three germ layers, for example cardiomyocytes, pancreatic cells or neurons. This technique offers a new approach to investigating disease pathogenesis and to the development of novel therapies. It may now be possible to generate iPS cells from somatic cells of patients who suffer from vascular genetic diseases, such as hereditary haemorrhagic telangiectasia (HHT). The iPS cells will have a similar genotype to that of the patient and can be differentiated in vitro into the cell type (s) that are affected in the patient. Thus they will serve as excellent models for a better understanding of mechanisms underlying the disease. This, together with the ability to test new drugs, could potentially lead to novel therapeutic concepts in the near future. Here we report the first derivation of three human iPS cell lines from two healthy individuals and one HHT patient in the Netherlands. The iPS cells resembled ESCs in morphology and expressed typical ESC markers. In vitro, iPS cells could be differentiated into cells of the three germ layers, including beating cardiomyocytes and vascular cells. With this technique it will be possible to establish human cardiovascular disease models from patient biopsies provided by the principal hospitals in the Netherlands. (Neth Heart J 2010;18:51-4.).

Fu, Q. L., et al. (2012). "Mesenchymal stem cells derived from human induced pluripotent stem cells modulate T-cell phenotypes in allergic rhinitis." Allergy **67**(10): 1215-1222.

BACKGROUND: Human induced pluripotent stem cells (iPSCs) possess remarkable self-renewal capacity and the potential to differentiate into novel cell types, such as mesenchymal stem cells (MSCs). iPSC-MSCs have been shown to enhance tissue regeneration and attenuate tissue ischaemia; however, their contribution to the immune regulation of Th2-skewed allergic rhinitis (AR) and asthma remains unclear. OBJECTIVE: This study compared the immunomodulatory effects of iPSC-MSCs and bone marrow-derived MSCs (BM-MSCs) on lymphocyte proliferation, T-cell phenotypes and cytokine production in peripheral blood mononuclear cells (PBMCs) in patients with AR, and investigated the possible molecular mechanisms underlying the immunomodulatory properties of iPSC-MSCs. METHODS: In co-cultures of PBMCs with iPSC-MSCs or BM-MSCs, lymphocyte proliferation was evaluated using 3H-thymidine (3H-TdR) uptake, carboxyfluorescein diacetate, succinimidyl ester (CFDA-SE) assays; the regulatory T-cell (Treg) phenotype was determined by flow cytometry, and cytokine levels were measured using an enzyme-linked immunosorbent assay. The immunomodulatory properties of both MSCs were further evaluated using NS398 and transwell experiments. RESULTS: Similar to BM-MSCs, we determined that iPSC-MSCs significantly inhibit lymphocyte proliferation and promote Treg response in PBMCs ($P < 0.05$). Accordingly, the cytokine milieu (IFN-gamma, IL-4, IL-5, IL-10 and IL-13) in the supernatants of PBMCs changed significantly ($P < 0.05$). The immunomodulatory properties of iPSC-MSCs and BM-MSCs were associated with prostaglandin E2 (PGE2) production and cell-cell contact. CONCLUSIONS: These data demonstrate that iPSC-MSCs are capable of modulating T-cell phenotypes towards Th2 suppression through inducing Treg expansion, suggesting that iPSC-MSCs can be used as an alternative candidate to adult MSCs to treat allergic airway diseases.

Fuerstenau-Sharp, M., et al. (2015). "Generation of highly purified human cardiomyocytes from peripheral blood mononuclear cell-derived induced pluripotent stem cells." PLoS One **10**(5): e0126596.

Induced pluripotent stem (iPS) cells have an enormous potential for physiological studies. A novel protocol was developed combining the derivation of iPS from peripheral blood with an optimized directed differentiation to cardiomyocytes and a subsequent metabolic selection. The human iPS cells were retrovirally dedifferentiated from activated T cells. The subsequent optimized directed differentiation protocol yielded 30-45% cardiomyocytes at day 16 of differentiation. The derived cardiomyocytes expressed appropriate structural markers like cardiac troponin T, alpha-actinin and myosin light chain 2 (MLC2V). In a

subsequent metabolic selection with lactate, the cardiomyocytes content could be increased to more than 90%. Loss of cardiomyocytes during metabolic selection were less than 50%, whereas alternative surface antibody-based selection procedures resulted in loss of up to 80% of cardiomyocytes. Electrophysiological characterization confirmed the typical cardiac features and the presence of ventricular, atrial and nodal-like action potentials within the derived cardiomyocyte population. Our combined and optimized protocol is highly robust and applicable for scalable cardiac differentiation. It provides a simple and cost-efficient method without expensive equipment for generating large numbers of highly purified, functional cardiomyocytes. It will further enhance the applicability of iPS cell-derived cardiomyocytes for disease modeling, drug discovery, and regenerative medicine.

Fuhrmann, T., et al. (2018). "Combined delivery of chondroitinase ABC and human induced pluripotent stem cell-derived neuroepithelial cells promote tissue repair in an animal model of spinal cord injury." Biomed Mater **13**(2): 024103.

The lack of tissue regeneration after traumatic spinal cord injury in animal models is largely attributed to the local inhibitory microenvironment. To overcome this inhibitory environment while promoting tissue regeneration, we investigated the combined delivery of chondroitinase ABC (chABC) with human induced pluripotent stem cell-derived neuroepithelial stem cells (NESCs). ChABC was delivered to the injured spinal cord at the site of injury by affinity release from a crosslinked methylcellulose (MC) hydrogel by injection into the intrathecal space. NESCs were distributed in a hydrogel comprised of hyaluronan and MC and injected into the spinal cord tissue both rostral and caudal to the site of injury. Cell transplantation led to reduced cavity formation, but did not improve motor function. While few surviving cells were found 2 weeks post injury, the majority of live cells were neurons, with only few astrocytes, oligodendrocytes, and progenitor cells. At 9 weeks post injury, there were more progenitor cells and a more even distribution of cell types compared to those at 2 weeks post injury, suggesting preferential survival and differentiation. Interestingly, animals that received cells and chABC had more neurons than animals that received cells alone, suggesting that chABC influenced the injury environment such that neuronal differentiation or survival was favoured.

Fujimoto, Y., et al. (2012). "Treatment of a mouse model of spinal cord injury by transplantation of human induced pluripotent stem cell-derived long-term self-renewing neuroepithelial-like stem cells." Stem Cells **30**(6): 1163-1173.

Because of their ability to self-renew, to differentiate into multiple lineages, and to migrate toward a damaged site, neural stem cells (NSCs), which can be derived from various sources such as fetal tissues and embryonic stem cells, are currently considered to be promising components of cell replacement strategies aimed at treating injuries of the central nervous system, including the spinal cord. Despite their efficiency in promoting functional recovery, these NSCs are not homogeneous and possess variable characteristics depending on their derivation protocols. The advent of induced pluripotent stem (iPS) cells has provided new prospects for regenerative medicine. We used a recently developed robust and stable protocol for the generation of long-term, self-renewing, neuroepithelial-like stem cells from human iPS cells (hiPS-lt-NES cells), which can provide a homogeneous and well-defined population of NSCs for standardized analysis. Here, we show that transplanted hiPS-lt-NES cells differentiate into neural lineages in the mouse model of spinal cord injury (SCI) and promote functional recovery of hind limb motor function. Furthermore, using two different neuronal tracers and ablation of the transplanted cells, we revealed that transplanted hiPS-lt-NES cell-derived neurons, together with the surviving endogenous neurons, contributed to restored motor function. Both types of neurons reconstructed the corticospinal tract by forming synaptic connections and integrating neuronal circuits. Our findings indicate that hiPS-lt-NES transplantation represents a promising avenue for effective cell-based treatment of SCI.

Fujita, A., et al. (2016). "beta-Globin-Expressing Definitive Erythroid Progenitor Cells Generated from Embryonic and Induced Pluripotent Stem Cell-Derived Sacs." Stem Cells **34**(6): 1541-1552.

Human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells represent a potential alternative source for red blood cell transfusion. However, when using traditional methods with embryoid bodies, ES cell-derived erythroid cells predominantly express embryonic type varepsilon-globin, with lesser fetal type gamma-globin and very little adult type beta-globin. Furthermore, no beta-globin expression is detected in iPS cell-derived erythroid cells. ES cell-derived sacs (ES sacs) have been recently used to generate functional platelets. Due to its unique structure, we hypothesized that ES sacs serve as hemangioblast-like progenitors capable to generate definitive erythroid cells that express beta-globin. With our ES sac-derived erythroid differentiation protocol, we obtained approximately

120 erythroid cells per single ES cell. Both primitive (varepsilon-globin expressing) and definitive (gamma- and beta-globin expressing) erythroid cells were generated from not only ES cells but also iPS cells. Primitive erythropoiesis is gradually switched to definitive erythropoiesis during prolonged ES sac maturation, concurrent with the emergence of hematopoietic progenitor cells. Primitive and definitive erythroid progenitor cells were selected on the basis of glycophorin A or CD34 expression from cells within the ES sacs before erythroid differentiation. This selection and differentiation strategy represents an important step toward the development of in vitro erythroid cell production systems from pluripotent stem cells. Further optimization to improve expansion should be required for clinical application. Stem Cells 2016;34:1541-1552.

Fujiwara, M., et al. (2011). "Induction and enhancement of cardiac cell differentiation from mouse and human induced pluripotent stem cells with cyclosporin-A." PLoS One **6**(2): e16734.

Induced pluripotent stem cells (iPSCs) are novel stem cells derived from adult mouse and human tissues by reprogramming. Elucidation of mechanisms and exploration of efficient methods for their differentiation to functional cardiomyocytes are essential for developing cardiac cell models and future regenerative therapies. We previously established a novel mouse embryonic stem cell (ESC) and iPSC differentiation system in which cardiovascular cells can be systematically induced from Flk1(+) common progenitor cells, and identified highly cardiogenic progenitors as Flk1(+)/CXCR4(+)/VE-cadherin (-) (FCV) cells. We have also reported that cyclosporin-A (CSA) drastically increases FCV progenitor and cardiomyocyte induction from mouse ESCs. Here, we combined these technologies and extended them to mouse and human iPSCs. Co-culture of purified mouse iPSC-derived Flk1(+) cells with OP9 stroma cells induced cardiomyocyte differentiation whilst addition of CSA to Flk1(+) cells dramatically increased both cardiomyocyte and FCV progenitor cell differentiation. Spontaneously beating colonies were obtained from human iPSCs by co-culture with END-2 visceral endoderm-like cells. Appearance of beating colonies from human iPSCs was increased approximately 4.3 times by addition of CSA at mesoderm stage. CSA-expanded human iPSC-derived cardiomyocytes showed various cardiac marker expressions, synchronized calcium transients, cardiomyocyte-like action potentials, pharmacological reactions, and ultra-structural features as cardiomyocytes. These results provide a technological basis to obtain functional cardiomyocytes from iPSCs.

Galat, V., et al. (2016). "Transgene Reactivation in Induced Pluripotent Stem Cell Derivatives and Reversion to Pluripotency of Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells." Stem Cells Dev **25**(14): 1060-1072.

Induced pluripotent stem cells (iPSCs) have enormous potential in regenerative medicine and disease modeling. It is now felt that clinical trials should be performed with iPSCs derived with nonintegrative constructs. Numerous studies, however, including those describing disease models, are still being published using cells derived from iPSCs generated with integrative constructs. Our experimental work presents the first evidence of spontaneous transgene reactivation in vitro in several cellular types. Our results show that the transgenes were predominantly silent in parent iPSCs, but in mesenchymal and endothelial iPSC derivatives, the transgenes experienced random upregulation of Nanog and c-Myc. Additionally, we provide evidence of spontaneous secondary reprogramming and reversion to pluripotency in mesenchymal stem cells derived from iPSCs. These findings strongly suggest that the studies, which use cellular products derived from iPSCs generated with retro- or lentiviruses, should be evaluated with consideration of the possibility of transgene reactivation. The in vitro model described here provides insight into the earliest events of culture transformation and suggests the hypothesis that reversion to pluripotency may be responsible for the development of tumors in cell replacement experiments. The main goal of this work, however, is to communicate the possibility of transgene reactivation in retro- or lenti-iPSC derivatives and the associated loss of cellular fidelity in vitro, which may impact the outcomes of disease modeling and related experimentation.

Gao, L., et al. (2018). "Large Cardiac Muscle Patches Engineered From Human Induced-Pluripotent Stem Cell-Derived Cardiac Cells Improve Recovery From Myocardial Infarction in Swine." Circulation **137**(16): 1712-1730.

BACKGROUND: Here, we generated human cardiac muscle patches (hCMPs) of clinically relevant dimensions (4 cm x 2 cm x 1.25 mm) by suspending cardiomyocytes, smooth muscle cells, and endothelial cells that had been differentiated from human induced-pluripotent stem cells in a fibrin scaffold and then culturing the construct on a dynamic (rocking) platform. METHODS: In vitro assessments of hCMPs suggest maturation in response to dynamic culture stimulation. In vivo assessments were conducted in a porcine model of myocardial infarction (MI). Animal groups included: MI hearts treated with 2 hCMPs (MI+hCMP, n=13), MI hearts treated with 2 cell-free

open fibrin patches (n=14), or MI hearts with neither experimental patch (n=15); a fourth group of animals underwent sham surgery (Sham, n=8). Cardiac function and infarct size were evaluated by MRI, arrhythmia incidence by implanted loop recorders, and the engraftment rate by calculation of quantitative polymerase chain reaction measurements of expression of the human Y chromosome. Additional studies examined the myocardial protein expression profile changes and potential mechanisms of action that related to exosomes from the cell patch. RESULTS: The hCMPs began to beat synchronously within 1 day of fabrication, and after 7 days of dynamic culture stimulation, in vitro assessments indicated the mechanisms related to the improvements in electronic mechanical coupling, calcium-handling, and force generation, suggesting a maturation process during the dynamic culture. The engraftment rate was 10.9+/-1.8% at 4 weeks after the transplantation. The hCMP transplantation was associated with significant improvements in left ventricular function, infarct size, myocardial wall stress, myocardial hypertrophy, and reduced apoptosis in the periscar boarder zone myocardium. hCMP transplantation also reversed some MI-associated changes in sarcomeric regulatory protein phosphorylation. The exosomes released from the hCMP appeared to have cytoprotective properties that improved cardiomyocyte survival. CONCLUSIONS: We have fabricated a clinically relevant size of hCMP with trilineage cardiac cells derived from human induced-pluripotent stem cells. The hCMP matures in vitro during 7 days of dynamic culture. Transplantation of this type of hCMP results in significantly reduced infarct size and improvements in cardiac function that are associated with reduction in left ventricular wall stress. The hCMP treatment is not associated with significant changes in arrhythmogenicity.

Garcia-Reitboeck, P., et al. (2018). "Human Induced Pluripotent Stem Cell-Derived Microglia-Like Cells Harboring TREM2 Missense Mutations Show Specific Deficits in Phagocytosis." Cell Rep **24**(9): 2300-2311.

Dysfunction of microglia, the brain's immune cells, is linked to neurodegeneration. Homozygous missense mutations in TREM2 cause Nasu-Hakola disease (NHD), an early-onset dementia. To study the consequences of these TREM2 variants, we generated induced pluripotent stem cell-derived microglia-like cells (iPSC-MGLCs) from patients with NHD caused by homozygous T66M or W50C missense mutations. iPSC-MGLCs expressed microglial markers and secreted higher levels of TREM2 than primary macrophages. TREM2 expression and secretion were reduced in variant lines. LPS-mediated cytokine secretion was comparable between control and TREM2 variant iPSC-MGLCs, whereas survival was markedly reduced in cells harboring missense mutations when compared with controls. Furthermore, TREM2 missense mutations caused a marked impairment in the phagocytosis of apoptotic bodies, but not in Escherichia coli or zymosan substrates. Coupled with changes in apoptotic cell-induced cytokine release and migration, these data identify specific deficits in the ability of iPSC-MGLCs harboring TREM2 missense mutations to respond to specific pathogenic signals.

Ge, X., et al. (2012). "Human amniotic mesenchymal stem cell-derived induced pluripotent stem cells may generate a universal source of cardiac cells." Stem Cells Dev **21**(15): 2798-2808.

Human amniotic mesenchymal stem cells (hAMSCs) demonstrated partially pluripotent characteristics with a strong expression of Oct4 and Nanog genes and immunomodulatory properties characterized by the absence of HLA-DR and the presence of HLA-G and CD59. The hAMSCs were reprogrammed into induced pluripotent stem cells (iPSCs) that generate a promising source of universal cardiac cells. The hAMSC-derived iPSCs (MiPSCs) successfully underwent robust cardiac differentiation to generate cardiomyocytes. This study investigated 3 key properties of the hAMSCs and MiPSCs: (1) the reprogramming efficiency of the partially pluripotent hAMSCs to generate MiPSCs; (2) immunomodulatory properties of the hAMSCs and MiPSCs; and (3) the cardiac differentiation potential of the MiPSCs. The characteristic iPSC colony formation was observed within 10 days after the transduction of the hAMSCs with a single integration polycistronic vector containing 4 Yamanaka factors. Immunohistology and reverse transcription-polymerase chain reaction assays revealed that the MiPSCs expressed stem cell surface markers and pluripotency-specific genes. Furthermore, the hAMSCs and MiPSCs demonstrated immunomodulatory properties enabling successful engraftment in the SVJ mice. Finally, the cardiac differentiation of MiPSCs exhibited robust spontaneous contractility, characteristic calcium transience across the membrane, a high expression of cardiac genes and mature cardiac phenotypes, and a contractile force comparable to cardiomyocytes. Our results demonstrated that the hAMSCs are reprogrammed with a high efficiency into MiPSCs, which possess pluripotent, immunomodulatory, and precardiac properties. The MiPSC-derived cardiac cells express a c-kit cell surface marker, which may be employed to purify the cardiac cell population and enable allogeneic cardiac stem cell therapy.

Ghasemi-Dehkordi, P., et al. (2015). "Comparison between the cultures of human induced pluripotent stem cells (hiPSCs) on feeder-and serum-free system (Matrigel matrix), MEF and HDF feeder cell lines." J Cell Commun Signal **9**(3): 233-246.

Human induced pluripotent stem cells (hiPSCs) are a type of pluripotent stem cells artificially derived from an adult somatic cell (typically human fibroblast) by forced expression of specific genes. In recent years, different feeders like inactivated mouse embryonic fibroblasts (MEFs), human dermal fibroblasts (HDFs), and feeder free system have commonly been used for supporting the culture of stem cells in undifferentiated state. In the present work, the culture of hiPSCs and their characterizations on BD Matrigel (feeder-and serum-free system), MEF and HDF feeders using cell culture methods and molecular techniques were evaluated and compared. The isolated HDFs from foreskin samples were reprogrammed to hiPSCs using gene delivery system. Then, the pluripotency ability of hiPSCs cultured on each layer was determined by teratoma formation and immunohistochemical staining. After EBs generation the expression level of three germ layers genes were evaluated by Q-real-time PCR. Also, the cytogenetic stability of hiPSCs cultured on each condition was analyzed by karyotyping and comet assay. Then, the presence of pluripotency antigens were confirmed by Immunocytochemistry (ICC) test and alkaline phosphatase staining. This study were showed culturing of hiPSCs on BD Matrigel, MEF and HDF feeders had normal morphology and could maintain in undifferentiated state for prolonged expansion. The hiPSCs cultured in each system had normal karyotype without any chromosomal abnormalities and the DNA lesions were not observed by comet assay. Moreover, up-regulation in three germ layers genes in cultured hiPSCs on each layer (same to ESCs) compare to normal HDFs were observed ($p < 0.05$). The findings of the present work were showed in stem cells culturing especially hiPSCs both MEF and HDF feeders as well as feeder free system like Matrigel are proper despite benefits and disadvantages. Although, MEFs is suitable for supporting of stem cell culturing but it can animal pathogens transferring and inducing immune response. Furthermore, HDFs have homologous source with hiPSCs and can be used as feeder instead of MEF but in therapeutic approaches the cells contamination is a problem. So, this study were suggested feeder free culturing of hiPSCs on Matrigel in supplemented media (without using MEF conditioned medium) resolves these problems and could prepare easy applications of hiPSCs in therapeutic approaches of regenerative medicine such as stem-cell therapy and somatic cell nuclear in further researches.

Ghosh, Z., et al. (2010). "Persistent donor cell gene expression among human induced pluripotent stem cells contributes to differences with human embryonic stem cells." PLoS One **5**(2): e8975.

Human induced pluripotent stem cells (hiPSCs) generated by de-differentiation of adult somatic cells offer potential solutions for the ethical issues surrounding human embryonic stem cells (hESCs), as well as their immunologic rejection after cellular transplantation. However, although hiPSCs have been described as "embryonic stem cell-like", these cells have a distinct gene expression pattern compared to hESCs, making incomplete reprogramming a potential pitfall. It is unclear to what degree the difference in tissue of origin may contribute to these gene expression differences. To answer these important questions, a careful transcriptional profiling analysis is necessary to investigate the exact reprogramming state of hiPSCs, as well as analysis of the impression, if any, of the tissue of origin on the resulting hiPSCs. In this study, we compare the gene profiles of hiPSCs derived from fetal fibroblasts, neonatal fibroblasts, adipose stem cells, and keratinocytes to their corresponding donor cells and hESCs. Our analysis elucidates the overall degree of reprogramming within each hiPSC line, as well as the "distance" between each hiPSC line and its donor cell. We further identify genes that have a similar mode of regulation in hiPSCs and their corresponding donor cells compared to hESCs, allowing us to specify core sets of donor genes that continue to be expressed in each hiPSC line. We report that residual gene expression of the donor cell type contributes significantly to the differences among hiPSCs and hESCs, and adds to the incompleteness in reprogramming. Specifically, our analysis reveals that fetal fibroblast-derived hiPSCs are closer to hESCs, followed by adipose, neonatal fibroblast, and keratinocyte-derived hiPSCs.

Ghule, P. N., et al. (2011). "Reprogramming the pluripotent cell cycle: restoration of an abbreviated G1 phase in human induced pluripotent stem (iPS) cells." J Cell Physiol **226**(5): 1149-1156.

Induced pluripotent stem (iPS) cells derived from terminally differentiated human fibroblasts are reprogrammed to possess stem cell like properties. However, the extent to which iPS cells exhibit unique properties of the human embryonic stem (hES) cell cycle remains to be established. hES cells are characterized by an abbreviated G1 phase (approximately 2.5 h) and accelerated organization of subnuclear domains that mediate the assembly of regulatory machinery for histone gene expression [i.e., histone locus bodies (HLBs)]. We therefore examined cell cycle parameters of iPS cells in comparison to hES cells. Analysis of DNA synthesis [5-bromo-2'-

deoxy-uridine (BrdU) incorporation], cell cycle distribution (FACS analysis and Ki67 staining) and subnuclear organization of HLBs [immunofluorescence microscopy and fluorescence in situ hybridization (FISH)] revealed that human iPS cells have a short G1 phase (approximately 2.5 h) and an abbreviated cell cycle (16-18 h). Furthermore, HLBs are formed and reorganized rapidly after mitosis (within 1.5-2 h). Thus, reprogrammed iPS cells have cell cycle kinetics and dynamic subnuclear organization of regulatory machinery that are principal properties of pluripotent hES cells. Our findings support the concept that the abbreviated cell cycle of hES and iPS cells is functionally linked to pluripotency.

Giordano, S., et al. (2017). "Induced Pluripotent Stem Cell-Derived Endothelial Cells Overexpressing Interleukin-8 Receptors A/B and/or C-C Chemokine Receptors 2/5 Inhibit Vascular Injury Response." Stem Cells Transl Med **6**(4): 1168-1177.

Recruitment of neutrophils and monocytes/macrophages to the site of vascular injury is mediated by binding of chemoattractants to interleukin (IL) 8 receptors RA and RB (IL8RA/B) C-C chemokine receptors (CCR) 2 and 5 expressed on neutrophil and monocyte/macrophage membranes. Endothelial cells (ECs) derived from rat-induced pluripotent stem cells (RiPS) were transduced with adenovirus containing cDNA of IL8RA/B and/or CCR2/5. We hypothesized that RiPS-ECs overexpressing IL8RA/B (RiPS-IL8RA/B-ECs), CCR2/5 (RiPS-CCR2/5-ECs), or both receptors (RiPS-IL8RA/B+CCR2/5-ECs) will inhibit inflammatory responses and neointima formation in balloon-injured rat carotid artery. Twelve-week-old male Sprague-Dawley rats underwent balloon injury of the right carotid artery and intravenous infusion of (a) saline vehicle, (b) control RiPS-Null-ECs (ECs transduced with empty virus), (c) RiPS-IL8RA/B-ECs, (d) RiPS-CCR2/5-ECs, or (e) RiPS-IL8RA/B+CCR2/5-ECs. Inflammatory mediator expression and leukocyte infiltration were measured in injured and uninjured arteries at 24 hours postinjury by enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry, respectively. Neointima formation was assessed at 14 days postinjury. RiPS-ECs expressing the IL8RA/B or CCR2/5 homing device targeted the injured arteries and decreased injury-induced inflammatory cytokine expression, neutrophil/macrophage infiltration, and neointima formation. Transfused RiPS-ECs overexpressing IL8RA/B and/or CCR2/5 prevented inflammatory responses and neointima formation after vascular injury. Targeted delivery of iPS-ECs with a homing device to inflammatory mediators in injured arteries provides a novel strategy for the treatment of cardiovascular diseases. Stem Cells Translational Medicine 2017;6:1168-1177.

Giuliani, M., et al. (2011). "Human mesenchymal stem cells derived from induced pluripotent stem cells down-regulate NK-cell cytolytic machinery." Blood **118**(12): 3254-3262.

A major issue in immunosuppressive biotherapy is the use of mesenchymal stem cells (MSCs) that harbor regulatory capacity. However, currently used bone marrow-derived MSCs (BM-MSCs) are short-lived and cannot assure long lasting immunoregulatory function both in vitro and in vivo. Consequently, we have generated MSCs from human induced pluripotent stem (IPS-MSCs) cells that share similar properties with embryonic stem cells (ES-MSCs). Herein, we compared the immunoregulatory properties of ES/IPS-MSCs with those of BM-MSCs and showed, for the first time, that IPS-derived MSCs display remarkable inhibition of NK-cell proliferation and cytolytic function in a similar way to ES-MSCs. Both MSCs disrupt NK-cell cytolytic machinery in the same fashion that BM-MSCs, by down-regulating the expression of different activation markers and ERK1/2 signaling, leading to an impairment to form immunologic synapses with target cells and, therefore, secretion of cytotoxic granules. In addition, they are more resistant than adult BM-MSCs to preactivated NK cells. IPS-MSCs could represent an attractive alternative source of immunoregulatory cells, and their capacity to impair NK-cell cytotoxicity constitutes a complex mechanism to prevent allograft rejection.

Glicksman, M. A. (2018). "Induced Pluripotent Stem Cells: The Most Versatile Source for Stem Cell Therapy." Clin Ther **40**(7): 1060-1065.

Cell therapy has existed since the first bone marrow transplant in the 1950s involving identical twins. The blood-forming stem cells were used to restore healthy blood cells for the twin with leukemia. It was not until 1968 that genetic matching (known as human leukocyte antigen matching) was known to be important, and not until 1973 that bone marrow transplants were performed from non-twin-related and nonrelated donors. The most important application of human stem cells is for the generation of cells and tissues for cell-based therapies. Currently, donated organs and tissues are often the only option to replace diseased, injured, or destroyed tissue. The availability for these transplantable tissues and organs is very limited, however. To satisfy the demand for a source for these cells and tissues, induced pluripotent stem cells that have been differentiated into specific cell types can serve as a renewable source of replacement cells and tissues. A bank of suitable human leukocyte

antigen-matched cells will be an important source providing immediate availability of cells that are readily scalable, economical, and well characterized. Areas of active pursuit with stem cell therapy is being investigated for treating diseases such as macular degeneration, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis, rheumatoid arthritis, and neurodegenerative diseases. This article describes the advantages and hurdles for the use of induced pluripotent cells as the starting material for a source of replacement cells for regenerative medicine.

Godfrey, K. J., et al. (2012). "Stem cell-based treatments for Type 1 diabetes mellitus: bone marrow, embryonic, hepatic, pancreatic and induced pluripotent stem cells." Diabet Med **29**(1): 14-23.

Type 1 diabetes mellitus--characterized by the permanent destruction of insulin-secreting beta-cells--is responsive to cell-based treatments that replace lost beta-cell populations. The current gold standard of pancreas transplantation provides only temporary independence from exogenous insulin and is fraught with complications, including increased mortality. Stem cells offer a number of theoretical advantages over current therapies. Our review will focus on the development of treatments involving tissue stem cells from bone marrow, liver and pancreatic cells, as well as the potential use of embryonic and induced pluripotent stem cells for Type 1 diabetes therapy. While the body of research involving stem cells is at once promising and inconsistent, bone marrow-derived mesenchymal stem cell transplantation seems to offer the most compelling evidence of efficacy. These cells have been demonstrated to increase endogenous insulin production, while partially mitigating the autoimmune destruction of newly formed beta-cells. However, recently successful experiments involving induced pluripotent stem cells could quickly move them into the foreground of therapeutic research. We address the limitations encountered by present research and look toward the future of stem cell treatments for Type 1 diabetes.

Gomez-Cabello, D., et al. (2017). "CtIP-Specific Roles during Cell Reprogramming Have Long-Term Consequences in the Survival and Fitness of Induced Pluripotent Stem Cells." Stem Cell Reports **8**(2): 432-445.

Acquired genomic instability is one of the major concerns for the clinical use of induced pluripotent stem cells (iPSCs). All reprogramming methods are accompanied by the induction of DNA damage, of which double-strand breaks are the most cytotoxic and mutagenic. Consequently, DNA repair genes seem to be relevant for accurate reprogramming to minimize the impact of such DNA damage. Here, we reveal that reprogramming is associated with high levels of DNA end resection, a critical step in homologous recombination. Moreover, the resection factor CtIP is essential for cell reprogramming and establishment of iPSCs, probably to repair reprogramming-induced DNA damage. Our data reveal a new role for DNA end resection in maintaining genomic stability during cell reprogramming, allowing DNA repair fidelity to be retained in both human and mouse iPSCs. Moreover, we demonstrate that reprogramming in a resection-defective environment has long-term consequences on stem cell self-renewal and differentiation.

Gong, J., et al. (2015). "Differentiation of Human Protein-Induced Pluripotent Stem Cells toward a Retinal Pigment Epithelial Cell Fate." PLoS One **10**(11): e0143272.

Compared with many induced pluripotent stem cell (iPSC) lines generated using retrovirus and other non-integrating methods, the utilization of human protein-induced iPSC (piPSC) lines may provide a safer alternative for the generation of retinal pigment epithelial (RPE) cells for transplantation in retinal degenerative diseases. Here we assess the ability of piPSCs to differentiate into RPE cells, and to perform native RPE cell behavior. piPSCs were seeded in 6-well low-attachment plates to allow embryoid body formation, and then analyzed for pluripotent stem cell markers NANOG, SSEA4 and TRA-1-60 by immunofluorescence. Following colony formation, piPSCs were assessed for confirmation of RPE cell differentiation by staining for zonula occludens (ZO-1), bestrophin, microphthalmia-associated transcription factor (MITF) and retinal pigment epithelium specific protein-65 (RPE65). To evaluate piPSC-RPE cell phagocytic ability, adult bovine photoreceptor rod outer segments (ROS) were fed to piPSC-RPE cells, which were analyzed by fluorescent microscopy and flow cytometry. Undifferentiated piPSCs expressed all pluripotent markers assessed and formed embryoid body aggregates after 7 days. Differentiated piPSC-RPE cells expressed ZO-1, bestrophin, MITF and RPE65, typical RPE cell markers. Flow cytometry revealed robust ingestion of fluorescently-labeled ROS by piPSC-RPE cells, which was over four-times greater than that of undifferentiated piPSCs and comparable to that of an immortalized RPE cell line. Phagocytosis activity by piPSC-RPE cells was significantly reduced after the addition of anti-integrin alphaVbeta5. In conclusion, piPSCs can be differentiated toward an RPE cell fate, expressing RPE cell markers and resembling native RPE cells in behavior. These results demonstrate that piPSCs can be differentiated into RPE-like cells using a method that has an increased safety profile, a critical

consideration for the development of better treatments for retinal degenerative diseases such as age-related macular degeneration (AMD).

Gong, Y., et al. (2015). "[Establishment of induced pluripotent stem cell lines from human amniotic fluid cells with 1q21.1 microdeletion]." Zhonghua Yi Xue Yi Chuan Xue Za Zhi **32**(2): 175-179.

OBJECTIVE: To reprogram the 1q21.1 microdeletion pluripotent stem cells in order to establish an ideal model for further studying its pathogenesis. METHODS: Human amniotic fluid-derived cells induced pluripotent stem cells (hAF-iPSCs) were induced from amniotic fluid cells harboring the 1q21.1 microdeletion by retroviral vectors encoding Oct4, Sox2, c-Myc and Klf4. Characteristics of the 1q21.1 microdeletion hAF-iPSCs were determined, which included in vitro pluripotency, karyotype, microarray analysis, the capacity of differentiation in vivo and in vitro, etc. RESULTS: hAF-iPSCs derived from amniotic fluid cells harboring the 1q21.1 microdeletion have maintained self renewal, with expression of pluripotency marker genes detectable at mRNA level. Stem cell surface antigens were tested by immunocytochemistry. The 1q21.1 microdeletion hAF-iPSCs showed a normal karyotype after long-term culturing in vitro, and harbored the same microdeletion as confirmed by microarray analysis. The cells have maintained their differentiation capacity both in vivo and in vitro. CONCLUSION: The hAF-iPSCs harboring the 1q21.1 microdeletion have all the characteristics of normal pluripotent stem cells, and can be used for directed differentiation into specific cells, which may provide an ideal model for studying the pathogenesis of 1q21.1 microdeletion in vitro.

Goyal, A., et al. (2013). "Generation of human induced pluripotent stem cells using epigenetic regulators reveals a germ cell-like identity in partially reprogrammed colonies." PLoS One **8**(12): e82838.

Previous studies have shown that induced pluripotent stem cells (iPSCs) can be derived from fibroblasts by ectopic expression of four transcription factors, OCT4, SOX2, KLF4 and c-MYC using various methods. More recent studies have focused on identifying alternative approaches and factors that can be used to increase reprogramming efficiency of fibroblasts to pluripotency. Here, we use nucleofection, morpholino technologies and novel epigenetic factors, which were chosen based on their expression profile in human embryos, fibroblasts and undifferentiated/differentiated human embryonic stem cells (hESCs) and conventionally generated iPSCs, to reprogram human fibroblasts into iPSCs. By over expressing DNMT3B, AURKB, PRMT5 and/or silencing SETD7 in human fibroblasts with and without NANOG, hTERT and/or SV40 overexpression, we observed the formation of colonies resembling iPSCs that were positive for certain pluripotency markers, but exhibited minimal proliferation. More importantly, we also demonstrate that these partially-reprogrammed colonies express high levels of early to mid germ cell-specific genes regardless of the transfection approach, which suggests conversion to a germ cell-like identity is associated with early reprogramming. These findings may provide an additional means to evaluate human germ cell differentiation in vitro, particularly in the context of pluripotent stem cell-derived germ cell development, and contribute to our understanding of the epigenetic requirements of the reprogramming process.

Griscelli, F., et al. (2012). "Malignant germ cell-like tumors, expressing Ki-1 antigen (CD30), are revealed during in vivo differentiation of partially reprogrammed human-induced pluripotent stem cells." Am J Pathol **180**(5): 2084-2096.

Because many of the genes used to produce induced pluripotent stem cells (iPSCs) from somatic cells are either outright established oncogenes, such as c-myc and Klf4, or potentially related to tumorigenesis in various cancers, both the safety and the risks of tumorigenesis linked to iPSC generation require evaluation. In this work, we generated, by lentivirus-mediated gene transfer of Oct4, Sox2, Nanog, and Lin28, two types of iPSCs from human mesenchymal stem cells and human amniotic fluid-derived cells: fully reprogrammed iPSCs with silencing of the four transgenes and partially reprogrammed iPSCs that still express one or several transgenes. We assessed the behavior of these cells during both their differentiation and proliferation using in vivo teratoma assays in nonobese diabetic mice with severe combined immunodeficiency. In contrast to fully reprogrammed iPSCs, 43% of partially reprogrammed iPSC cases (6 of 14 teratomas) generated major dysplasia and malignant tumors, with yolk sac tumors and embryonal carcinomas positive for alpha-fetoprotein, cytokeratin AE1/AE3, and CD30. This correlated with the expression of one or several transgenes used for the reprogramming, down-regulation of CDK 1A mRNA (p21/CDKN1A), and up-regulation of antiapoptotic Bcl-2 mRNA. Therefore, the oncogenicity of therapeutically valuable patient-specific iPSC-derived cells should be scrupulously evaluated before they are used for any clinical applications.

Gu, M., et al. (2015). "Pravastatin reverses obesity-induced dysfunction of induced pluripotent stem cell-derived endothelial cells via a nitric oxide-dependent mechanism." Eur Heart J **36**(13): 806-816.

AIMS: High-fat diet-induced obesity (DIO) is a major contributor to type II diabetes and micro- and macro-vascular complications leading to peripheral vascular disease (PVD). Metabolic abnormalities of induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) from obese individuals could potentially limit their therapeutic efficacy for PVD. The aim of this study was to compare the function of iPSC-ECs from normal and DIO mice using comprehensive in vitro and in vivo assays. METHODS AND RESULTS: Six-week-old C57Bl/6 mice were fed with a normal or high-fat diet. At 24 weeks, iPSCs were generated from tail tip fibroblasts and differentiated into iPSC-ECs using a directed monolayer approach. In vitro functional analysis revealed that iPSC-ECs from DIO mice had significantly decreased capacity to form capillary-like networks, diminished migration, and lower proliferation. Microarray and ELISA confirmed elevated apoptotic, inflammatory, and oxidative stress pathways in DIO iPSC-ECs. Following hindlimb ischaemia, mice receiving intramuscular injections of DIO iPSC-ECs had significantly decreased reperfusion compared with mice injected with control healthy iPSC-ECs. Hindlimb sections revealed increased muscle atrophy and presence of inflammatory cells in mice receiving DIO iPSC-ECs. When pravastatin was co-administered to mice receiving DIO iPSC-ECs, a significant increase in reperfusion was observed; however, this beneficial effect was blunted by co-administration of the nitric oxide synthase inhibitor, N (omega)-nitro-l-arginine methyl ester. CONCLUSION: This is the first study to provide evidence that iPSC-ECs from DIO mice exhibit signs of endothelial dysfunction and have suboptimal efficacy following transplantation in a hindlimb ischaemia model. These findings may have important implications for future treatment of PVD using iPSC-ECs in the obese population.

Gu, M., et al. (2012). "Microfluidic single-cell analysis shows that porcine induced pluripotent stem cell-derived endothelial cells improve myocardial function by paracrine activation." Circ Res **111**(7): 882-893.

RATIONALE: Induced pluripotent stem cells (iPSCs) hold great promise for the development of patient-specific therapies for cardiovascular disease. However, clinical translation will require preclinical optimization and validation of large-animal iPSC models. OBJECTIVE: To successfully derive endothelial cells from porcine iPSCs and demonstrate their potential utility for the treatment of myocardial ischemia. METHODS AND RESULTS: Porcine adipose stromal cells were reprogrammed to generate porcine iPSCs (piPSCs). Immunohistochemistry, quantitative PCR, microarray hybridization, and angiogenic assays confirmed that piPSC-derived endothelial cells (piPSC-ECs) shared similar morphological and functional properties as endothelial cells isolated from the autologous pig aorta. To demonstrate their therapeutic potential, piPSC-ECs were transplanted into mice with myocardial infarction. Compared with control, animals transplanted with piPSC-ECs showed significant functional improvement measured by echocardiography (fractional shortening at week 4: 27.2+/-1.3% versus 22.3+/-1.1%; P<0.001) and MRI (ejection fraction at week 4: 45.8+/-1.3% versus 42.3+/-0.9%; P<0.05). Quantitative protein assays and microfluidic single-cell PCR profiling showed that piPSC-ECs released proangiogenic and antiapoptotic factors in the ischemic microenvironment, which promoted neovascularization and cardiomyocyte survival, respectively. Release of paracrine factors varied significantly among subpopulations of transplanted cells, suggesting that transplantation of specific cell populations may result in greater functional recovery. CONCLUSIONS: In summary, this is the first study to successfully differentiate piPSCs-ECs from piPSCs and demonstrate that transplantation of piPSC-ECs improved cardiac function after myocardial infarction via paracrine activation. Further development of these large animal iPSC models will yield significant insights into their therapeutic potential and accelerate the clinical translation of autologous iPSC-based therapy.

Guan, L., et al. (2014). "[Study on the induced differentiation of induced pluripotent stem cells into cochlear hair cell-like cells and spiral ganglion neuron-like cells in vitro]." Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi **49**(8): 680-686.

OBJECTIVE: In this study, we investigated the potential of mouse induced pluripotent stem cells (iPSC) for use as a source of transplants for the restoration of auditory hair cells and spiral ganglion neurons. METHODS: We co-cultured the mouse iPSC with the cells of the cochlear organ of Corti or the modiolus in vitro. The cochlear organ of Corti (which contains cochlear hair cells) and the modiolus (which contains auditory spiral ganglion neurons) were obtained from postnatal day 3 (P3) CD-1 ICR mice. After 18 days of coculture with the cells of newborn mouse cochleae. The expressions of hair cell markers (Myosin VIIa, Math1, Calretinin, Espin) and Spiral ganglion neuron markers [Nestin, Neurofilament-M, beta-III Tubulin, Vesicular glutamate transporter 1(VGluT1)] were detected by immunocytochemical

analysis. RESULTS: Immunocytochemical analysis results indicated that the differentiated iPSC expressed auditory hair cell markers (MyosinVIIa,Math1, Calretinin, Espin) and spiral ganglion markers (Nestin, Neurofilament-M,beta-III Tubulin,VGluT1). CONCLUSION: Mouse iPSC in virto cultured could successfully be induced to differentiate into hair cell-like cells and spiral ganglion-like cells with hair cell and spiral ganglion molecular markers.

Gunaratne, P. H. (2009). "Embryonic stem cell microRNAs: defining factors in induced pluripotent (iPS) and cancer (CSC) stem cells?" Curr Stem Cell Res Ther **4**(3): 168-177.

The discovery of microRNAs (miRNAs - small non-coding RNAs of approximately 22 nt) heralded a new and exciting era in biology. During this period miRNAs have gone from ignominy due to their origin mainly in 'junk DNA' to notoriety where they can be at once characterized as being all powerful (a single miRNA can target and potentially silence several hundred genes) and yet marginal (a given gene can be targeted by several miRNAs such that a given miRNA typically exerts a modest repression) [1-4]. The emerging paradox is exemplified by miRNAs that are prominently expressed in embryonic stem (ES) cells. The collective importance of miRNAs is firmly established by the fact that Dicer-/- mouse embryos die on day 7.5 due to defects in differentiation [5]. However, oppositely correlated expression that is expected of conventional repressors is increasingly being defied in multiple systems in relation to miRNA-mRNA target pairs. This is most evident in ES cells where miR-290-295 and 302 clusters the most abundant ES cell miRNAs are found to be driven by pluripotency genes Oct4, Nanog and Sox2 and also target these genes in 'incoherent feed-forward loops' [7]. Here the miRNAs are co-expressed and positively correlated with these targets that they repress suggesting that one of their primary roles is to fine tune gene expression rather than act as ON/OFF switches. On the other hand, let-7 family members that are notably low in ES cells and rapidly induced upon differentiation exhibit more conventional anti-correlated expression patterns with their targets [7-8]. In an intricately designed auto-regulatory loop, LIN28, a key 'keeper' of the pluripotent state binds and represses the processing of let-7 (a key 'keeper' of the differentiated state) [9-11]. One of the let-7 family members, let-7g targets and represses LIN28 through four 3'-UTR binding sites [12]. We propose that LIN28/let-7 pair has the potential to act as a 'toggle switch' that balances the decision to maintain pluripotency vs. differentiation. We also propose that the c-Myc/E2F driven miR17-92 cluster that together controls the G1 to S transition is fundamental for ES self-renewal and cell proliferation [13-18]. In that context it is no surprise that LIN28 and c-Myc (and therefore let-7 and miR-17-92 by association) and more recently Oct4/Sox2 regulated miR-302 has been shown to be among a handful of factors shown to be necessary and sufficient to convert differentiated cells to induced pluripotent stem (iPS) cells [19-29]. It is also no surprise that activation of miR-17-92 (OncomiRs) and down-regulation of let-7 (tumor suppressors) is a recurring theme in relation to cancers from multiple systems [30-48]. We speculate that the LIN28/let-7; c-MYC-E2F/miR-17-92 and Oct4/Sox2/miR-302-cyclin D1 networks are fundamental to properties of pluripotency and self-renewal associated with embryonic stem cells. We also speculate that ES cell miRNA-mRNA associations may also regulate tissue homeostasis and regeneration in the fully developed adult. Consequently, the appropriate regulation of LIN28/let-7; c-MYC-E2F/miR-17-92 and Oct4/Sox2/miR-302-cyclin D1 gene networks will be critical for the success of regenerative strategies that involve iPS cells. Any perturbation in key ES cell miRNA-mRNA networks during any of the above processes maybe a hallmark of (CSCs).

Gunewardene, N., et al. (2016). "Innervation of Cochlear Hair Cells by Human Induced Pluripotent Stem Cell-Derived Neurons In Vitro." Stem Cells Int **2016**: 1781202.

Induced pluripotent stem cells (iPSCs) may serve as an autologous source of replacement neurons in the injured cochlea, if they can be successfully differentiated and reconnected with residual elements in the damaged auditory system. Here, we explored the potential of hiPSC-derived neurons to innervate early postnatal hair cells, using established in vitro assays. We compared two hiPSC lines against a well-characterized hESC line. After ten days' coculture in vitro, hiPSC-derived neural processes contacted inner and outer hair cells in whole cochlear explant cultures. Neural processes from hiPSC-derived neurons also made contact with hair cells in denervated sensory epithelia explants and expressed synapsin at these points of contact. Interestingly, hiPSC-derived neurons cocultured with hair cells at an early stage of differentiation formed synapses with a higher number of hair cells, compared to hiPSC-derived neurons cocultured at a later stage of differentiation. Notable differences in the innervation potentials of the hiPSC-derived neurons were also observed and variations existed between the hiPSC lines in their innervation efficiencies. Collectively, these data illustrate the promise of hiPSCs for auditory neuron replacement and highlight the need to develop methods to mitigate

variabilities observed amongst hiPSC lines, in order to achieve reliable clinical improvements for patients.

Hackett, C. H., et al. (2012). "Comparison of gene-specific DNA methylation patterns in equine induced pluripotent stem cell lines with cells derived from equine adult and fetal tissues." Stem Cells Dev **21**(10): 1803-1811.

Cellular pluripotency is associated with expression of the homeobox transcription factor genes NANOG, SOX2, and POU5F1 (OCT3/4 protein). Some reports suggest that mesenchymal progenitor cells (MPCs) may express increased quantities of these genes, creating the possibility that MPCs are more "pluripotent" than other adult cell types. The objective of this study was to determine whether equine bone marrow-derived MPCs had gene expression or DNA methylation patterns that differed from either early fetal-derived or terminally differentiated adult cells. Specifically, this study compared DNA methylation of the NANOG and SOX2 promoter regions and concurrent gene expression of NANOG, SOX2, and POU5F1 in equine induced pluripotent stem (iPS) cells, fetal fibroblasts, fetal brain cells, adult chondrocytes, and MPCs. Results indicate that NANOG and POU5F1 were not detectable in appreciable quantities in tissues other than the equine iPS cell lines. Equine iPS cells expressed large quantities of all three genes examined. Significantly increased quantities of SOX2 were noted in iPS cells and both fetal-derived cell types compared with adult cells. MPCs and adult chondrocytes expressed equivalent, low quantities of SOX2. Further, NANOG and SOX2 expression inversely correlated with the DNA methylation pattern in the promoter region, such that as gene expression increased, DNA methylation decreased. The equine iPS cell lines examined demonstrated DNA methylation and gene expression patterns that were consistent with pluripotency features described in other species. Results do not support previous reports that NANOG, SOX2, and POU5F1 are poised for increased activity in MPCs compared with other adult cells.

Hamano, S., et al. (2018). "Extracellular Matrix from Periodontal Ligament Cells Could Induce the Differentiation of Induced Pluripotent Stem Cells to Periodontal Ligament Stem Cell-Like Cells." Stem Cells Dev **27**(2): 100-111.

The periodontal ligament (PDL) plays an important role in anchoring teeth in the bone socket. Damage to the PDL, such as after severe inflammation, can be treated with a therapeutic strategy that uses stem cells derived from PDL tissue (PDLSCs), a strategy that has received intense scrutiny over the past decade. However, there is an insufficient number of PDLSCs within the PDL for treating such damage. Therefore, we sought to induce the differentiation of induced pluripotent stem (iPS) cells into PDLSCs as an initial step toward PDL therapy. To this end, we first induced iPS cells into neural crest (NC)-like cells. We then captured the p75 neurotrophic receptor-positive cells (iPS-NC cells) and cultured them on an extracellular matrix (ECM) produced by human PDL cells (iPS-NC-PDL cells). These iPS-NC-PDL cells showed reduced expression of embryonic stem cell and NC cell markers as compared with iPS and iPS-NC cells, and enrichment of mesenchymal stem cell markers. The cells also had a higher proliferative capacity, multipotency, and elevated expression of PDL-related markers than iPS-NC cells cultured on fibronectin and laminin (iPS-NC-FL cells) or ECM produced by human skin fibroblast cells (iPS-NC-SF cells). Overall, we present a culture method to produce high number of PDLSC-like cells from iPS cells as a first step toward a strategy for PDL regeneration.

Haynes, J. M., et al. (2018). "Induced Pluripotent Stem Cell-Derived Podocyte-Like Cells as Models for Assessing Mechanisms Underlying Heritable Disease Phenotype: Initial Studies Using Two Alport Syndrome Patient Lines Indicate Impaired Potassium Channel Activity." J Pharmacol Exp Ther **367**(2): 335-347.

Renal podocyte survival depends upon the dynamic regulation of a complex cell architecture that links the glomerular basement membrane to integrins, ion channels, and receptors. Alport syndrome is a heritable chronic kidney disease where mutations in alpha3, alpha4, or alpha5 collagen genes promote podocyte death. In rodent models of renal failure, activation of the calcium-sensing receptor (CaSR) can protect podocytes from stress-related death. In this study, we assessed CaSR function in podocyte-like cells derived from induced-pluripotent stem cells from two patients with Alport Syndrome (AS1 & AS2) and a renal disease free individual [normal human mesangial cell (NHMC)], as well as a human immortalized podocyte-like (HIP) cell line. Extracellular calcium elicited concentration-dependent elevations of intracellular calcium in all podocyte-like cells. NHMC and HIP, but not AS1 or AS2 podocyte-like cells, also showed acute reductions in intracellular calcium prior to elevation. In NHMC podocyte-like cells this acute reduction was blocked by the large-conductance potassium channel (KCNMA1) inhibitors iberiotoxin (10 nM) and tetraethylammonium (5 mM), as well as the focal adhesion kinase inhibitor PF562271 (N-methyl-N-(3-((2-(2-oxo-2,3-dihydro-1H-indol-5-ylamino)-5-trifluoromethyl-pyrim idin-4-ylamino)-methyl)-pyridin-2-yl)-methanesulfonamide, 10 nM). Quantitative polymerase chain reaction

(qPCR) and immunolabeling showed the presence of KCNMA1 transcript and protein in all podocyte-like cells tested. Cultivation of AS1 podocytes on decellularized plates of NHMC podocyte-like cells partially restored acute reductions in intracellular calcium in response to extracellular calcium. We conclude that the AS patient-derived podocyte-like cells used in this study showed dysfunctional integrin signaling and potassium channel function, which may contribute to podocyte death seen in Alport syndrome.

Hermanson, D. L., et al. (2016). "Induced Pluripotent Stem Cell-Derived Natural Killer Cells for Treatment of Ovarian Cancer." Stem Cells **34**(1): 93-101.

Natural killer (NK) cells can provide effective immunotherapy for ovarian cancer. Here, we evaluated the ability of NK cells isolated from peripheral blood (PB) and NK cells derived from induced pluripotent stem cell (iPSC) to mediate killing of ovarian cancer cells in a mouse xenograft model. A mouse xenograft model was used to evaluate the intraperitoneal delivery of three different NK cell populations: iPSC-derived NK cells, PB-NK cells that had been activated and expanded in long-term culture, and overnight activated PB-NK cells that were isolated through CD3/CD19 depletion of PB B and T cells. Bioluminescent imaging was used to monitor tumor burden of luciferase expressing tumor lines. Tumors were allowed to establish prior to administering NK cells via intraperitoneal injection. These studies demonstrate a single dose of any of the three NK cell populations significantly reduced tumor burden. When mice were given three doses of either iPSC-NK cells or expanded PB-NK cells, the median survival improved from 73 days in mice untreated to 98 and 97 days for treated mice, respectively. From these studies, we conclude iPSC-derived NK cells mediate antiovarian cancer killing at least as well as PB-NK cells, making these cells a viable resource for immunotherapy for ovarian cancer. Due to their ability to be easily differentiated into NK cells and their long-term expansion potential, iPSCs can be used to produce large numbers of well-defined NK cells that can be banked and used to treat a large number of patients including treatment with multiple doses if necessary.

Heslop, J. A., et al. (2017). "Donor-Dependent and Other Nondefined Factors Have Greater Influence on the Hepatic Phenotype Than the Starting Cell Type in Induced Pluripotent Stem Cell Derived Hepatocyte-Like Cells." Stem Cells Transl Med **6**(5): 1321-1331.

Drug-induced liver injury is the greatest cause of post-marketing drug withdrawal; therefore, substantial resources are directed toward triaging potentially dangerous new compounds at all stages of drug development. One of the major factors preventing effective screening of new compounds is the lack of a predictive in vitro model of hepatotoxicity. Primary human hepatocytes offer a metabolically relevant model for which the molecular initiating events of hepatotoxicity can be examined; however, these cells vary greatly between donors and dedifferentiate rapidly in culture. Induced pluripotent stem cell (iPSC)-derived hepatocyte-like cells (HLCs) offer a reproducible, physiologically relevant and genotypically normal model cell; however, current differentiation protocols produce HLCs with a relatively immature phenotype. During the reprogramming of somatic cells, the epigenome undergoes dramatic changes; however, this "resetting" is a gradual process, resulting in an altered differentiation propensity, skewed toward the lineage of origin, particularly in early passage cultures. We, therefore, performed a comparison of human hepatocyte- and dermal fibroblast-derived iPSCs, assessing the impact of epigenetic memory at all stages of HLC differentiation. These results provide the first isogenic assessment of the starting cell type in human iPSC-derived HLCs. Despite a trend toward improvement in hepatic phenotype in albumin secretion and gene expression, few significant differences in hepatic differentiation capacity were found between hepatocyte and fibroblast-derived iPSCs. We conclude that the donor and inter-clonal differences have a greater influence on the hepatocyte phenotypic maturity than the starting cell type. Therefore, it is not necessary to use human hepatocytes for generating iPSC-derived HLCs. Stem Cells Translational Medicine 2017;6:1321-1331.

Hibaoui, Y. and A. Feki (2015). "Concise Review: Methods and Cell Types Used to Generate Down Syndrome Induced Pluripotent Stem Cells." J Clin Med **4**(4): 696-714.

Down syndrome (DS, trisomy 21), is the most common viable chromosomal disorder, with an incidence of 1 in 800 live births. Its phenotypic characteristics include intellectual impairment and several other developmental abnormalities, for the majority of which the pathogenetic mechanisms remain unknown. Several models have been used to investigate the mechanisms by which the extra copy of chromosome 21 leads to the DS phenotype. In the last five years, several laboratories have been successful in reprogramming patient cells carrying the trisomy 21 anomaly into induced pluripotent stem cells, i.e., T21-iPSCs. In this review, we summarize the different T21-iPSCs that have been generated with a particular interest in the technical procedures and the somatic cell types used for the reprogramming.

Hidema, S., et al. (2016). "Transgenic expression of Telomerase reverse transcriptase (Tert) improves cell proliferation of primary cells and enhances reprogramming efficiency into the induced pluripotent stem cell." Biosci Biotechnol Biochem **80**(10): 1925-1933.

The enzymatic activity of telomerase is important for the extension of the telomere repeat sequence and overcoming cellular senescence. We generated a conditional transgenic mouse line, carrying the telomerase reverse transcriptase (Tert) expression cassette, controlled by the Cre-loxP-mediated recombination. In our study, Cre recombinase expression efficiently activated Tert expression, resulting in its increased enzymatic activity, which extended the period of cellular proliferation until the keratinocytes entered senescence. This suggests that transgenic Tert expression is effective in enhancing primary cell proliferation. Notably, Tert expression increased colony formation of induced pluripotent stem (iPS) cells after the introduction of four reprogramming factors, Oct-4, klf4, SOX-2, and c-Myc into the transgenic fibroblasts. To the best of our knowledge, this is the first study to show that the transgenic Tert expression enhances reprogramming efficiency of iPS cells, which indicates a critical role for Tert in the reprogramming process.

Hildebrand, L., et al. (2015). "Selective cell targeting and lineage tracing of human induced pluripotent stem cells using recombinant avian retroviruses." Cell Mol Life Sci **72**(23): 4671-4680.

Human induced pluripotent stem cells (hiPSC) differentiate into multiple cell types. Selective cell targeting is often needed for analyzing gene function by overexpressing proteins in a distinct population of hiPSC-derived cell types and for monitoring cell fate in response to stimuli. However, to date, this has not been possible, as commonly used viruses enter the hiPSC via ubiquitously expressed receptors. Here, we report for the first time the application of a heterologous avian receptor, the tumor virus receptor A (TVA), to selectively transduce TVA (+) cells in a mixed cell population. Expression of the TVA surface receptor via genetic engineering renders cells susceptible for infection by avian leucosis virus (ALV). We generated hiPSC lines with this stably integrated, ectopic TVA receptor gene that expressed the receptor while retaining pluripotency. The undifferentiated hiPSC (TVA+) as well as their differentiating progeny could be infected by recombinant ALV (so-called RCAS virus) with high efficiency. Due to incomplete receptor blocking, even sequential infection of differentiating or undifferentiated TVA (+) cells was possible. In conclusion, the TVA/RCAS system provides an efficient and gentle gene transfer system for hiPSC and extends our possibilities for selective cell targeting and lineage tracing studies.

Himeno, T., et al. (2013). "Mesenchymal stem cell-like cells derived from mouse induced pluripotent stem cells ameliorate diabetic polyneuropathy in mice." Biomed Res Int **2013**: 259187.

BACKGROUND: Although pathological involvements of diabetic polyneuropathy (DPN) have been reported, no dependable treatment of DPN has been achieved. Recent studies have shown that mesenchymal stem cells (MSCs) ameliorate DPN. Here we demonstrate a differentiation of induced pluripotent stem cells (iPSCs) into MSC-like cells and investigate the therapeutic potential of the MSC-like cell transplantation on DPN. RESEARCH DESIGN AND METHODS: For induction into MSC-like cells, GFP-expressing iPSCs were cultured with retinoic acid, followed by adherent culture for 4 months. The MSC-like cells, characterized with flow cytometry and RT-PCR analyses, were transplanted into muscles of streptozotocin-diabetic mice. Three weeks after the transplantation, neurophysiological functions were evaluated. RESULTS: The MSC-like cells expressed MSC markers and angiogenic/neurotrophic factors. The transplanted cells resided in hindlimb muscles and peripheral nerves, and some transplanted cells expressed S100 beta in the nerves. Impairments of current perception thresholds, nerve conduction velocities, and plantar skin blood flow in the diabetic mice were ameliorated in limbs with the transplanted cells. The capillary number-to-muscle fiber ratios were increased in transplanted hindlimbs of diabetic mice. CONCLUSIONS: These results suggest that MSC-like cell transplantation might have therapeutic effects on DPN through secreting angiogenic/neurotrophic factors and differentiation to Schwann cell-like cells.

Hirata, M. and T. Yamaoka (2018). "Effect of stem cell niche elasticity/ECM protein on the self-beating cardiomyocyte differentiation of induced pluripotent stem (iPS) cells at different stages." Acta Biomater **65**: 44-52.

Stem cell-based myocardial regeneration therapies have emerged as alternative strategies to heart transplantation for serious heart diseases, but autologous beating mature cardiomyocytes are not available. Here we investigated the effect of culture substrates on the cardiomyocyte differentiation of induced pluripotent stem cells (iPSs) in vitro by separately evaluating the following continuous three steps: (1) cardiac marker gene expression, (2) contractile gene expression and self-beating, and (3) beating duration. To this end, we used iPS cells to study the cardiac differentiation, and neonatal rat

cardiomyocytes (NCMs) to study beating behavior. These cells were cultured on substrates with different natures, i.e., an elastic substrate (Es) with the modulus of 9, 20, or 180kPa, and hard tissue culture polystyrene dishes (TCPS) coated with collagen type I (Col), gelatin (Gel), or fibronectin (FN). The results revealed that the effective niches in each step were very different. The cardiac marker gene (GATA4, Tbx5, MEF2C) expression of iPSs at the 1st step was very high on the TCPS coated with FN or Gel, whereas on the FN-coated Es (especially with the 9kPa modulus), the undifferentiated marker gene (Nanog) expression of iPSs was maintained. The expression of the contractile genes alpha-MHC, TnC1, and TnT2 and the self-beating (the 2nd step) of the NCMs were high on FN-coated TCPS and Col-coated Es. The 3rd step (beating duration) of the NCMs was effective on the Es, and at 21 days both the iPSs and NCMs stopped beating on the TCPS but were still beating on the Es. Overall, cardiac differentiation 'preferred' ECM-rigid culture substrates, and beating-behavior 'preferred' Col-soft culture substrates. These results are important for understanding and designing cardiac differentiation niches for regenerative medicine, and they suggest that a single culture substrate is not suitable for preparing self-beating cardiomyocytes. STATEMENT OF SIGNIFICANCE: The transplantation of beating cardiomyocytes (BCMs) is expected to be made more effective for serious heart diseases. The identification of the appropriate engineering processes and suitable culture substrates for inducing stem cell differentiation into BCMs is thus indispensable. The differentiation can be divided into three major processes, the cardiac differentiation step, the beating-induction step and the beating-duration step. A protocol with the higher efficiency in all of the steps must be useful. In this study, we separately evaluated the effect of culture substrates at each three step. We clarified that the biological and the physical properties of the culture substrates required at these steps were different. We found useful criteria for effective cardiac cell niche systems design.

Hitomi, T., et al. (2013). "Downregulation of Securin by the variant RNF213 R4810K (rs112735431, G>A) reduces angiogenic activity of induced pluripotent stem cell-derived vascular endothelial cells from moyamoya patients." Biochem Biophys Res Commun **438**(1): 13-19.

Moyamoya disease (MMD) is a cerebrovascular disease characterized by occlusive lesions in the circle of Willis. The RNF213 R4810K polymorphism increases susceptibility to MMD. Induced pluripotent stem cells (iPSCs) were established from unaffected fibroblast donors with wild-type RNF213 alleles, and from carriers/patients with one or two RNF213 R4810K alleles. Angiogenic activities of iPSC-derived vascular endothelial cells (iPSECs) from patients and carriers were lower (49.0 +/- 19.4%) than from wild-type subjects ($p<0.01$). Gene expression profiles in iPSECs showed that Securin was down-regulated ($p<0.01$) in carriers and patients. Overexpression of RNF213 R4810K downregulated Securin, inhibited angiogenic activity (36.0 +/- 16.9%) and proliferation of humanumbilical vein endothelial cells (HUVECs) while overexpression of RNF213 wild type did not. Securin expression was downregulated using RNA interference techniques, which reduced the level of tube formation in iPSECs and HUVECs without inhibition of proliferation. RNF213 R4810K reduced angiogenic activities of iPSECs from patients with MMD, suggesting that it is a promising in vitro model for MMD.

Hossini, A. M., et al. (2015). "Induced pluripotent stem cell-derived neuronal cells from a sporadic Alzheimer's disease donor as a model for investigating AD-associated gene regulatory networks." BMC Genomics **16**: 84.

BACKGROUND: Alzheimer's disease (AD) is a complex, irreversible neurodegenerative disorder. At present there are neither reliable markers to diagnose AD at an early stage nor therapy. To investigate underlying disease mechanisms, induced pluripotent stem cells (iPSCs) allow the generation of patient-derived neuronal cells in a dish. RESULTS: In this study, employing iPS technology, we derived and characterized iPSCs from dermal fibroblasts of an 82-year-old female patient affected by sporadic AD. The AD-iPSCs were differentiated into neuronal cells, in order to generate disease-specific protein association networks modeling the molecular pathology on the transcriptome level of AD, to analyse the reflection of the disease phenotype in gene expression in AD-iPS neuronal cells, in particular in the ubiquitin-proteasome system (UPS), and to address expression of typical AD proteins. We detected the expression of p-tau and GSK3B, a physiological kinase of tau, in neuronal cells derived from AD-iPSCs. Treatment of neuronal cells differentiated from AD-iPSCs with an inhibitor of gamma-secretase resulted in the down-regulation of p-tau. Transcriptome analysis of AD-iPS derived neuronal cells revealed significant changes in the expression of genes associated with AD and with the constitutive as well as the inducible subunits of the proteasome complex. The neuronal cells expressed numerous genes associated with sub-regions within the brain thus suggesting the usefulness of our in-vitro model. Moreover, an AD-related protein interaction network composed of APP and GSK3B among others could be generated using neuronal cells differentiated from two AD-iPS cell lines. CONCLUSIONS: Our

study demonstrates how an iPSC-based model system could represent (i) a tool to study the underlying molecular basis of sporadic AD, (ii) a platform for drug screening and toxicology studies which might unveil novel therapeutic avenues for this debilitating neuronal disorder.

Hou, L., et al. (2016). "Combinatorial extracellular matrix microenvironments promote survival and phenotype of human induced pluripotent stem cell-derived endothelial cells in hypoxia." Acta Biomater **44**: 188-199.

UNLABELLED: Recent developments in cell therapy using human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) hold great promise for treating ischemic cardiovascular tissues. However, poor post-transplantation viability largely limits the potential of stem cell therapy. Although the extracellular matrix (ECM) has become increasingly recognized as an important cell survival factor, conventional approaches primarily rely on single ECMs for in vivo co-delivery with cells, even though the endothelial basement membrane is comprised of a milieu of different ECMs. To address this limitation, we developed a combinatorial ECM microarray platform to simultaneously interrogate hundreds of micro-scale multi-component chemical compositions of ECMs on iPSC-EC response. After seeding iPSC-ECs onto ECM microarrays, we performed high-throughput analysis of the effects of combinatorial ECMs on iPSC-EC survival, endothelial phenotype, and nitric oxide production under conditions of hypoxia (1% O2) and reduced nutrients (1% fetal bovine serum), as is present in ischemic injury sites. Using automated image acquisition and analysis, we identified combinatorial ECMs such as collagen IV+gelatin+heparan sulfate+laminin and collagen IV+fibronectin+gelatin+heparan sulfate+laminin that significantly improved cell survival, nitric oxide production, and CD31 phenotypic expression, in comparison to single-component ECMs. These results were further validated in conventional cell culture platforms and within three-dimensional scaffolds. Furthermore, this approach revealed complex ECM interactions and non-intuitive cell behavior that otherwise could not be easily determined using conventional cell culture platforms. Together these data suggested that iPSC-EC delivery within optimal combinatorial ECMs may improve their survival and function under the condition of hypoxia with reduced nutrients. STATEMENT OF SIGNIFICANCE: Human endothelial cells (ECs) derived from induced pluripotent stem cells (iPSC-ECs) are promising for treating diseases associated with reduced nutrient and oxygen supply like heart failure. However, diminished iPSC-EC survival after implantation into diseased environments limits their therapeutic potential. Since native ECs interact with numerous extracellular matrix (ECM) proteins for functional maintenance, we hypothesized that combinatorial ECMs may improve cell survival and function under conditions of reduced oxygen and nutrients. We developed a high-throughput system for simultaneous screening of iPSC-ECs cultured on multi-component ECM combinations under the condition of hypoxia and reduced serum. Using automated image acquisition and analytical algorithms, we identified combinatorial ECMs that significantly improved cell survival and function, in comparison to single ECMs. Furthermore, this approach revealed complex ECM interactions and non-intuitive cell behavior that otherwise could not be easily determined.

Hoveizi, E., et al. (2015). "In vitro comparative survey of cell adhesion and proliferation of human induced pluripotent stem cells on surfaces of polymeric electrospun nanofibrous and solution-cast film scaffolds." J Biomed Mater Res A **103**(9): 2952-2958.

Extracellular matrix (ECM) components play a critical role in regulating cell behaviors. Interactions between ECM components and cells are important in various biological processes, including cell attachment, survival, morphogenesis, spreading, proliferation, and gene expression. In this study the in vitro responses of human induced pluripotent stem cells (hiPSCs) on polycaprolactone (PCL) electrospun nanofibrous scaffold were reported in comparison with those of the cells on corresponding solution-cast film scaffold. Our results demonstrated that the nanofibrous scaffold showed better support for the attachment and proliferation of hiPSCs than their corresponding film scaffold. Consequently, we emphasize that hiPSCs can sense the physical properties and chemical composition of the materials and regulate their behaviors accordingly.

Hu, G. W., et al. (2015). "Exosomes secreted by human-induced pluripotent stem cell-derived mesenchymal stem cells attenuate limb ischemia by promoting angiogenesis in mice." Stem Cell Res Ther **6**: 10.

INTRODUCTION: 'Patient-specific' induced pluripotent stem cells (iPSCs) are attractive because they can generate abundant cells without the risk of immune rejection for cell therapy. Studies have shown that iPSC-derived mesenchymal stem cells (iMSCs) possess powerful proliferation, differentiation, and therapeutic effects. Recently, most studies indicate that stem cells exert their therapeutic effect mainly through a paracrine mechanism other than transdifferentiation, and exosomes have emerged as an important paracrine

factor for stem cells to reprogram injured cells. The objective of this study was to evaluate whether exosomes derived from iMSCs (iMSCs-Exo) possess the ability to attenuate limb ischemia and promote angiogenesis after transplantation into limbs of mice with femoral artery excision. METHODS: Human iPSCs (iPS-S-01, C1P33, and PCKDSF001C1) were used to differentiate into iMSCs in a modified one-step method. iMSCs were characterized by flow cytometry and multipotent differentiation potential analysis. Ultrafiltration combined with a purification method was used to isolate iMSCs-Exo, and transmission electron microscopy and Western blotting were used to identify iMSCs-Exo. After establishment of mouse hind-limb ischemia with excision of femoral artery and iMSCs-Exo injection, blood perfusion was monitored at days 0, 7, 14, and 21; microvessel density in ischemic muscle was also analyzed. In vitro migration, proliferation, and tube formation experiments were used to analyze the ability of pro-angiogenesis in iMSCs-Exo, and quantitative reverse-transcriptase polymerase chain reaction and enzyme-linked immunosorbent assay were used to identify expression levels of angiogenesis-related molecules in human umbilical vein endothelial cells (HUVECs) after being cultured with iMSCs-Exo. RESULTS: iPSCs were efficiently induced into iMSC- with MSC-positive and -negative surface antigens and osteogenesis, adipogenesis, and chondrogenesis differentiation potential. iMSCs-Exo with a diameter of 57 +/- 11 nm and expressed CD63, CD81, and CD9. Intramuscular injection of iMSCs-Exo markedly enhanced microvessel density and blood perfusion in mouse ischemic limbs, consistent with an attenuation of ischemic injury. In addition, iMSCs-Exo could activate angiogenesis-related molecule expression and promote HUVEC migration, proliferation, and tube formation. CONCLUSION: Implanted iMSCs-Exo was able to protect limbs from ischemic injury via the promotion of angiogenesis, which indicated that iMSCs-Exo may be a novel therapeutic approach in the treatment of ischemic diseases.

Hu, J., et al. (2018). "Embryonic germ cell extracts erase imprinted genes and improve the efficiency of induced pluripotent stem cells." Sci Rep **8**(1): 10955.

Patient-specific induced pluripotent stem cells (iPSCs) have the potential to be useful in the treatment of human diseases. While prior studies have reported multiple methods to generate iPSCs, DNA methylation continues to limit the totipotency and reprogramming efficiency of iPSCs. Here, we first show the competency of embryonic germ cells (EGCs) as a reprogramming catalyst capable of effectively promoting reprogramming induced by four defined factors, including Oct4, Sox2, Klf4 and c-Myc. Combining EGC extracts with these four factors resulted in formation of more embryonic stem cell-like colonies than did factors alone. Notably, expression of imprinted genes was higher with combined induction than with factors alone. Moreover, iPSCs derived from the combined inductors tended to have more global hypomethylation. Our research not only provides evidence that EGC extracts could activate DNA demethylation and reprogram imprinted genes, but also establishes a new way to enhance reprogramming of iPSCs, which remains a critical safety concern for potential use of iPSCs in regenerative medicine.

Hu, S., et al. (2016). "Effects of cellular origin on differentiation of human induced pluripotent stem cell-derived endothelial cells." JCI Insight **1**(8).

Human induced pluripotent stem cells (iPSCs) can be derived from various types of somatic cells by transient overexpression of 4 Yamanaka factors (OCT4, SOX2, C-MYC, and KLF4). Patient-specific iPSC derivatives (e.g., neuronal, cardiac, hepatic, muscular, and endothelial cells [ECs]) hold great promise in drug discovery and regenerative medicine. In this study, we aimed to evaluate whether the cellular origin can affect the differentiation, in vivo behavior, and single-cell gene expression signatures of human iPSC-derived ECs. We derived human iPSCs from 3 types of somatic cells of the same individuals: fibroblasts (FB-iPSCs), ECs (EC-iPSCs), and cardiac progenitor cells (CPC-iPSCs). We then differentiated them into ECs by sequential administration of Activin, BMP4, bFGF, and VEGF. EC-iPSCs at early passage ($10 < P < 20$) showed higher EC differentiation propensity and gene expression of EC-specific markers (PECAM1 and NOS3) than FB-iPSCs and CPC-iPSCs. In vivo transplanted EC-iPSC-ECs were recovered with a higher percentage of CD31(+) population and expressed higher EC-specific gene expression markers (PECAM1, KDR, and ICAM) as revealed by microfluidic single-cell quantitative PCR (qPCR). In vitro EC-iPSC-ECs maintained a higher CD31(+) population than FB-iPSC-ECs and CPC-iPSC-ECs with long-term culturing and passaging. These results indicate that cellular origin may influence lineage differentiation propensity of human iPSCs; hence, the somatic memory carried by early passage iPSCs should be carefully considered before clinical translation.

Huang, H. P., et al. (2010). "Factors from human embryonic stem cell-derived fibroblast-like cells promote topology-dependent hepatic differentiation in primate embryonic and induced pluripotent stem cells." J Biol Chem **285**(43): 33510-33519.

The future clinical use of embryonic stem cell (ESC)-based hepatocyte replacement therapy depends on the development of an efficient procedure for differentiation of hepatocytes from ESCs. Here we report that a high density of human ESC-derived fibroblast-like cells (hESdFs) supported the efficient generation of hepatocyte-like cells with functional and mature hepatic phenotypes from primate ESCs and human induced pluripotent stem cells. Molecular and immunocytochemistry analyses revealed that hESdFs caused a rapid loss of pluripotency and induced a sequential endoderm-to-hepatocyte differentiation in the central area of ESC colonies. Knockdown experiments demonstrated that pluripotent stem cells were directed toward endodermal and hepatic lineages by FGF2 and activin A secreted from hESdFs. Furthermore, we found that the central region of ESC colonies was essential for the hepatic endoderm-specific differentiation, because its removal caused a complete disruption of endodermal differentiation. In conclusion, we describe a novel in vitro differentiation model and show that hESdF-secreted factors act in concert with regional features of ESC colonies to induce robust hepatic endoderm differentiation in primate pluripotent stem cells.

Huang, N. F., et al. (2013). "Chemotaxis of human induced pluripotent stem cell-derived endothelial cells." Am J Transl Res **5**(5): 510-520.

This study examined the homing capacity of human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) and their response to chemotactic gradients of stromal derived factor-1alpha (SDF). We have previously shown that EC derived from murine pluripotent stem cells can home to the ischemic hindlimb of the mouse. In the current study, we were interested to understand if ECs derived from human induced pluripotent stem cells are capable of homing. The homing capacity of iPSC-ECs was assessed after systemic delivery into immunodeficient mice with unilateral hindlimb ischemia. Furthermore, the iPSC-ECs were evaluated for their expression of CXCR4 and their ability to respond to SDF chemotactic gradients in vitro. Upon systemic delivery, the iPSC-ECs transiently localized to the lungs but did not home to the ischemic limb over the course of 14 days. To understand the mechanism of the lack of homing, the expression levels of the homing receptor, CXCR4, was examined at the transcriptional and protein levels. Furthermore, their ability to migrate in response to chemokines was assessed using microfluidic and scratch assays. Unlike ECs derived from syngeneic mouse pluripotent stem cells, human iPSC-ECs do not home to the ischemic mouse hindlimb. This lack of functional homing may represent an impairment of interspecies cellular communication or a difference in the differentiation state of the human iPSC-ECs. These results may have important implications in therapeutic delivery of iPSC-ECs.

Hynes, K., et al. (2014). "Generation of functional mesenchymal stem cells from different induced pluripotent stem cell lines." Stem Cells Dev **23**(10): 1084-1096.

The therapeutic potential of mesenchymal stem cells (MSC) has highlighted the need for identifying easily accessible and reliable sources of these cells. An alternative source for obtaining large populations of MSC is through the controlled differentiation of induced pluripotent stem cells (iPSC). In the present study, colonies of iPSC were cultured in MSC culture media for 2 weeks. Serial passaging then selected for fast growing MSC-like cells with a typical fibroblastic morphology and the capacity to proliferate on standard culture flasks without feeder cells. MSC-like cells were developed from iPSC lines arising from three different somatic tissues: gingiva, periodontal ligament (PDL), and lung. The iPSC-MSC like cells expressed key MSC-associated markers (CD73, CD90, CD105, CD146, and CD166) and lacked expression of pluripotent markers (TRA160, TRA181, and alkaline phosphatase) and hematopoietic markers (CD14, CD34, and CD45). In vitro iPSC-MSC-like cells displayed the capacity to differentiate into osteoblasts, adipocytes, and chondrocytes. In vivo subcutaneous implantation of the iPSC-MSC-like cells into NOD/SCID mice demonstrated that only the PDL-derived iPSC-MSC-like cells exhibited the capacity to form mature mineralized structures which were histologically similar to mature bone. These findings demonstrate that controlled induction of iPSC into fibroblastic-like cells that phenotypically and functionally resemble adult MSC is an attractive approach to obtain a readily available source of progenitor cells for orthopedic and dental-related tissue-engineering applications. However, a detailed characterization of the iPSC-MSC-like cells will be important, as MSC-like cells derived from different iPSC lines exhibit variability in their differentiation capacity.

Ikeda, K., et al. (2017). "Cell fiber-based three-dimensional culture system for highly efficient expansion of human induced pluripotent stem cells." Sci Rep **7**(1): 2850.

Human pluripotent stem cells are a potentially powerful cellular resource for application in regenerative medicine. Because such applications require large numbers of human pluripotent stem cell-derived cells, a scalable culture system of human pluripotent stem cell needs to be developed. Several

suspension culture systems for human pluripotent stem cell expansion exist; however, it is difficult to control the thickness of cell aggregations in these systems, leading to increased cell death likely caused by limited diffusion of gases and nutrients into the aggregations. Here, we describe a scalable culture system using the cell fiber technology for the expansion of human induced pluripotent stem (iPS) cells. The cells were encapsulated and cultured within the core region of core-shell hydrogel microfibers, resulting in the formation of rod-shaped or fiber-shaped cell aggregations with sustained thickness and high viability. By encapsulating the cells with type I collagen, we demonstrated a long-term culture of the cells by serial passaging at a high expansion rate (14-fold in four days) while retaining its pluripotency. Therefore, our culture system could be used for large-scale expansion of human pluripotent stem cells for use in regenerative medicine.

Ikeda, M., et al. (2017). "Implantation of Induced Pluripotent Stem Cell-Derived Tracheal Epithelial Cells." Ann Otol Rhinol Laryngol **126**(7): 517-524.

OBJECTIVES: Compared with using autologous tissue, the use of artificial materials in the regeneration of tracheal defects is minimally invasive. However, this technique requires early epithelialization on the inner side of the artificial trachea. After differentiation from induced pluripotent stem cells (iPSCs), tracheal epithelial tissues may be used to produce artificial tracheas. Herein, we aimed to demonstrate that after differentiation from fluorescent protein-labeled iPSCs, tracheal epithelial tissues survived in nude rats with tracheal defects. METHODS: Red fluorescent tdTomato protein was electroporated into mouse iPSCs to produce tdTomato-labeled iPSCs. Embryoid bodies derived from these iPSCs were then cultured in differentiation medium supplemented with growth factors, followed by culture on air-liquid interfaces for further differentiation into tracheal epithelium. The cells were implanted with artificial tracheas into nude rats with tracheal defects on day 26 of cultivation. On day 7 after implantation, the tracheas were exposed and examined histologically. RESULTS: Tracheal epithelial tissue derived from tdTomato-labeled iPSCs survived in the tracheal defects. Moreover, immunochemical analyses showed that differentiated tissues had epithelial structures similar to those of proximal tracheal tissues. CONCLUSIONS: After differentiation from iPSCs, tracheal epithelial tissues survived in rat bodies, warranting the use of iPSCs for epithelial regeneration in tracheal defects.

Ikemoto, Y., et al. (2017). "Somatic mosaicism containing double mutations in PTCH1 revealed by generation of induced pluripotent stem cells from nevoid basal cell carcinoma syndrome." J Med Genet **54**(8): 579-584.

BACKGROUND: Nevoid basal cell carcinoma syndrome (NBCCS) is an autosomal dominant disorder characterised by developmental defects and tumorigenesis, such as medulloblastomas and basal cell carcinomas, caused by mutations of the patched-1 (PTCH1) gene. In this article, we seek to demonstrate a mosaicism containing double mutations in PTCH1 in an individual with NBCCS. METHODS AND RESULTS: A de novo germline mutation of PTCH1 (c.272delG) was detected in a 31-year-old woman with NBCCS. Gene analysis of two out of four induced pluripotent stem cell (iPSC) clones established from the patient unexpectedly revealed an additional mutation, c.274delT. Deep sequencing confirmed a low-prevalence somatic mutation (5.5%-15.6% depending on the tissue) identical to the one found in iPSC clones. CONCLUSIONS: This is the first case of mosaicism unequivocally demonstrated in NBCCS. Furthermore, the mosaicism is unique in that the patient carries one normal and two mutant alleles. Because these mutations are located in close proximity, reversion error is likely to be involved in this event rather than a spontaneous mutation. In addition, this study indicates that gene analysis of iPSC clones can contribute to the detection of mosaicism containing a minor population carrying a second mutation.

Imagawa, K., et al. (2017). "Generation of a bile salt export pump deficiency model using patient-specific induced pluripotent stem cell-derived hepatocyte-like cells." Sci Rep **7**: 41806.

Bile salt export pump (BSEP) plays an important role in hepatic secretion of bile acids and its deficiency results in severe cholestasis and liver failure. Mutation of the ABCB11 gene encoding BSEP induces BSEP deficiency and progressive familial intrahepatic cholestasis type 2 (PFIC2). Because liver transplantation remains standard treatment for PFIC2, the development of a novel therapeutic option is desired. However, a well reproducible model, which is essential for the new drug development for PFIC2, has not been established. Therefore, we attempted to establish a PFIC2 model by using iPSC technology. Human iPSCs were generated from patients with BSEP-deficiency (BD-iPSC), and were differentiated into hepatocyte-like cells (HLCs). In the BD-iPSC derived HLCs (BD-HLCs), BSEP was not expressed on the cell surface and the biliary excretion capacity was significantly impaired. We also identified a novel mutation in the 5'-untranslated region of the ABCB11 gene that led to aberrant RNA splicing in BD-HLCs. Furthermore, to evaluate the drug efficacy, BD-HLCs were treated with 4-phenylbutyrate (4PBA). The membrane BSEP expression level and the biliary

excretion capacity in BD-HLCs were rescued by 4PBA treatment. In summary, we succeeded in establishing a PFIC2 model, which may be useful for its pathophysiological analysis and drug development.

Itakura, G., et al. (2017). "Low immunogenicity of mouse induced pluripotent stem cell-derived neural stem/progenitor cells." Sci Rep **7**(1): 12996.

Resolving the immunogenicity of cells derived from induced pluripotent stem cells (iPSCs) remains an important challenge for cell transplant strategies that use banked allogeneic cells. Thus, we evaluated the immunogenicity of mouse fetal neural stem/progenitor cells (fetus-NSPCs) and iPSC-derived neural stem/progenitor cells (iPSC-NSPCs) both in vitro and in vivo. Flow cytometry revealed the low expression of immunological surface antigens, and these cells survived in all mice when transplanted syngeneically into subcutaneous tissue and the spinal cord. In contrast, an allogeneic transplantation into subcutaneous tissue was rejected in all mice, and allogeneic cells transplanted into intact and injured spinal cords survived for 3 months in approximately 20% of mice. In addition, cell survival was increased after co-treatment with an immunosuppressive agent. Thus, the immunogenicity and post-transplantation immunological dynamics of iPSC-NSPCs resemble those of fetus-NSPCs.

Itoh, M., et al. (2016). "Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a healthy individual: WT-iPSC1." Stem Cell Res **17**(1): 22-24.

Expanded human T cells from a Japanese healthy male were used to generate integration-free induced pluripotent stem cells (iPSCs) by exogenous expression of four reprogramming factors, OCT3/4, SOX2, cMYC, KLF4, using Sendai virus vector (SeVdp). The authenticity of established iPSC line, WT-iPSC1, was confirmed by the expressions of stem cell markers and the differentiation capability into three germ layers. WT-iPSC1 may be a useful cell resource as a normal control for the comparative study using disease-specific iPSCs.

Iwamoto, H., et al. (2013). "[Cancer vaccine therapy using genetically modified induced pluripotent stem cell-derived dendritic cells expressing the TAA gene]." Gan To Kagaku Ryoho **40**(12): 1575-1577.

It is generally accepted that the difficulty in obtaining a sufficient number of functional dendritic cells (DCs) poses a serious problem in DC-based immunotherapy. Therefore, we used induced pluripotent stem (iPS) cell-derived DCs (iPSDCs) instead. If the therapeutic efficacy of iPSDCs was equivalent to that of bone marrow-derived DCs (BMDCs), then the above-mentioned problems may be solved. In this study, we generated iPSDCs from iPS cells and compared their capacity to mature and migrate to the regional lymph nodes with that of BMDCs. We adenovirally transduced the hgp100 gene, which codes for a natural tumor antigen, into the DCs and immunized the mice with these genetically modified DCs. The cytotoxic activity of CD8(+) cytotoxic T lymphocytes (CTLs) was assayed using a 51Cr-release assay. The therapeutic efficacy of the vaccination was examined in a subcutaneous tumor model. Our results demonstrated that iPSDCs equaled BMDCs in terms of their maturation and migration capacity. Furthermore, hgp100-specific CTLs were generated in mice that were immunized with the genetically modified iPSDCs. These CTLs exhibited a high level of cytotoxicity against B16 cells, which is similar to that exhibited by CTLs generated in BMDCs immunized mice. Moreover, vaccination with genetically modified iPSDCs elicited a high level of therapeutic efficacy equaling that of vaccination with BMDCs. This study clarified experimentally that genetically modified iPSDCs are equivalent to BMDCs in terms of tumor-associated antigen-specific therapeutic antitumor immunity. This vaccination strategy may therefore be useful for future clinical application as a cancer vaccine.

Iwao, T. (2018). "[Development of an In Vitro System for Evaluating Intestinal Drug Disposition Using Human Induced Pluripotent Stem Cell-Derived Intestinal Epithelial Cells]." Yakugaku Zasshi **138**(10): 1241-1247.

Tissues of the small intestine are crucial to understanding drug disposition because these tissues regulate the bioavailability of drugs. However, no evaluation system is currently available for precise and comprehensive analysis of intestinal pharmacokinetics. To address this, functional intestinal epithelial cells were generated from human induced pluripotent stem (iPS) cells for use in pharmacokinetic studies. An improved intestinal differentiation method was established by screening a variety of small molecule compounds against cells during differentiation. The mRNA expression levels of intestinal markers, drug transporters, and CYP3A4 were found to increase following treatment with compounds that act as inhibitors of mitogen-activated protein kinase, DNA methyltransferase, and transforming growth factor-beta. Therefore, we inferred that these compounds enhanced differentiation into intestinal epithelial cells. The differentiated intestinal epithelial cells in the presence of these compounds possessed drug-metabolizing enzyme activities, such as those of CYPs, UDP-glucuronosyltransferase, and sulfotransferase. In addition, these cells had the ability to induce CYP3A4

in the presence of 1alpha,25-dihydroxyvitamin D3. The differentiated intestinal epithelial cells seeded on cell culture inserts formed loose-tight junctions, similar to those in the human small intestine, rather than Caco-2 cells. The cells exhibited polarity, such as apical and basal sides. We also demonstrated that the uptake and efflux transport activities in the cells occurred via peptide transporter and breast cancer resistance protein, respectively. Taken together, it was suggested that human iPS cell-derived intestinal epithelial cells are pharmacokinetically functional, and represent a promising model system for pharmacokinetic studies of drug candidates.

Iwata, Y., et al. (2017). "High-Content Assay Multiplexing for Vascular Toxicity Screening in Induced Pluripotent Stem Cell-Derived Endothelial Cells and Human Umbilical Vein Endothelial Cells." Assay Drug Dev Technol **15**(6): 267-279.

Endothelial cells (ECs) play a major role in blood vessel formation and function. While there is longstanding evidence for the potential of chemical exposures to adversely affect EC function and vascular development, the hazard potential of chemicals with respect to vascular effects is not routinely evaluated in safety assessments. Induced pluripotent stem cell (iPSC)-derived ECs promise to provide a physiologically relevant, organotypic culture model that is amenable for high-throughput (HT) EC toxicant screening and may represent a viable alternative to traditional in vitro models, including human umbilical vein endothelial cells (HUVECs). To evaluate the utility of iPSC-ECs for multidimensional HT toxicity profiling of chemicals, both iPSC-ECs and HUVECs were exposed to selected positive (angiogenesis inhibitors, cytotoxic agents) and negative compounds in concentration response for either 16 or 24 h in a 384-well plate format. Furthermore, chemical effects on vascularization were quantified using EC angiogenesis on biological (Geltrex) and synthetic (SP-105 angiogenesis hydrogel) extracellular matrices. Cellular toxicity was assessed using high-content live cell imaging and the CellTiter-Glo ((R)) assay. Assay performance indicated good to excellent assay sensitivity and reproducibility for both cell types investigated. Both iPSC-derived ECs and HUVECs formed tube-like structures on Geltrex and hydrogel, an effect that was inhibited by angiogenesis inhibitors and cytotoxic agents in a concentration-dependent manner. The quality of HT assays in HUVECs was generally higher than that in iPSC-ECs. Altogether, this study demonstrates the capability of ECs for comprehensive assessment of the biological effects of chemicals on vasculature in a HT compatible format.

Javed, M. S., et al. (2014). "Generation of hepatocyte-like cells from human induced pluripotent stem (iPS) cells by co-culturing embryoid body cells with liver non-parenchymal cell line TWNT-1." J Coll Physicians Surg Pak **24**(2): 91-96.

OBJECTIVE: To generate a homogeneous population of patient-specific hepatocyte-like cells (HLCs) from human iPS cells those show the morphologic and phenotypic properties of primary human hepatocytes. STUDY DESIGN: An experimental study. PLACE AND DURATION OF STUDY: Department of Surgery, Okayama University, Graduate School of Medicine, Japan, from April to December 2011. METHODOLOGY: Human iPS cells were generated and maintained on ES qualified matrigel coated plates supplemented with mTeSR medium or alternatively on mitotically inactivated MEF feeder layer in DMEM/F12 medium containing 20% KOSR, 4ng/ml bFGF-2, 1 x 10-4 M 2-mercaptoethanol, 1 mmol/L NEAA, 2mM L-glutamine and 1% penicillin-streptomycin. iPS cells were differentiated to HLCs by sequential culture using a four step differentiation protocol: (I) Generation of embryoid bodies (EBs) in suspension culture; (II) Induction of definitive endoderm (DE) from 2 days old EBs by growth in human activin-A (100 ng/ml) and basic fibroblasts growth factor (bFGF2) (100 ng/ml) on matrigel coated plates; (III) Induction of hepatic progenitors by co-culture with non-parenchymal human hepatic stellate cell line (TWNT-1); and (IV) Maturation by culture in dexamethasone. Characterization was performed by RT-PCR and functional assays. RESULTS: The generated HLCs showed microscopically morphological phenotype of human hepatocytes, expressed liver-specific genes (ASGPR, Albumin, AFP, Sox17, Fox A2), secreted human liver-specific proteins such as albumin, synthesized urea and metabolized ammonia. CONCLUSION: Functional HLCs were generated from human iPS cells, which could be used for autologus hepatocyte transplantation for liver failure and as in vitro model for determining the metabolic and toxicological properties of drug compounds.

Jerebtsova, M., et al. (2012). "HIV-1 Resistant CDK2-Knockdown Macrophage-Like Cells Generated from 293T Cell-Derived Human Induced Pluripotent Stem Cells." Biology (Basel) **1**(2): 175-195.

A major challenge in studies of human diseases involving macrophages is low yield and heterogeneity of the primary cells and limited ability of these cells for transfections and genetic manipulations. To address this issue, we developed a simple and efficient three steps method for somatic 293T cells reprogramming into monocytes and macrophage-like cells. First, 293T cells were reprogrammed into

induced pluripotent stem cells (iPSCs) through a transfection-mediated expression of two factors, Oct-4 and Sox2, resulting in a high yield of iPSC. Second, the obtained iPSC were differentiated into monocytes using IL-3 and M-CSF treatment. And third, monocytes were differentiated into macrophage-like cells in the presence of M-CSF. As an example, we developed HIV-1-resistant macrophage-like cells from 293T cells with knockdown of CDK2, a factor critical for HIV-1 transcription. Our study provides a proof-of-principle approach that can be used to study the role of host cell factors in HIV-1 infection of human macrophages.

Ji, H., et al. (2017). "Application of induced pluripotent stem cells to model smooth muscle cell function in vascular diseases." Curr Opin Biomed Eng **1**: 38-44.

Vascular smooth muscle cells (SMC) play an essential role in remodeling the vasculature during disease progression. Induced pluripotent stem cells (iPSC) provide an attractive approach to obtain autologous SMC source for patient-specific disease modeling. Here we discuss the current methods to 1) derive functional SMC from iPSC, 2) model vascular diseases using SMC generated from patient-derived iPSC, and 3) modulate microenvironmental cues to enhance cellular differentiation and functionality and better mimic the physiological environment. We emphasize that continuous exploration of biomaterial technologies to engineer a more SMC-specific microenvironment will provide further insight on complex vascular diseases.

Jiang, B., et al. (2015). "SIRT1 Overexpression Maintains Cell Phenotype and Function of Endothelial Cells Derived from Induced Pluripotent Stem Cells." Stem Cells Dev **24**(23): 2740-2745.

Endothelial cells (ECs) that are differentiated from induced pluripotent stem cells (iPSCs) can be used in establishing disease models for personalized drug discovery or developing patient-specific vascularized tissues or organoids. However, a number of technical challenges are often associated with iPSC-ECs in culture, including instability of the endothelial phenotype and limited cell proliferative capacity over time. Early senescence is believed to be the primary mechanism underlying these limitations. Sirtuin1 (SIRT1) is an NAD (+)-dependent deacetylase involved in the regulation of cell senescence, redox state, and inflammatory status. We hypothesize that overexpression of the SIRT1 gene in iPSC-ECs will maintain EC phenotype, function, and proliferative capacity by overcoming early cell senescence. SIRT1 gene was packaged into a lentiviral vector (LV-SIRT1) and transduced into iPSC-ECs at passage 4. Beginning with passage 5, iPSC-ECs exhibited a fibroblast-like morphology, whereas iPSC-ECs overexpressing SIRT1 maintained EC cobblestone morphology. SIRT1 overexpressing iPSC-ECs also exhibited a higher percentage of canonical markers of endothelia (LV-SIRT1 61.8% CD31(+) vs. LV-empty 31.7% CD31(+), $P < 0.001$; LV-SIRT1 46.3% CD144(+) vs. LV-empty 20.5% CD144(+), $P < 0.02$), with a higher nitric oxide synthesis, lower beta-galactosidase production indicating decreased senescence (3.4% for LV-SIRT1 vs. 38.6% for LV-empty, $P < 0.001$), enhanced angiogenesis, increased deacetylation activity, and higher proliferation rate. SIRT1 overexpressing iPSC-ECs continued to proliferate through passage 9 with high purity of EC-like characteristics, while iPSC-ECs without SIRT1 overexpression became senescent after passage 5. Taken together, SIRT1 overexpression in iPSC-ECs maintains EC phenotype, improves EC function, and extends cell lifespan, overcoming critical hurdles associated with the use of iPSC-ECs in translational research.

Jiang, G., et al. (2014). "Human transgene-free amniotic-fluid-derived induced pluripotent stem cells for autologous cell therapy." Stem Cells Dev **23**(21): 2613-2625.

The establishment of a reliable prenatal source of autologous, transgene-free progenitor cells has enormous potential in the development of regenerative-medicine-based therapies for infants born with devastating birth defects. Here, we show that a largely CD117-negative population of human amniotic fluid mesenchymal stromal cells (AF-MSCs) obtained from fetuses with or without prenatally diagnosed anomalies are readily abundant and have limited baseline differentiation potential when compared with bone-marrow-derived MSCs and other somatic cell types. Nonetheless, the AF-MSCs could be easily reprogrammed into induced pluripotent stem cells (iPSCs) using nonintegrating Sendai viral vectors encoding for OCT4, SOX2, KLF4, and cMYC. The iPSCs were virtually indistinguishable from human embryonic stem cells in multiple assays and could be used to generate a relatively homogeneous population of neural progenitors, expressing PAX6, SOX2, SOX3, Musashi-1, and PSA-NCAM, for potential use in neurologic diseases. Further, these neural progenitors showed engraftment potential in vivo and were capable of differentiating into mature neurons and astrocytes in vitro. This study demonstrates the usefulness of AF-MSCs as an excellent source for the generation of human transgene-free iPSCs ideally suited for autologous perinatal regenerative medicine applications.

Joyce, D., et al. (2018). "Induced pluripotent stem cells-derived myeloid-derived suppressor cells regulate the CD8(+) T cell response." Stem Cell Res **29**: 32-41.

Myeloid-derived suppressor cells (MDSCs) are markedly increased in cancer patients and tumor-bearing mice and promote tumor growth and survival by inhibiting host innate and adaptive immunity. In this study, we generated and characterized MDSCs from murine-induced pluripotent stem cells (iPSCs). The iPSCs were co-cultured with OP9 cells, stimulated with GM-CSF, and became morphologically heterologous under co-culturing with hepatic stellate cells. Allogeneic and OVA-specific antigen stimulation demonstrated that iPS-MDSCs have a T-cell regulatory function. Furthermore, a popliteal lymph node assay and autoimmune hepatitis model showed that iPS-MDSCs also regulate immune responsiveness in vivo and have a therapeutic effect against hepatitis. Taken together, our results demonstrated a method of generating functional MDSCs from iPSCs and highlighted the potential of iPS-MDSCs as a key cell therapy resource for transplantation and autoimmune diseases.

Jozefczuk, J., et al. (2011). "Comparative analysis of human embryonic stem cell and induced pluripotent stem cell-derived hepatocyte-like cells reveals current drawbacks and possible strategies for improved differentiation." Stem Cells Dev **20**(7): 1259-1275.

Hepatocytes derived from human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs) could provide a defined and renewable source of human cells relevant for cell replacement therapies and toxicology studies. However, before patient-specific iPSCs can be routinely used for these purposes, there is a dire need to critically compare these cells to the golden standard--hESCs. In this study, we aimed at investigating the differences and similarities at the transcriptional level between hepatocyte-like cells (HLCs) derived from both hESCs and iPSCs. Two independent protocols for deriving HLCs from hESCs and iPSCs were adopted and further characterization included immunocytochemistry, real-time (RT)-polymerase chain reaction, and in vitro functional assays. Comparative microarray-based gene expression profiling was conducted on these cells and compared to the transcriptomes of human fetal liver and adult liver progenitors. HLCs derived from hESCs and human iPSCs showed significant functional similarities, similar expression of genes important for liver physiology and common pathways. However, specific differences between the 2 cell types could be observed. For example, among the cytochrome P450 gene family, CYP19A1, CYP1A1, and CYP11A1 were enriched in hESC-derived HLCs, and CYP46A1 and CYP26A1 in iPSC-derived HLCs. HLCs derived from hESCs and human iPSCs exhibited broad similarities but as well meaningful differences. We identified common upregulated transcription factors, which might serve as a source for generating a cocktail of factors able to directly transdifferentiate somatic cells into HLCs. The findings may be vital to the refinement of protocols for the efficient derivation of functional patient-specific HLCs for regenerative and toxicology studies.

Jung, D. W., et al. (2014). "Reprogram or reboot: small molecule approaches for the production of induced pluripotent stem cells and direct cell reprogramming." ACS Chem Biol **9**(1): 80-95.

Stem cell transplantation is a potential therapy for regenerative medicine, which aims to restore tissues damaged by trauma, aging, and diseases. Since its conception in the late 1990s, chemical biology has provided powerful and diverse small molecule tools for modulating stem cell function. Embryonic stem cells could be an ideal source for transplantation, but ethical concerns restrict their development for cell therapy. The seminal advance of induced pluripotent stem cell (iPSC) technology provided an attractive alternative to human embryonic stem cells. However, iPSCs are not yet considered an ideal stem cell source, due to limitations associated with the reprogramming process and their potential tumorigenic behavior. This is an area of research where chemical biology has made a significant contribution to facilitate the efficient production of high quality iPSCs and elucidate the biological mechanisms governing their phenotype. In this review, we summarize these advances and discuss the latest progress in developing small molecule modulators. Moreover, we also review a new trend in stem cell research, which is the direct reprogramming of readily accessible cell types into clinically useful cells, such as neurons and cardiac cells. This is a research area where chemical biology is making a pivotal contribution and illustrates the many advantages of using small molecules in stem cell research.

Jung, Y., et al. (2012). "Concise review: Induced pluripotent stem cell-derived mesenchymal stem cells: progress toward safe clinical products." Stem Cells **30**(1): 42-47.

Adult stem cell therapies have provided success for more than 50 years, through reconstitution of the hematopoietic system using bone marrow, umbilical cord blood, and mobilized peripheral blood transplantation. Mesenchymal stem cell (MSC)-mediated therapy is a fast-growing field that has

proven safe and effective in the treatment of various degenerative diseases and tissue injuries. Since the first derivation of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), there has been impressive progress toward developing safe clinical applications from PSCs. Recent successes in transgene-free iPSC reprogramming have brought attention to the potential of clinical applications of these pluripotent cells, but key hurdles must be overcome, which are discussed in this review. Looking to the future, it could be advantageous to derive MSC from iPSC or human ESC in cases where genetic engineering is needed, since in the PSCs, clones with "safe harbor" vector integration could be selected, expanded, and differentiated. Here, we describe the status of the progress of the use of MSC and PSCs in clinical trials and analyze the challenges that should be overcome before iPSC-derived MSC therapy can be used widely in the clinic.

Kaichi, S., et al. (2010). "Cell line-dependent differentiation of induced pluripotent stem cells into cardiomyocytes in mice." Cardiovasc Res **88**(2): 314-323.

AIMS: Mouse and human fibroblasts can be directly reprogrammed to pluripotency by the ectopic expression of four transcription factors (Oct3/4, Sox2, Klf4, and c-Myc) to yield induced pluripotent stem (iPS) cells. iPS cells can be generated even without the expression of c-Myc. The present study examined patterns of differentiation of mouse iPS cells into cardiomyocytes in three different cell lines reprogrammed by three or four factors. METHODS AND RESULTS: During the induction of differentiation on feeder-free gelatinized dishes, genes involved in cardiogenesis were expressed as in embryonic stem cells and myogenic contraction occurred in two iPS cell lines. However, in one iPS cell line (20D17) generated by four factors, the expression of cardiac-specific genes and the beating activity were extremely low. Treating iPS cells with trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor, increased Nkx2.5 expression in all iPS cell lines. While the basal Nkx2.5 expression was very low in 20D17, the TSA-induced increase was the greatest. TSA also induced the expression of contractile proteins in 20D17. Furthermore, we demonstrated the increased mRNA level of Oct3/4 and nuclear protein level of HDAC4 in 20D17 compared with the other two iPS cell lines. DNA microarray analysis identified genes whose expression is up- or down-regulated in 20D17. CONCLUSIONS: Mouse iPS cells differentiate into cardiomyocytes in a cell line-dependent manner. TSA induces myocardial differentiation in mouse iPS cells and might be useful to overcome cell line variation in the differentiation efficiency.

Kaitsuka, T. and K. Tomizawa (2015). "Cell-Penetrating Peptide as a Means of Directing the Differentiation of Induced-Pluripotent Stem Cells." Int J Mol Sci **16**(11): 26667-26676.

Protein transduction using cell-penetrating peptides (CPPs) is useful for the delivery of large protein molecules, including some transcription factors. This method is safer than gene transfection methods with a viral vector because there is no risk of genomic integration of the exogenous DNA. Recently, this method was reported as a means for the induction of induced pluripotent stem (iPS) cells, directing the differentiation into specific cell types and supporting gene editing/correction. Furthermore, we developed a direct differentiation method to obtain a pancreatic lineage from mouse and human pluripotent stem cells via the protein transduction of three transcription factors, Pdx1, NeuroD, and MafA. Here, we discuss the possibility of using CPPs as a means of directing the differentiation of iPS cells and other stem cell technologies.

Kajiwara, K., et al. (2017). "Fetal Therapy Model of Myelomeningocele with Three-Dimensional Skin Using Amniotic Fluid Cell-Derived Induced Pluripotent Stem Cells." Stem Cell Reports **8**(6): 1701-1713.

Myelomeningocele (MMC) is a congenital disease without genetic abnormalities. Neurological symptoms are irreversibly impaired after birth, and no effective treatment has been reported to date. Only surgical repairs have been reported so far. In this study, we performed antenatal treatment of MMC with an artificial skin using induced pluripotent stem cells (iPSCs) generated from a patient with Down syndrome (AF-T21-iPSCs) and twin-twin transfusion syndrome (AF-TTTS-iPSCs) to a rat model. We manufactured three-dimensional skin with epidermis generated from keratinocytes derived from AF-T21-iPSCs and AF-TTTS-iPSCs and dermis of human fibroblasts and collagen type I. For generation of epidermis, we developed a protocol using Y-27632 and epidermal growth factor. The artificial skin was successfully covered over MMC defect sites during pregnancy, implying a possible antenatal surgical treatment with iPSC technology.

Kamada, M., et al. (2014). "Tumorigenic risk of human induced pluripotent stem cell explants cultured on mouse SNL76/7 feeder cells." Biochem Biophys Res Commun **453**(3): 668-673.

The potential for tumor formation from transplanted human induced pluripotent stem cell

(hiPSC) derivatives represents a high risk in their application to regenerative medicine. We examined the genetic origin and characteristics of tumors, that were formed when 13 hiPSC lines, established by ourselves, and 201B7 hiPSC from Kyoto University were transplanted into severe combined immune-deficient (SCID) mice. Though teratomas formed in 58% of mice, five angiosarcomas, one malignant solitary fibrous tumor and one undifferentiated pleomorphic sarcoma formed in the remaining mice. Three malignant cell lines were established from the tumors, which were derived from mitomycin C (MMC)-treated SNL76/7 (MMC-SNL) feeder cells, as tumor development from fusion cells between MMC-SNL and hiPSCs was negative by genetic analysis. While parent SNL76/7 cells produced malignant tumors, neither MMC-SNL nor MMC-treated mouse embryo fibroblast (MEF) produced malignant tumors. When MMC-SNL feeder cells were co-cultured with hiPSCs, growing cell lines were generated, that expressed genes similar to the parent SNL76/7 cells. Thus, hiPSCs grown on MMC-SNL feeder cells have a high risk of generating feeder-derived malignant tumors. The possible mechanism (s) of growth restoration and the formation of multiple tumor types are discussed with respect of the interactions between MMC-SNL and hiPSC.

Kamada, M., et al. (2016). "Reversible transformation and de-differentiation of human cells derived from induced pluripotent stem cell teratomas." Hum Cell **29**(1): 1-9.

We first aimed to generate transformed cell lines from a human induced pluripotent stem cell (hiPSC)-teratoma, and then examined the tumorigenic risks of the differentiated cells from hiPSC explant, because hiPSC-derivatives give rise to tumors in immune-deficient mice when transplanted. The colonies isolated from sparse cultures of hiPSC-teratoma cells expressed NANOG and OCT3/4 strongly, and telomerase reverse transcriptase (TERT) weakly. However, soft agar assay demonstrated that only one of them generated colonies in the gel, though hiPSCs, hTERT-transfected immortal cells, and its oncogene-transfected cells did not form any colonies. Furthermore, none of colonies isolated from the soft agar gel on primary culture (passage 0) of teratoma cells, expressed NANOG and OCT3/4 in the expanded cultures. The second soft agar assay on the colony-derived cells was unexpectedly negative. The cumulative growth curve, telomere shortening, and senescence-associated beta-galactosidase (SA beta-gal) staining confirmed the mortality of these cells, suggesting their reversible transformation. By using medium for embryonic stem cell (ESC medium) after MCDB 131 (MCDB) medium, the differentiated culture cells derived from hiPSC-teratoma converted into the cells expressing undifferentiated marker proteins, which lost afterwords even in ESC medium with feeder SNL76/7. The reversibility of transformation and de-differentiation suggest that tumorigenic risks of differentiated cells arise when they are exposed to suitable niches in vivo. Thus, removal of only the undifferentiated cells from iPSC-derivatives before transplantation does not solve the problem. Elucidation of mechanisms of reversibility and control of epigenetic changes is discussed as a safety bottleneck for hiPSC therapy.

Kambal, A., et al. (2011). "Generation of HIV-1 resistant and functional macrophages from hematopoietic stem cell-derived induced pluripotent stem cells." Mol Ther **19**(3): 584-593.

Induced pluripotent stem cells (iPSCs) have radically advanced the field of regenerative medicine by making possible the production of patient-specific pluripotent stem cells from adult individuals. By developing iPSCs to treat HIV, there is the potential for generating a continuous supply of therapeutic cells for transplantation into HIV-infected patients. In this study, we have used human hematopoietic stem cells (HSCs) to generate anti-HIV gene expressing iPSCs for HIV gene therapy. HSCs were dedifferentiated into continuously growing iPSC lines with four reprogramming factors and a combination anti-HIV lentiviral vector containing a CCR5 short hairpin RNA (shRNA) and a human/rhesus chimeric TRIM5alpha gene. Upon directed differentiation of the anti-HIV iPSCs toward the hematopoietic lineage, a robust quantity of colony-forming CD133(+) HSCs were obtained. These cells were further differentiated into functional end-stage macrophages which displayed a normal phenotypic profile. Upon viral challenge, the anti-HIV iPSC-derived macrophages exhibited strong protection from HIV-1 infection. Here, we demonstrate the ability of iPSCs to develop into HIV-1 resistant immune cells and highlight the potential use of iPSCs for HIV gene and cellular therapies.

Kandasamy, K., et al. (2015). "Prediction of drug-induced nephrotoxicity and injury mechanisms with human induced pluripotent stem cell-derived cells and machine learning methods." Sci Rep **5**: 12337.

The renal proximal tubule is a main target for drug-induced toxicity. The prediction of proximal tubular toxicity during drug development remains difficult. Any in vitro methods based on induced pluripotent stem cell-derived renal cells had not been developed, so far. Here, we developed a rapid 1-step protocol for the differentiation of human induced pluripotent stem cells (hiPSC) into proximal tubular-like cells. These proximal tubular-like cells had a

purity of >90% after 8 days of differentiation and could be directly applied for compound screening. The nephrotoxicity prediction performance of the cells was determined by evaluating their responses to 30 compounds. The results were automatically determined using a machine learning algorithm called random forest. In this way, proximal tubular toxicity in humans could be predicted with 99.8% training accuracy and 87.0% test accuracy. Further, we studied the underlying mechanisms of injury and drug-induced cellular pathways in these hiPSC-derived renal cells, and the results were in agreement with human and animal data. Our methods will enable the development of personalized or disease-specific hiPSC-based renal in vitro models for compound screening and nephrotoxicity prediction.

Kaneko, S. (2016). "In Vitro Generation of Antigen-Specific T Cells from Induced Pluripotent Stem Cells of Antigen-Specific T Cell Origin." Methods Mol Biol **1393**: 67-73.

Induced pluripotent stem (iPS) cells derived from T lymphocyte (T-iPS cells) preserve the T cell receptor (TCR) alpha and beta gene rearrangements identical to the original T cell clone. Re-differentiated CD8 single positive alphabeta T cells from the T-iPS cells exhibited antigen-specific cytotoxicity, improved proliferative response, and elongation of telomere indicating rejuvenation of antigen specific T cell immunity in vitro. To regenerate antigen specific cytotoxic T lymphocytes (CTL), first, we have optimized a method for reprogramming-resistant CD8 T cell clones into T-iPS cells by using sendaiviral vectors. Second, we have optimized stepwise differentiation methods for inducing hematopoietic progenitor cells, T cell progenitors, and functionally matured CD8 single positive CTL. These protocols provide useful in vitro tools and models both for research of antigen-specific T cell immunotherapy and for research of normal and pathological thymopoiesis.

Kasuda, S., et al. (2011). "Expression of coagulation factors from murine induced pluripotent stem cell-derived liver cells." Blood Coagul Fibrinolysis **22**(4): 271-279.

A protocol to differentiate liver cells from induced pluripotent stem (iPS) cells is being established. However, the ability of these differentiated iPS cells to express liver-specific proteins, such as coagulation cascade and related factors, has yet to be assessed. This study evaluated whether liver-like populations differentiated from murine iPS cells gain the ability to produce coagulation-related factors. Following differentiation of murine iPS cells into hematopoietic-like and liver-like embryoid bodies, we assessed gene expression profiles for coagulation-related markers, including fibrinogen, factors II, V, VII, VIII, IX, X, XI, XII, and XIIIbeta, protein C, protein S, antithrombin, plasminogen, von Willebrand factor, and ADAMTS13 by real-time reverse transcription PCR. Liver-like embryoid bodies demonstrated strong expression levels of nearly all the coagulation-related genes assessed, compared with undifferentiated iPS cells and hematopoietic-like embryoid bodies. We also confirmed efficient translation and secretion of fibrinogen and albumin (hepatocyte-specific marker proteins) into the conditioned medium by these differentiated cells, suggesting successful differentiation of iPS cells into the liver lineage. These findings suggest that iPS cells can be differentiated into liver-like populations that express coagulation-related factors. Liver-like embryoid bodies may provide a source for cell-based therapies directed toward liver diseases, including coagulation factor deficiencies in the future.

Kawamura, T., et al. (2014). "N-glycans: phenotypic homology and structural differences between myocardial cells and induced pluripotent stem cell-derived cardiomyocytes." PLoS One **9**(10): e111064.

Cell surface glycans vary widely, depending on cell properties. We hypothesized that glycan expression on induced pluripotent stem cells (iPSCs) might change during cardiomyogenic differentiation toward the myocardial phenotype. N-glycans were isolated from iPSCs, iPSC-derived cardiomyocytes (iPSC-CM), and original C57BL/6 mouse myocardium (Heart). Their structures were analyzed by a mapping technique based on HPLC elution times and MALDI-TOF/MS spectra. Sixty-eight different N-glycans were isolated; the structures of 60 of these N-glycans were identified. The quantity of high-mannose type (immature) N-glycans on the iPSCs decreased with cardiomyogenic differentiation, but did not reach the low levels observed in the heart. We observed a similar reduction in neutral N-glycans and an increase in fucosylated or sialyl N-glycans. Some structural differences were detected between iPSC-CM and Heart. No N-glycolyl neuraminic acid (NeuGc) structures were detected in iPSC-CM, whereas the heart contained numerous NeuGc structures, corresponding to the expression of cytidine monophosphate-N-acetylneuraminic acid hydroxylase. Furthermore, several glycans containing Galalpha1-6 Gal, rarely identified in the other cells, were detected in the iPSC-CM. The expression of N-glycan on murine iPSCs changed toward the myocardial phenotype during cardiomyogenic differentiation, leaving the structural differences of NeuGc content or Galalpha1-6 Gal structures. Further studies will be

warranted to reveal the meaning of the difference of N-glycans between the iPSC-CM and the myocardium.

Kim, B. J., et al. (2017). "Modeling Group B Streptococcus and Blood-Brain Barrier Interaction by Using Induced Pluripotent Stem Cell-Derived Brain Endothelial Cells." mSphere **2**(6).

Bacterial meningitis is a serious infection of the central nervous system (CNS) that occurs after bacteria interact with and penetrate the blood-brain barrier (BBB). The BBB is comprised of highly specialized brain microvascular endothelial cells (BMECs) that function to separate the circulation from the CNS and act as a formidable barrier for toxins and pathogens. Certain bacteria, such as Streptococcus agalactiae (group B Streptococcus [GBS]), possess the ability to interact with and penetrate the BBB to cause meningitis. Modeling bacterial interaction with the BBB in vitro has been limited to primary and immortalized BMEC culture. While useful, these cells often do not retain BBB-like properties, and human primary cells have limited availability. Recently, a human induced pluripotent stem cell (iPSC)-derived BMEC model has been established that is readily renewable and retains key BBB phenotypes. Here, we sought to evaluate whether the iPSC-derived BMECs were appropriate for modeling bacterial interaction with the BBB. Using GBS as a model meningeal pathogen, we demonstrate that wild-type GBS adhered to, invaded, and activated the iPSC-derived BMECs, while GBS mutants known to have diminished BBB interaction were attenuated in the iPSC-derived model. Furthermore, bacterial infection resulted in the disruption of tight junction components ZO-1, occludin, and claudin-5. Thus, we show for the first time that the iPSC-derived BBB model can be utilized to study BBB interaction with a bacterial CNS pathogen. IMPORTANCE Here for the first time, human iPSC-derived BMECs were used to model bacterial interaction with the BBB. Unlike models previously used to study these interactions, iPSC-derived BMECs possess robust BBB properties, such as the expression of complex tight junctions that are key components for the investigation of bacterial effects on the BBB. Here, we demonstrated that GBS interacts with the iPSC-derived BMECs and specifically disrupts these tight junctions. Thus, using this BBB model may allow researchers to uncover novel mechanisms of BBB disruption during meningitis that are inaccessible to immortalized or primary cell models that lack substantial tight junctions.

Kim, E. M., et al. (2017). "Induced pluripotent stem cell-derived gamete-associated proteins incite rejection of induced pluripotent stem cells in syngeneic mice." Immunology **151**(2): 191-197.

The safety of induced pluripotent stem cells (iPSCs) in autologous recipients has been questioned after iPSCs, but not embryonic stem cells (ESCs), were reported to be rejected in syngeneic mice. This important topic has remained controversial because there has not been a mechanistic explanation for this phenomenon. Here, we hypothesize that iPSCs, but not ESCs, readily differentiate into gamete-forming cells that express meiotic antigens normally found in immune-privileged gonads. Because peripheral blood T cells are not tolerized to these antigens in the thymus, gamete-associated-proteins (GAPs) sensitize T cells leading to rejection. Here, we provide evidence that GAPs expressed in iPSC teratomas, but not in ESC teratomas, are responsible for the immunological rejection of iPSCs. Furthermore, silencing the expression of Stra8, 'the master regulator of meiosis', in iPSCs, using short hairpin RNA led to significant abrogation of the rejection of iPSCs, supporting our central hypothesis that GAPs expressed after initiation of meiosis in iPSCs were responsible for rejection. In contrast to iPSCs, iPSC-derivatives, such as haematopoietic progenitor cells, are able to engraft long-term into syngeneic recipients because they no longer express GAPs. Our findings, for the first time, provide a unifying explanation of why iPSCs, but not ESCs, are rejected in syngeneic recipients, ending the current controversy on the safety of iPSCs and their derivatives.

Kim, K., et al. (2011). "Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells." Nat Biotechnol **29**(12): 1117-1119.

We compared bona fide human induced pluripotent stem cells (iPSCs) derived from umbilical cord blood (CB) cells and neonatal keratinocytes (K). As a consequence of both incomplete erasure of tissue-specific methylation and aberrant de novo methylation, CB-iPSCs and K-iPSCs were distinct in genome-wide DNA methylation profiles and differentiation potential. Extended passage of some iPSC clones in culture did not improve their epigenetic resemblance to embryonic stem cells, implying that some human iPSCs retain a residual 'epigenetic memory' of their tissue of origin.

Kim, M. H. and M. Kino-oka (2014). "Switching between self-renewal and lineage commitment of human induced pluripotent stem cells via cell-substrate and cell-cell interactions on a dendrimer-immobilized surface." Biomaterials **35**(22): 5670-5678.

Understanding mechanisms that govern cell fate determination of human induced pluripotent stem cells

(hiPSCs) could assist in maintenance of the undifferentiated state during cell expansion. We used polyamidoamine dendrimer surfaces with first-generation (G1), third-generation (G3) and fifth-generation (G5) of dendron structure in cultures of hiPSCs with SNL feeder cells. Cells on the G1 surface formed tightly packed colony with close cell-cell contacts during division and migration; those on the G3 surface exhibited loose or dispersed colony pattern by enhanced migration. On the G5 surface, formation of aggregated colony with ring-like structures occurred spontaneously. We found that the substrate-adsorbed fibronectin and feeder cell-secreted fibronectin appeared elevated levels with the varied generation numbers of dendrimer surfaces. This subsequently resulted in cell migration and in activation of paxillin of hiPSCs. Location-dependent expression of Rac1 induced rearrangement of E-cadherin-mediated cell-cell interactions on dendrimer surfaces, and was associated with alterations in the cell and colony morphology, and migratory behavior. Furthermore, caspase-3 occurred in apoptotic cells on dendrimer surfaces, concomitant with the loss of E-cadherin-mediated cell-cell interactions. Cells on the G1 surface were maintained in an undifferentiated state, while those on the G5 surface exhibited the early commitment to differentiation toward endodermal fates. We conclude that morphological changes associated with altered migration on the dendrimer surfaces were responsible for the coordinated regulation of balance between cell-cell and cell-substrate interactions, thereby switching their transition from self-renewal state to early endoderm differentiation in hiPSCs.

Kim, M. H. and M. Kino-oka (2015). "Maintenance of an undifferentiated state of human induced pluripotent stem cells through migration-dependent regulation of the balance between cell-cell and cell-substrate interactions." J Biosci Bioeng **119**(6): 617-622.

We present an outlook on the current strategies for maintaining and culturing human induced pluripotent stem cells (hiPSCs) in an undifferentiated state without affecting their pluripotency. First, cell structures and function are described in relation to interactions between hiPSCs and their surroundings. Second, the phenomenon of spontaneous deviation from undifferentiated hiPSCs in cultures with feeder cells is addressed, with a summary of current topics that are of particular interest to our studies. The key regulatory factors that can contribute to the growth and maintenance of undifferentiated hiPSCs are also discussed, with a summary of recent work toward a culture strategy to control hiPSC fate through balancing cell-cell and cell-substrate interactions. Finally, we discuss culture process design in our previous studies with respect to maintaining and expanding cultures of undifferentiated and pluripotent hiPSCs. We focus on the regulation of migration-dependent balance between cell-cell and cell-substrate interactions. This review offers novel insights into the regulation and processing of stem cells for research in regenerative medicine.

Kim, M. H., et al. (2018). "Role of cell-secreted extracellular matrix formation in aggregate formation and stability of human induced pluripotent stem cells in suspension culture." J Biosci Bioeng.

Clinical and industrial applications require large quantities of human induced pluripotent stem cells (hiPSCs); however, little is known regarding the mechanisms governing aggregate formation and stability in suspension culture. To address this, we determined differences in growth processes among hiPSC lines in suspension culture. Using an hiPSC aggregate suspension culture system, hiPSCs from different lines formed multicellular aggregates classified as large compact or small loose based on their size and morphology. Time-lapse observation of the growth processes of two different hiPSC lines revealed that the balance between cell division and the extent of subsequent cell death determined the final size and morphology of aggregates. Comparison of the cell survival and death of two hiPSC lines showed that the formation of small, loose aggregates was due to continued cell death during the exponential phase of growth, with apoptotic cells extruded from growing hiPSC aggregates by the concerted contraction of their neighbors. Western blot and immunofluorescent staining revealed that aggregate morphology and proliferative ability relied to a considerable extent upon secretion of the extracellular matrix (ECM). hiPSCs forming large compact and stable aggregates showed enhanced production of collagen type I in suspension culture at 120 h. Furthermore, these aggregates exhibited higher expression of E-cadherin and proliferation marker Ki-67 as compared with levels observed in small and loose aggregates at 120 h. These findings indicated that differences in both aggregate formation and stability in suspension culture among hiPSC lines were caused by differences in ECM secretion capacity.

Kim, S., et al. (2018). "Exosomes Secreted from Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells Accelerate Skin Cell Proliferation." Int J Mol Sci **19**(10).

Induced pluripotent stem cell (iPSC)-derived mesenchymal stem cells (iMSCs) serve as a unique source for cell therapy. We investigated whether exosomes from iMSCs promote the proliferation of

human keratinocytes (HaCaT) and human dermal fibroblasts (HDFs). iPSCs were established from human Wharton's jelly MSCs and were allowed to differentiate into iMSCs. Exosomes were collected from the culture supernatant of MSCs (MSC-exo) and iMSCs (iMSC-exo), and their characteristics were investigated. Both exosome types possessed basic characteristics of exosomes and were taken up by skin cells in vitro and in vivo. A significant increase in HaCaT proliferation was observed with iMSC-exo, although both exosomes increased the viability and cell cycle progression in HaCaT and HDFs. No significant difference was observed in the closure of wound scratch and the expression of reparative genes between cells treated with the two exosome types. Both exosomes enhanced the secretion of collagen in HaCaT and HDFs; however, an increase in fibronectin level was observed only in HaCaT, and this effect was better with iMSC-exo treatment. Only iMSC-exo increased the phosphorylation of extracellular signal-regulated kinase (ERK)-1/2. Our results indicate that iMSC-exo promote the proliferation of skin cells by stimulating ERK1/2 and highlight the application of iMSCs for producing exosomes.

Kimura, H., et al. (2018). "Stem cells purified from human induced pluripotent stem cell-derived neural crest-like cells promote peripheral nerve regeneration." Sci Rep **8**(1): 10071.

Strategies for therapeutic cell transplantation have been assessed for use in the treatment of massive peripheral nerve defects. To support safe and efficient cell transplantation, we have focused on the purification of cells using cell surface markers. Our group previously reported low-affinity nerve growth factor receptor (LNGFR)- and thymocyte antigen-1 (THY-1)-positive neural crest-like cells (LT-NCLCs), generated from human induced pluripotent stem cells (hiPSCs). In the present study, we investigated the efficacy of transplantation of hiPSC-derived LT-NCLCs in a murine massive peripheral nerve defect model. Animals with a sciatic nerve defect were treated with a bridging silicone tube prefilled with LT-NCLCs or medium in the transplantation (TP) and negative control (NC) groups, respectively. The grafted LT-NCLCs survived and enhanced myelination and angiogenesis, as compared to the NC group. Behavioral analysis indicated that motor functional recovery in the TP group was superior to that in the NC group, and similar to that in the autograft (Auto) group. LT-NCLCs promoted axonal regrowth and remyelination by Schwann cells. Transplantation of LT-NCLCs is a promising approach for nerve regeneration treatment of massive peripheral nerve defects.

Kitada, M., et al. (2012). "Muse cells and induced pluripotent stem cell: implication of the elite model." Cell Mol Life Sci **69**(22): 3739-3750.

Induced pluripotent stem (iPS) cells have attracted a great deal attention as a new pluripotent stem cell type that can be generated from somatic cells, such as fibroblasts, by introducing the transcription factors Oct3/4, Sox2, Klf4, and c-Myc. The mechanism of generation, however, is not fully understood. Two mechanistic theories have been proposed; the stochastic model purports that every cell type has the potential to be reprogrammed to become an iPS cell and the elite model proposes that iPS cell generation occurs only from a subset of cells. Some reports have provided theoretical support for the stochastic model, but a recent publication demonstrated findings that support the elite model, and thus the mechanism of iPS cell generation remains under debate. To enhance our understanding of iPS cells, it is necessary to clarify the properties of the original cell source, i.e., the components of the original populations and the potential of each population to become iPS cells. In this review, we discuss the two theories and their implications in iPS cell research.

Kitadani, J., et al. (2016). "[Cancer Immunotherapy Using Human Induced Pluripotent Stem Cell-Derived Dendritic Cells (iPSDCs)Expressing Carcinoembryonic Antigen]." Gan To Kagaku Ryoho **43**(9): 1071-1073.

The difficulty in obtaining a sufficient number of functional dendritic cells (DCs)is a well-known serious problem in DCbased immunotherapy. Therefore, we used induced pluripotent stem cell-derived DCs (iPSDCs). We have reported that mouse iPSDCs are equivalent to BMDCs, in terms of maturation and antigen presentation. In this study, the antitumor immune response of human iPSDCs expressing the carcinoembryonic antigen was examined, to determine its clinical application in gastrointestinal cancer. Human iPS cells were established from healthy human fibroblasts using a Sendai virus vector, and human iPSDCs were differentiated under a feeder-free culture. Additionally, the surface marker expression, cytokine production, and migratory capacity of human iPSDCs were equivalent to those of monocyte-derived DCs (MoDCs). After 3 cycles of stimulation of autologous PBMCs by genetically modified DCs, the 51Cr-release assay was performed. The lymphocytes stimulated by iPSDCs-CEA showed cytotoxic activity against LCL-CEA and CEA652-pulsed LCL, but showed no cytotoxicity against LCL-LacZ. In addition, they showed cytotoxic activity against CEA-positive human cancer cell lines, MKN45 and HT29, but showed no

cytotoxicity against CEA-negative human cancer cell line MKN1. In conclusion, CEA-specific CTLs responses could be induced by iPSDCs-CEA. This vaccination strategy may be useful in future clinical applications of cancer vaccines.

Koch, L., et al. (2018). "Laser bioprinting of human induced pluripotent stem cells-the effect of printing and biomaterials on cell survival, pluripotency, and differentiation." Biofabrication **10**(3): 035005.

Research on human induced pluripotent stem cells (hiPSCs) is one of the fastest growing fields in biomedicine. Generated from patient's own somatic cells, hiPSCs can be differentiated towards all functional cell types and returned to the patient without immunological concerns. 3D printing of hiPSCs could enable the generation of functional organs for replacement therapies or realization of organ-on-chip systems for individualized medicine. Printing of living cells was demonstrated with immortalized cell lines, primary cells, and adult stem cells with different printing technologies and biomaterials. However, hiPSCs are more sensitive to handling procedures, in particular, when dissociated into single cells. Both pluripotency and directed differentiation are influenced by numerous environmental factors including culture media, biomaterials, and cell density. Notably, existing literature on the effect of applied biomaterials on pluripotency is rather ambiguous. In this study, laser bioprinting of undifferentiated hiPSCs in combination with different biomaterials was performed and the impact on cells' behavior, pluripotency, and differentiation was investigated. Our findings suggest that hiPSCs are indeed more sensitive to the applied biomaterials, but not to laser printing itself. With appropriate biomaterials, such as the hyaluronic acid based solutions applied in this study, hiPSCs can be successfully laser printed without losing their pluripotency.

Konagaya, S., et al. (2015). "Long-term maintenance of human induced pluripotent stem cells by automated cell culture system." Sci Rep **5**: 16647.

Pluripotent stem cells, such as embryonic stem cells and induced pluripotent stem (iPS) cells, are regarded as new sources for cell replacement therapy. These cells can unlimitedly expand under undifferentiated conditions and be differentiated into multiple cell types. Automated culture systems enable the large-scale production of cells. In addition to reducing the time and effort of researchers, an automated culture system improves the reproducibility of cell cultures. In the present study, we newly designed a fully automated cell culture system for human iPS maintenance. Using an automated culture system, hiPS cells maintained their undifferentiated state for 60 days. Automatically prepared hiPS cells had a potency of differentiation into three germ layer cells including dopaminergic neurons and pancreatic cells.

Kuise, T., et al. (2014). "Establishment of a pancreatic stem cell line from fibroblast-derived induced pluripotent stem cells." Biomed Eng Online **13**: 64.

BACKGROUND: For cell therapies to treat diabetes, it is important to produce a sufficient number of pancreatic endocrine cells that function similarly to primary islets. Induced pluripotent stem (iPS) cells represent a potentially unlimited source of functional pancreatic endocrine cells. However, the use of iPS cells for laboratory studies and cell-based therapies is hampered by their high tumorigenic potential and limited ability to generate pure populations of differentiated cell types in vitro. The purpose of this study was to establish a pancreatic stem cell line from iPS cells derived from mouse fibroblasts. METHODS: Mouse iPS cells were induced to differentiate into insulin-producing cells by a multi-step differentiation protocol, which was conducted as described previously with minor modifications. Selection of the pancreatic stem cell was based on morphology and Pdx1 expression. The pancreatic potential of the pancreatic stem cells was evaluated using a reverse transcription PCR, real-time PCR, immunofluorescence, and a glucose challenge test. To assess potential tumorigenicity of the pancreatic stem cells, the cells were injected into the quadriceps femoris muscle of the left hindlimb of nude mice. RESULTS: The iPS-derived pancreatic stem cells expressed the transcription factor--Pdx1--a marker of pancreatic development, and continued to divide actively beyond passage 80. Endocrine cells derived from these pancreatic stem cells expressed insulin and pancreatic genes, and they released insulin in response to glucose stimulation. Mice injected with the pancreatic stem cells did not develop tumors, in contrast to mice injected with an equal number of iPS cells. CONCLUSION: This strategy provides a new approach for generation of insulin-producing cells that is more efficient and safer than using iPS cells. We believe that this approach will help to develop a patient-specific cell transplantation therapy for diabetes in the near future.

Kumar, S. S., et al. (2014). "Recent developments in beta-cell differentiation of pluripotent stem cells induced by small and large molecules." Int J Mol Sci **15**(12): 23418-23447.

Human pluripotent stem cells, including human embryonic stem cells (hESCs) and human induced

pluripotent stem cells (hiPSCs), hold promise as novel therapeutic tools for diabetes treatment because of their self-renewal capacity and ability to differentiate into beta (beta)-cells. Small and large molecules play important roles in each stage of beta-cell differentiation from both hESCs and hiPSCs. The small and large molecules that are described in this review have significantly advanced efforts to cure diabetic disease. Lately, effective protocols have been implemented to induce hESCs and human mesenchymal stem cells (hMSCs) to differentiate into functional beta-cells. Several small molecules, proteins, and growth factors promote pancreatic differentiation from hESCs and hMSCs. These small molecules (e.g., cyclopamine, wortmannin, retinoic acid, and sodium butyrate) and large molecules (e.g. activin A, betacellulin, bone morphogentic protein (BMP4), epidermal growth factor (EGF), fibroblast growth factor (FGF), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), noggin, transforming growth factor (TGF-alpha), and WNT3A) are thought to contribute from the initial stages of definitive endoderm formation to the final stages of maturation of functional endocrine cells. We discuss the importance of such small and large molecules in uniquely optimized protocols of beta-cell differentiation from stem cells. A global understanding of various small and large molecules and their functions will help to establish an efficient protocol for beta-cell differentiation.

Kurokawa, Y. K., et al. (2017). "Human Induced Pluripotent Stem Cell-Derived Endothelial Cells for Three-Dimensional Microphysiological Systems." Tissue Eng Part C Methods **23**(8): 474-484.

Microphysiological systems (MPS), or "organ-on-a-chip" platforms, aim to recapitulate in vivo physiology using small-scale in vitro tissue models of human physiology. While significant efforts have been made to create vascularized tissues, most reports utilize primary endothelial cells that hinder reproducibility. In this study, we report the use of human induced pluripotent stem cell-derived endothelial cells (iPS-ECs) in developing three-dimensional (3D) microvascular networks. We established a CDH5-mCherry reporter iPS cell line, which expresses the vascular endothelial (VE)-cadherin fused to mCherry. The iPS-ECs demonstrate physiological functions characteristic of primary endothelial cells in a series of in vitro assays, including permeability, response to shear stress, and the expression of endothelial markers (CD31, von Willibrand factor, and endothelial nitric oxide synthase). The iPS-ECs form stable, perfusable microvessels over the course of 14 days when cultured within 3D microfluidic devices. We also demonstrate that inhibition of TGF-beta signaling improves vascular network formation by the iPS-ECs. We conclude that iPS-ECs can be a source of endothelial cells in MPS providing opportunities for human disease modeling and improving the reproducibility of 3D vascular networks.

Kwon, D. J., et al. (2017). "Effects of Cell Cycle Regulators on the Cell Cycle Synchronization of Porcine induced Pluripotent Stem Cells." Dev Reprod **21**(1): 47-54.

Unlike mouse results, cloning efficiency of nuclear transfer from porcine induced pluripotent stem cells (piPSCs) is very low. The present study was performed to investigate the effect of cell cycle inhibitors on the cell cycle synchronization of piPSCs. piPSCs were generated using combination of six human transcriptional factors under stem cell culture condition. To examine the efficiency of cell cycle synchronization, piPSCs were cultured on a matrigel coated plate with stem cell media and they were treated with staurosporine (STA, 20 nM), daidzein (DAI, 100 muM), roscovitine (ROSC, 10 muM), or olomoucine (OLO, 200 muM) for 12 h. Flow Cytometry (FACs) data showed that piPSCs in control were in G1 (37.5+/-0.2%), S (34.0+/-0.6%) and G2/M (28.5+/-0.4%). The proportion of cells at G1 in DAI group was significantly higher than that in control, while STA, ROSC and OLO treatments could not block the cell cycle of piPSCs. Both of viability and apoptosis were affected by STA and ROSC treatment, but there were no significantly differences between control and DAI groups. Real-Time qPCR and FACs results revealed that DAI treatment did not affect the expression of pluripotent gene, Oct4. In case of OLO, it did not affect both of viability and apoptosis, but Oct4 expression was significantly decreased. Our results suggest that DAI could be used for synchronizing piPSCs at G1 stage and has any deleterious effect on survival and pluripotency sustaining of piPSCs.

Lachmann, N., et al. (2015). "Large-scale hematopoietic differentiation of human induced pluripotent stem cells provides granulocytes or macrophages for cell replacement therapies." Stem Cell Reports **4**(2): 282-296.

Interleukin-3 (IL-3) is capable of supporting the proliferation of a broad range of hematopoietic cell types, whereas granulocyte colony-stimulating factor (G-CSF) and macrophage CSF (M-CSF) represent critical cytokines in myeloid differentiation. When this was investigated in a pluripotent-stem-cell-based hematopoietic differentiation model, IL-3/G-CSF or IL-3/M-CSF exposure resulted in the continuous generation of myeloid cells from an intermediate

myeloid-cell-forming complex containing CD34(+) clonogenic progenitor cells for more than 2 months. Whereas IL-3/G-CSF directed differentiation toward CD45(+)CD11b (+)CD15(+)CD16(+)CD66b (+) granulocytic cells of various differentiation stages up to a segmented morphology displaying the capacity of cytokine-directed migration, respiratory burst response, and neutrophil-extracellular-trap formation, exposure to IL-3/M-CSF resulted in CD45(+)CD11b (+)CD14(+)CD163(+)CD68(+) monocyte/macrophage-type cells capable of phagocytosis and cytokine secretion. Hence, we show here that myeloid specification of human pluripotent stem cells by IL-3/G-CSF or IL-3/M-CSF allows for prolonged and large-scale production of myeloid cells, and thus is suited for cell-fate and disease-modeling studies as well as gene- and cell-therapy applications.

Lagrutta, A., et al. (2016). "Interaction between amiodarone and hepatitis-C virus nucleotide inhibitors in human induced pluripotent stem cell-derived cardiomyocytes and HEK-293 Cav1.2 over-expressing cells." Toxicol Appl Pharmacol **308**: 66-76.

Several clinical cases of severe bradyarrhythmias have been reported upon co-administration of the Hepatitis-C NS5B Nucleotide Polymerase Inhibitor (HCV-NI) direct-acting antiviral agent, sofosbuvir (SOF), and the Class-III anti-arrhythmic amiodarone (AMIO). We model the cardiac drug-drug interaction (DDI) between AMIO and SOF, and between AMIO and a closely-related SOF analog, MNI-1 (Merck Nucleotide Inhibitor #1), in functional assays of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), to provide mechanistic insights into recently reported clinical cases. AMIO co-applied with SOF or MNI-1 increased beating rate or field potential (FP) rate and decreased impedance (IMP) and Ca (2+) transient amplitudes in hiPSC-CM syncytia. This action resembled that of Ca (2+) channel blockers (CCBs) in the model, but CCBs did not substitute for AMIO in the DDI. AMIO analog dronedarone (DRON) did not substitute for, but competed with AMIO in the DDI. Ryanodine and thapsigargin, decreasing intracellular Ca (2+) stores, and SEA-0400, a Na (+)/Ca (2+) exchanger-1 (NCX1) inhibitor, partially antagonized or suppressed DDI effects. Other agents affecting FP rate only exerted additive or subtractive effects, commensurate with their individual effects. We also describe an interaction between AMIO and MNI-1 on Cav1.2 ion channels in an over-expressing HEK-293 cell line. MNI-1 enhanced Cav1.2 channel inhibition by AMIO, but did not affect inhibition of Cav1.2 by DRON, verapamil, nifedipine, or diltiazem. Our data in hiPSC-CMs indicate that HCV-NI agents such as SOF and MNI-1 interact with key intracellular Ca (2+)-handling mechanisms. Additional study in a Cav1.2 HEK-293 cell-line suggests that HCV-NIs potentiate the inhibitory action of AMIO on L-type Ca (2+) channels.

Lahlou, H., et al. (2018). "Modeling human early otic sensory cell development with induced pluripotent stem cells." PLoS One **13**(6): e0198954.

The inner ear represents a promising system to develop cell-based therapies from human induced pluripotent stem cells (hiPSCs). In the developing ear, Notch signaling plays multiple roles in otic region specification and for cell fate determination. Optimizing hiPSC induction for the generation of appropriate numbers of otic progenitors and derivatives, such as hair cells, may provide an unlimited supply of cells for research and cell-based therapy. In this study, we used monolayer cultures, otic-inducing agents, Notch modulation, and marker expression to track early and otic sensory lineages during hiPSC differentiation. Otic/placodal progenitors were derived from hiPSC cultures in medium supplemented with FGF3/FGF10 for 13 days. These progenitor cells were then treated for 7 days with retinoic acid (RA) and epidermal growth factor (EGF) or a Notch inhibitor. The differentiated cultures were analyzed in parallel by qPCR and immunocytochemistry. After the 13 day induction, hiPSC-derived cells displayed an upregulated expression of a panel of otic/placodal markers. Strikingly, a subset of these induced progenitor cells displayed key-otic sensory markers, the percentage of which was increased in cultures under Notch inhibition as compared to RA/EGF-treated cultures. Our results show that modulating Notch pathway during in vitro differentiation of hiPSC-derived otic/placodal progenitors is a valuable strategy to promote the expression of human otic sensory lineage genes.

Lai, F. P., et al. (2017). "Correction of Hirschsprung-Associated Mutations in Human Induced Pluripotent Stem Cells Via Clustered Regularly Interspaced Short Palindromic Repeats/Cas9, Restores Neural Crest Cell Function." Gastroenterology **153**(1): 139-153 e138.

BACKGROUND & AIMS: Hirschsprung disease is caused by failure of enteric neural crest cells (ENCCs) to fully colonize the bowel, leading to bowel obstruction and megacolon. Heterozygous mutations in the coding region of the RET gene cause a severe form of Hirschsprung disease (total colonic aganglionosis). However, 80% of HSCR patients have short-segment Hirschsprung disease (S-HSCR), which has not been associated with genetic factors. We sought to identify mutations associated with S-HSCR, and used the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 gene editing

system to determine how mutations affect ENCC function. METHODS: We created induced pluripotent stem cell (iPSC) lines from 1 patient with total colonic aganglionosis (with the G731del mutation in RET) and from 2 patients with S-HSCR (without a RET mutation), as well as RET (+/-) and RET (-/-) iPSCs. IMR90-iPSC cells were used as the control cell line. Migration and differentiation capacities of iPSC-derived ENCCs were analyzed in differentiation and migration assays. We searched for mutation (s) associated with S-HSCR by combining genetic and transcriptome data from patient blood- and iPSC-derived ENCCs, respectively. Mutations in the iPSCs were corrected using the CRISPR/Cas9 system. RESULTS: ENCCs derived from all iPSC lines, but not control iPSCs, had defects in migration and neuronal lineage differentiation. RET mutations were associated with differentiation and migration defects of ENCCs in vitro. Genetic and transcriptome analyses associated a mutation in the vinculin gene (VCL M209L) with S-HSCR. CRISPR/Cas9 correction of the RET G731del and VCL M209L mutations in iPSCs restored the differentiation and migration capacities of ENCCs. CONCLUSIONS: We identified mutations in VCL associated with S-HSCR. Correction of this mutation in iPSC using CRISPR/Cas9 editing, as well as the RET G731del mutation that causes Hirschsprung disease with total colonic aganglionosis, restored ENCC function. Our study demonstrates how human iPSCs can be used to identify disease-associated mutations and determine how they affect cell functions and contribute to pathogenesis.

Lapillonne, H., et al. (2010). "Red blood cell generation from human induced pluripotent stem cells: perspectives for transfusion medicine." Haematologica **95**(10): 1651-1659.

BACKGROUND: Ex vivo manufacture of red blood cells from stem cells is a potential means to ensure an adequate and safe supply of blood cell products. Advances in somatic cell reprogramming of human induced pluripotent stem cells have opened the door to generating specific cells for cell therapy. Human induced pluripotent stem cells represent a potentially unlimited source of stem cells for erythroid generation for transfusion medicine. DESIGN AND METHODS: We characterized the erythroid differentiation and maturation of human induced pluripotent stem cell lines obtained from human fetal (IMR90) and adult fibroblasts (FD-136) compared to those of a human embryonic stem cell line (H1). Our protocol comprises two steps: (i) differentiation of human induced pluripotent stem cells by formation of embryoid bodies with indispensable conditioning in the presence of cytokines and human plasma to obtain early erythroid commitment, and (ii) differentiation/maturation to the stage of cultured red blood cells in the presence of cytokines. The protocol dispenses with major constraints such as an obligatory passage through a hematopoietic progenitor, co-culture on a cellular stroma and use of proteins of animal origin. RESULTS: We report for the first time the complete differentiation of human induced pluripotent stem cells into definitive erythrocytes capable of maturation up to enucleated red blood cells containing fetal hemoglobin in a functional tetrameric form. CONCLUSIONS: Red blood cells generated from human induced pluripotent stem cells pave the way for future development of allogeneic transfusion products. This could be done by banking a very limited number of red cell phenotype combinations enabling the safe transfusion of a great number of immunized patients.

Lee, E. X., et al. (2011). "Glioma gene therapy using induced pluripotent stem cell derived neural stem cells." Mol Pharm **8**(5): 1515-1524.

Using neural stem cells (NSCs) with tumor tropic migratory capacity to deliver therapeutic genes is an attractive strategy in eliminating metastatic or disseminated tumors. While different methods have been developed to isolate or generate NSCs, it has not been assessed whether induced pluripotent stem (iPS) cells, a type of pluripotent stem cells that hold great potential for regenerative medicine, can be used as a source for derivation of NSCs with tumor tropism. In this study, we used a conventional lentivirus transduction method to derive iPS cells from primary mouse embryonic fibroblasts and then generated NSCs from the iPS cells. To investigate whether the iPS cell derived NSCs can be used in the treatment of disseminated brain tumors, the cells were transduced with a baculoviral vector containing the herpes simplex virus thymidine kinase suicide gene and injected into the cerebral hemisphere contralateral to a tumor inoculation site in a mouse intracranial human glioma xenograft model. We observed that NSCs expressing the suicide gene were, in the presence of ganciclovir, effective in inhibiting the growth of the glioma xenografts and prolonging survival of tumor-bearing mice. Our findings provide evidence for the feasibility of using iPS cell derived NSCs as cellular vehicles for targeted anticancer gene therapy.

Lee, H. K., et al. (2016). "Peripheral blood mononuclear cell-converted induced pluripotent stem cells (iPSCs) from an early onset Alzheimer's patient." Stem Cell Res **16**(2): 213-215.

Improvement in transduction efficiency makes it possible to convert blood cells into induced pluripotent stem cells (iPSC). In this study, we generated an iPSC line from peripheral blood mononuclear cells (PBMC)

donated by a patient who exhibited memory deficit at age 59; outcome of positron emission tomography scan is consistent with a diagnosis of Alzheimer's disease. Integration-free CytoTune-iPS Sendai Reprogramming factors which include Sendai virus particles of the four Yamanaka factors Oct4, Sox2, Klf4, and c-Myc were introduced to PBMC to convert them to iPSCs without retention of virus. Three germ layer differentiation was induced to demonstrate the pluripotency of these iPSCs.

Lee, J., et al. (2018). "Human induced pluripotent stem cell line with cytochrome P450 enzyme polymorphism (CYP2C19*2/CYP3A5*3C) generated from lymphoblastoid cells." Stem Cell Res **27**: 34-37.

Cytochrome P450 (CYP) comprises a superfamily of monooxygenase responsible for the metabolism of xenobiotics and approximately 75% of drugs in use today. Thus, genetic polymorphisms in CYP genes contribute to interindividual differences in hepatic metabolism of drugs, affecting on individual drug efficacy and may cause adverse effects. Here, we generated a human induced pluripotent stem cell (hiPSC) line with pharmacologically important traits (CYP2C19*2/CYP3A5*3C), which are highly polymorphic in Asian from lymphoblastoid cells. This hiPSC line could be a valuable source for predicting individual drug responses in the drug screening process that uses hiPSC-derived somatic cells, including hepatocytes.

Ma, R., et al. (2015). "Thyroid cell differentiation from murine induced pluripotent stem cells." Front Endocrinol (Lausanne) **6**: 56.

BACKGROUND: Here, we demonstrate the successful differentiation of induced pluripotent stem (iPS) cells into functional thyroid cells indicating the therapeutic potential of this approach when applied to individuals with thyroid deficiency. RESEARCH DESIGN AND METHODS: Using embryonic murine fibroblasts, we generated iPS cells with a single lentiviral "stem cell cassette" vector and then differentiated these iPS cells into thyroid cells after transfection with PAX8 and NKX2-1 by Activin A and TSH stimulation. RESULTS: The generated iPS cells expressed pluripotent stem cell markers as assessed using both reverse transcription quantitative PCRs and immunofluorescence staining with ~0.5% reprograming efficiency. Compared to control cells, the expression of thyroid-specific genes NIS, TSHR, Tg, and TPO were greatly enhanced in PAX8(+)NKX2-1(+) iPS cells after differentiation. On stimulation with TSH, these differentiated iPS cells were also capable of dose-dependent cAMP generation and radioiodine uptake indicative of functional thyroid epithelial cells. Furthermore, the cells formed three-dimensional follicles in culture, and "thyroid organoids" formed after PAX8(+)NKX2-1(+) iPS cells transplanted into nude mice, and all expressed Tg protein as judged immunohistochemically. Taken together, thyroid epithelial cells differentiated from iPS cells, which were themselves derived from murine fibroblasts, exhibited very similar properties to thyroid cells previously developed from traditional murine embryonic stem cells. CONCLUSION: Thyroid cells differentiated from iPS cells offer the opportunity to examine the detailed transcriptional regulation of thyroid cell differentiation and may provide a useful future source for individualized regenerative cell therapy.

Magdy, T., et al. (2018). "Human Induced Pluripotent Stem Cell (hiPSC)-Derived Cells to Assess Drug Cardiotoxicity: Opportunities and Problems." Annu Rev Pharmacol Toxicol **58**: 83-103.

Billions of US dollars are invested every year by the pharmaceutical industry in drug development, with the aim of introducing new drugs that are effective and have minimal side effects. Thirty percent of in-pipeline drugs are excluded in an early phase of preclinical and clinical screening owing to cardiovascular safety concerns, and several lead molecules that pass the early safety screening make it to market but are later withdrawn owing to severe cardiac side effects. Although the current drug safety screening methodologies can identify some cardiotoxic drug candidates, they cannot accurately represent the human heart in many aspects, including genomics, transcriptomics, and patient- or population-specific cardiotoxicity. Despite some limitations, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are a powerful and evolving technology that has been shown to recapitulate many attributes of human cardiomyocytes and their drug responses. In this review, we discuss the potential impact of the inclusion of the hiPSC-CM platform in premarket candidate drug screening.

Maguire, E. M., et al. (2017). "Differentiation and Application of Induced Pluripotent Stem Cell-Derived Vascular Smooth Muscle Cells." Arterioscler Thromb Vasc Biol **37**(11): 2026-2037.

Vascular smooth muscle cells (VSMCs) play a role in the development of vascular disease, for example, neointimal formation, arterial aneurysm, and Marfan syndrome caused by genetic mutations in VSMCs, but little is known about the mechanisms of the disease process. Advances in induced pluripotent stem cell technology have now made it possible to derive VSMCs from several different somatic cells using a selection of protocols. As such, researchers have set out to delineate key signaling processes

involved in triggering VSMC gene expression to grasp the extent of gene regulatory networks involved in phenotype commitment. This technology has also paved the way for investigations into diseases affecting VSMC behavior and function, which may be treatable once an identifiable culprit molecule or gene has been repaired. Moreover, induced pluripotent stem cell-derived VSMCs are also being considered for their use in tissue-engineered blood vessels as they may prove more beneficial than using autologous vessels. Finally, while several issues remains to be clarified before induced pluripotent stem cell-derived VSMCs can become used in regenerative medicine, they do offer both clinicians and researchers hope for both treating and understanding vascular disease. In this review, we aim to update the recent progress on VSMC generation from stem cells and the underlying molecular mechanisms of VSMC differentiation. We will also explore how the use of induced pluripotent stem cell-derived VSMCs has changed the game for regenerative medicine by offering new therapeutic avenues to clinicians, as well as providing researchers with a new platform for modeling of vascular disease.

Maldonado, M., et al. (2015). "The effects of electrospun substrate-mediated cell colony morphology on the self-renewal of human induced pluripotent stem cells." Biomaterials **50**: 10-19.

The development of xeno-free, chemically defined stem cell culture systems has been a primary focus in the field of regenerative medicine to enhance the clinical application of pluripotent stem cells (PSCs). In this regard, various electrospun substrates with diverse physiochemical properties were synthesized utilizing various polymer precursors and surface treatments. Human induced pluripotent stem cells (IPSCs) cultured on these substrates were characterized by their gene and protein expression to determine the effects of the substrate physiochemical properties on the cells' self-renewal, i.e., proliferation and the maintenance of pluripotency. The results showed that surface chemistry significantly affected cell colony formation via governing the colony edge propagation. More importantly, when surface chemistry of the substrates was uniformly controlled by collagen conjugation, the stiffness of substrate was inversely related to the sphericity, a degree of three dimensionality in colony morphology. The differences in sphericity subsequently affected spontaneous differentiation of IPSCs during a long-term culture, implicating that the colony morphology is a deciding factor in the lineage commitment of PSCs. Overall, we show that the capability of controlling IPSC colony morphology by electrospun substrates provides a means to modulate IPSC self-renewal.

Malecki, M., et al. (2013). "Safeguarding Stem Cell-Based Regenerative Therapy against Iatrogenic Cancerogenesis: Transgenic Expression of DNASE1, DNASE1L3, DNASE2, DFFB Controlled By POLA1 Promoter in Proliferating and Directed Differentiation Resisting Human Autologous Pluripotent Induced Stem Cells Leads to their Death." J Stem Cell Res Ther **Suppl 9**(5).

INTRODUCTION: The worst possible complication of using stem cells for regenerative therapy is iatrogenic cancerogenesis. The ultimate goal of our work is to develop a self-triggering feedback mechanism aimed at causing death of all stem cells, which resist directed differentiation, keep proliferating, and can grow into tumors. SPECIFIC AIM: The specific aim was threefold: (1) to genetically engineer the DNA constructs for the human, recombinant DNASE1, DNASE1L3, DNASE2, DFFB controlled by POLA promoter; (2) to bioengineer anti-SSEA-4 antibody guided vectors delivering transgenes to human undifferentiated and proliferating pluripotent stem cells; (3) to cause death of proliferating and directed differentiation resisting stem cells by transgenic expression of the human recombinant the DNases (hrDNases). METHODS: The DNA constructs for the human, recombinant DNASE1, DNASE1L3, DNASE2, DFFB controlled by POLA promoter were genetically engineered. The vectors targeting specifically SSEA-4 expressing stem cells were bioengineered. The healthy volunteers' bone marrow mononuclear cells (BMMCs) were induced into human, autologous, pluripotent stem cells with non-integrating plasmids. Directed differentiation of the induced stem cells into endothelial cells was accomplished with EGF and BMP. The anti-SSEA 4 antibodies' guided DNA vectors delivered the transgenes for the human recombinant DNases' into proliferating stem cells. RESULTS: Differentiation of the pluripotent induced stem cells into the endothelial cells was verified by highlighting formation of tight and adherens junctions through transgenic expression of recombinant fluorescent fusion proteins: VE cadherin, claudin, zona occludens 1, and catenin. Proliferation of the stem cells was determined through highlighting transgenic expression of recombinant fluorescent proteins controlled by POLA promoter, while also reporting expression of the transgenes for the hrDNases. Expression of the transgenes for the DNases resulted in complete collapse of the chromatin architecture and degradation of the proliferating cells' genomic DNA. The proliferating stem cells, but not the differentiating ones, were effectively induced to die. CONCLUSION: Herein, we describe attaining the proof-of-concept for the strategy, whereby transgenic expression of the genetically engineered human recombinant DNases in proliferating and directed

differentiation resisting stem cells leads to their death. This novel strategy reduces the risk of iatrogenic neoplasms in stem cell therapy.

Marion, R. M., et al. (2009). "Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells." Cell Stem Cell **4**(2): 141-154.

Telomere shortening is associated with organismal aging. iPS cells have been recently derived from old patients; however, it is not known whether telomere chromatin acquires the same characteristics as in ES cells. We show here that telomeres are elongated in iPS cells compared to the parental differentiated cells both when using four (Oct3/4, Sox2, Klf4, cMyc) or three (Oct3/4, Sox2, Klf4) reprogramming factors and both from young and aged individuals. We demonstrate genetically that, during reprogramming, telomere elongation is usually mediated by telomerase and that iPS telomeres acquire the epigenetic marks of ES cells, including a low density of trimethylated histones H3K9 and H4K20 and increased abundance of telomere transcripts. Finally, reprogramming efficiency of cells derived from increasing generations of telomerase-deficient mice shows a dramatic decrease in iPS cell efficiency, a defect that is restored by telomerase reintroduction. Together, these results highlight the importance of telomere biology for iPS cell generation and functionality.

Matsumoto, T., et al. (2016). "Functional Neurons Generated from T Cell-Derived Induced Pluripotent Stem Cells for Neurological Disease Modeling." Stem Cell Reports **6**(3): 422-435.

Modeling of neurological diseases using induced pluripotent stem cells (iPSCs) derived from the somatic cells of patients has provided a means of elucidating pathogenic mechanisms and performing drug screening. T cells are an ideal source of patient-specific iPSCs because they can be easily obtained from samples. Recent studies indicated that iPSCs retain an epigenetic memory relating to their cell of origin that restricts their differentiation potential. The classical method of differentiation via embryoid body formation was not suitable for T cell-derived iPSCs (TiPSCs). We developed a neurosphere-based robust differentiation protocol, which enabled TiPSCs to differentiate into functional neurons, despite differences in global gene expression between TiPSCs and adult human dermal fibroblast-derived iPSCs. Furthermore, neurons derived from TiPSCs generated from a juvenile patient with Parkinson's disease exhibited several Parkinson's disease phenotypes. Therefore, we conclude that TiPSCs are a useful tool for modeling neurological diseases.

Matsumura, T., et al. (2014). "Single-cell cloning and expansion of human induced pluripotent stem cells by a microfluidic culture device." Biochem Biophys Res Commun **453**(1): 131-137.

The microenvironment of cells, which includes basement proteins, shear stress, and extracellular stimuli, should be taken into consideration when examining physiological cell behavior. Although microfluidic devices allow cellular responses to be analyzed with ease at the single-cell level, few have been designed to recover cells. We herein demonstrated that a newly developed microfluidic device helped to improve culture conditions and establish a clonality-validated human pluripotent stem cell line after tracing its growth at the single-cell level. The device will be a helpful tool for capturing various cell types in the human body that have not yet been established in vitro.

Matsushita, N., et al. (2014). "[Establishment of induced pluripotent stem cells from adipose tissue-derived stem cells for dendritic cell-based cancer vaccines]." Gan To Kagaku Ryoho **41**(4): 467-470.

Recently, studies on regenerative stem cell therapy are being encouraged, and efforts to generate dendritic cells, which play important roles in cancer immunotherapy, from stem cells are being made in the field of tumor immunology. Therapeutic acquisition of stem cells has important clinical applications. Studies on induced pluripotent stem (iPS)cells generated from somatic cells with pluripotent genes have advanced in recent years. Stem cells are reported to be found in adipose tissue (adipose-derived stem cells, ADSC). Our goal is to develop a new cancer vaccine by using dendritic cells generated from ADSC. In a preliminary study, we examined whether iPS cells can be generated from ADSC to serve as a source of dendritic cells.We introduced a plasmid with pluripotent genes (OCT3/4, KLF4, SOX2, L-MYC, LIN28, p53-shRNA)into an ADSC strain derived from adipose tissue by electroporation and subsequently cultured the cells for further examination. A colony sugges- tive of iPS cells from ADSC was observed. OCT3/4, KLF4, SOX2, L-MYC, and LIN28 mRNAs were expressed in the cultured cells, as confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR). On the basis of these results, we confirmed that iPS cells were generated from ADSC. The method of inducing dendritic cells from iPS cells has already been reported, and the results of this study suggest that ADSC is a potential source of dendritic cells.

Matsuura, K., et al. (2015). "Elimination of remaining undifferentiated induced pluripotent stem cells in the process of human cardiac cell sheet

fabrication using a methionine-free culture condition." Tissue Eng Part C Methods **21**(3): 330-338.

Cardiac tissue engineering is a promising method for regenerative medicine. Although we have developed human cardiac cell sheets by integration of cell sheet-based tissue engineering and scalable bioreactor culture, the risk of contamination by induced pluripotent stem (iPS) cells in cardiac cell sheets remains unresolved. In the present study, we established a novel culture method to fabricate human cardiac cell sheets with a decreased risk of iPS cell contamination while maintaining viabilities of iPS cell-derived cells, including cardiomyocytes and fibroblasts, using a methionine-free culture condition. When cultured in the methionine-free condition, human iPS cells did not survive without feeder cells and could not proliferate or form colonies on feeder cells or in coculture with cells for cardiac cell sheet fabrication. When iPS cell-derived cells after the cardiac differentiation were transiently cultured in the methionine-free condition, gene expression of OCT3/4 and NANOG was downregulated significantly compared with that in the standard culture condition. Furthermore, in fabricated cardiac cell sheets, spontaneous and synchronous beating was observed in the whole area while maintaining or upregulating the expression of various cardiac and extracellular matrix genes. These findings suggest that human iPS cells are methionine dependent and a methionine-free culture condition for cardiac cell sheet fabrication might reduce the risk of iPS cell contamination.

Mauritz, C., et al. (2011). "Induced pluripotent stem cell (iPSC)-derived Flk-1 progenitor cells engraft, differentiate, and improve heart function in a mouse model of acute myocardial infarction." Eur Heart J **32**(21): 2634-2641.

AIMS: Induced pluripotent stem cell (iPSC)-derived cardiovascular progenitor cells represent a suitable autologous cell source for myocardial regeneration as they have the capability to form myocardial cells and to contribute to revascularization. As a first proof of concept we evaluated the potential of a murine iPSC-derived cardiovascular progenitor population, which expresses the surface marker foetal liver kinase-1 (Flk-1), to restore myocardial tissue and improve cardiac function after acute myocardial infarction (MI) in mice. METHODS AND RESULTS: iPSC-derived Flk-1(pos) vs. Flk-1(neg) cells were selected by fluorescence activated cell sorting (FACS) and injected into the ischaemic myocardium of left anterior descending coronary artery (LAD)-ligated mice. Addressing safety aspects we used an octamer binding factor 4 (Oct4)-enhanced green fluorescent protein (eGFP) expressing iPSC clone from the transgenic Oct4-eGFP reporter mouse strain OG2 to enable FACS-based depletion of undifferentiated cells prior to transplantation. Infarcted animals were treated with placebo (phosphate-buffered saline, n = 13), Flk-1(neg) cells (n = 14), or Flk-1(pos) cells (n = 11; 5 x 10(5) cells each). Heart function was evaluated by magnetic resonance imaging and conductance catheter analysis 2 weeks postoperatively. Cardiovascular in vitro and in vivo differentiations were investigated by immunofluorescence staining. Treatment with Flk-1(pos) and Flk-1(neg) cells resulted in a favourable myocardial remodelling and improved left ventricular function. Engraftment and functional benefits were superior after transplantation of Flk-1(pos) compared with Flk-1(neg) cells. Furthermore, Flk-1(pos) grafts contained considerably more vascular structures in relation to Flk-1(neg) grafts. CONCLUSION: iPSC-derived Flk-1(pos) progenitor cells differentiate into cardiovascular lineages in vitro and in vivo and improve cardiac function after acute MI. This proof of concept study paves the way for an autologous iPSC-based therapy of MI.

McLaren, D., et al. (2013). "Automated large-scale culture and medium-throughput chemical screen for modulators of proliferation and viability of human induced pluripotent stem cell-derived neuroepithelial-like stem cells." J Biomol Screen **18**(3): 258-268.

The aim of this study was to demonstrate proof-of-concept feasibility for the use of human neural stem cells (NSCs) for high-throughput screening (HTS) applications. For this study, an adherent human induced pluripotent stem (iPS) cell-derived long-term, self-renewing, neuroepithelial-like stem (lt-NES) cell line was selected as a representative NSC. Here, we describe the automated large-scale serum-free culture ("scale-up") of human lt-NES cells on the CompacT SelecT cell culture robotic platform, followed by their subsequent automated "scale-out" into a microwell plate format. We also report a medium-throughput screen of 1000 compounds to identify modulators of neural stem cell proliferation and/or survival. The screen was performed on two independent occasions using a cell viability assay with end-point reading resulting in the identification of 24 potential hit compounds, 5 of which were found to increase the proliferation and/or survival of human lt-NES on both occasions. Follow-up studies confirmed a dose-dependent effect of one of the hit compounds, which was a Cdk-2 modulator. This approach could be further developed as part of a strategy to screen compounds to either improve the procedures for the in vitro expansion of neural stem cells or to potentially modulate endogenous neural stem cell behavior in the diseased nervous system.

Medrano, J. V., et al. (2014). "Human germ cell differentiation from pluripotent embryonic stem cells and induced pluripotent stem cells." Methods Mol Biol **1154**: 563-578.

Although 10-15 % of couples are infertile, little is known of the diverse, underlying pathologies in men and women with poor germ cell production; furthermore, for those with few or no high-quality germ cells, there are few options available for treatment. Thus, over the last decade, concerted efforts have been aimed at developing a biological system to probe the fundamentals of human egg and sperm production via pluripotent stem cell cells with the hopes of informing clinical decisions and ultimately providing alternative methods for therapy which may include developing a source of germ cells ultimately for reproductive purposes.

Meneghini, V., et al. (2017). "Generation of Human Induced Pluripotent Stem Cell-Derived Bona Fide Neural Stem Cells for Ex Vivo Gene Therapy of Metachromatic Leukodystrophy." Stem Cells Transl Med **6**(2): 352-368.

Allogeneic fetal-derived human neural stem cells (hfNSCs) that are under clinical evaluation for several neurodegenerative diseases display a favorable safety profile, but require immunosuppression upon transplantation in patients. Neural progenitors derived from patient-specific induced pluripotent stem cells (iPSCs) may be relevant for autologous ex vivo gene-therapy applications to treat genetic diseases with unmet medical need. In this scenario, obtaining iPSC-derived neural stem cells (NSCs) showing a reliable "NSC signature" is mandatory. Here, we generated human iPSC (hiPSC) clones via reprogramming of skin fibroblasts derived from normal donors and patients affected by metachromatic leukodystrophy (MLD), a fatal neurodegenerative lysosomal storage disease caused by genetic defects of the arylsulfatase A (ARSA) enzyme. We differentiated hiPSCs into NSCs (hiPS-NSCs) sharing molecular, phenotypic, and functional identity with hfNSCs, which we used as a "gold standard" in a side-by-side comparison when validating the phenotype of hiPS-NSCs and predicting their performance after intracerebral transplantation. Using lentiviral vectors, we efficiently transduced MLD hiPSCs, achieving supraphysiological ARSA activity that further increased upon neural differentiation. Intracerebral transplantation of hiPS-NSCs into neonatal and adult immunodeficient MLD mice stably restored ARSA activity in the whole central nervous system. Importantly, we observed a significant decrease of sulfatide storage when ARSA-overexpressing cells were used, with a clear advantage in those mice receiving neonatal as compared with adult intervention. Thus, we generated a renewable source of ARSA-overexpressing iPSC-derived bona fide hNSCs with improved features compared with clinically approved hfNSCs. Patient-specific ARSA-overexpressing hiPS-NSCs may be used in autologous ex vivo gene therapy protocols to provide long-lasting enzymatic supply in MLD-affected brains. Stem Cells Translational Medicine 2017;6:352-368.

Meng, F. X. and W. Y. Guo (2010). "[Present status on studies of differentiation into retinal neurons and pigmented cell from induced pluripotent stem cells]." Zhonghua Yan Ke Za Zhi **46**(12): 1139-1142.

Somatic cells could be induced into pluripotent stem (iPS) cells through transferring special genes (Oct4, Sox2, c-myc and Klf4). This has brought a revolutionary change in stem cell study and application. The generation of iPS cells has great potential and enormous significance as it can resolve some insurmountable problems in stem cells research, such as ethical dilemma, immune rejection, etc. Because of these characteristics, it plays an important role in the repair of various tissues and organs. Rapid progress in this field during the past 3 years convinced us that iPS cells will be more and more applicable in tissue engineering. The present paper reviews the progress of pre-clinical study on iPS cells in the treatment of retinal and optic nerve diseases.

Mikhailova, A., et al. (2014). "Small-molecule induction promotes corneal epithelial cell differentiation from human induced pluripotent stem cells." Stem Cell Reports **2**(2): 219-231.

Human induced pluripotent stem cells (hiPSCs) offer unique opportunities for developing novel cell-based therapies and disease modeling. In this study, we developed a directed differentiation method for hiPSCs toward corneal epithelial progenitor cells capable of terminal differentiation toward mature corneal epithelial-like cells. In order to improve the efficiency and reproducibility of our method, we replicated signaling cues active during ocular surface ectoderm development with the help of two small-molecule inhibitors in combination with basic fibroblast growth factor (bFGF) in serum-free and feeder-free conditions. First, small-molecule induction downregulated the expression of pluripotency markers while upregulating several transcription factors essential for normal eye development. Second, protein expression of the corneal epithelial progenitor marker p63 was greatly enhanced, with up to 95% of cells being p63 positive after 5 weeks of differentiation. Third, corneal epithelial-like cells were obtained upon further maturation.

Milani, P., et al. (2016). "Cell freezing protocol suitable for ATAC-Seq on motor neurons derived from

human induced pluripotent stem cells." Sci Rep **6**: 25474.

In recent years, the assay for transposase-accessible chromatin using sequencing (ATAC-Seq) has become a fundamental tool of epigenomic research. However, it is difficult to perform this technique on frozen samples because freezing cells before extracting nuclei can impair nuclear integrity and alter chromatin structure, especially in fragile cells such as neurons. Our aim was to develop a protocol for freezing neuronal cells that is compatible with ATAC-Seq; we focused on a disease-relevant cell type, namely motor neurons differentiated from induced pluripotent stem cells (iMNs) from a patient affected by spinal muscular atrophy. We found that while flash-frozen iMNs are not suitable for ATAC-Seq, the assay is successful with slow-cooled cryopreserved cells. Using this method, we were able to isolate high quality, intact nuclei, and we verified that epigenetic results from fresh and cryopreserved iMNs quantitatively agree.

Miyagawa, S., et al. (2016). "Building A New Treatment For Heart Failure-Transplantation of Induced Pluripotent Stem Cell-derived Cells into the Heart." Curr Gene Ther **16**(1): 5-13.

Advanced cardiac failure is a progressive intractable disease and is the main cause of mortality and morbidity worldwide. Since this pathology is represented by a definite decrease in cardiomyocyte number, supplementation of functional cardiomyocytes into the heart would hypothetically be an ideal therapeutic option. Recently, unlimited in vitro production of human functional cardiomyocytes was established by using induced pluripotent stem cell (iPSC) technology, which avoids the use of human embryos. A number of basic studies including ours have shown that transplantation of iPSCderived cardiomyocytes (iPSC-CMs) into the damaged heart leads to recovery of cardiac function, thereby establishing "proof-of-concept" of this iPSC-transplantation therapy. However, considering clinical application of this therapy, its feasibility, safety, and therapeutic efficacy need to be further investigated in the pre-clinical stage. This review summarizes up-to-date important topics related to safety and efficacy of iPSC-CMs transplantation therapy for cardiac disease and discusses the prospects for this treatment in clinical studies.

Mobarra, N., et al. (2014). "Efficient Differentiation of Human Induced Pluripotent Stem Cell (hiPSC) Derived Hepatocyte-Like Cells on hMSCs Feeder." Int J Hematol Oncol Stem Cell Res **8**(4): 20-29.

BACKGROUND: The use of stem cells is considered as an appropriate source in cell therapy and tissue engineering. Differentiation of human induced Pluripotent Stem Cells (hiPSCs) to Hepatocyte-like Cells (HLCs) on mouse embryonic fibroblasts (MEFs) feeders is confronted with several problems that hinder the clinical applications of these differentiated cells for the treatment of liver injuries. Safe appropriate cells for stem cell-based therapies could create new hopes for liver diseases. This work focused on the determination of a capacity/efficiency for the differentiation of the hiPSCs into Hepatocyte-like Cells on a novel human adult bone marrow mesenchymal stem cells (hMSCs) feeder. MATERIALS AND METHODS: Undifferentiated human iPSCs were cultured on mitotically inactivated human adult bone marrow mesenchymal stem cells. A three-step differentiation process has been performed in presence of activin A which added for 3 days to induce a definitive endoderm formation. In the second step, medium was exchanged for six days. Subsequently, cells were treated with oncostatin M plus dexamethasone for 9 days to generate hepatic cells. Endodermic and liver-specific genes were assessed via quantitative reverse transcription-polymerase chain reaction and RT-PCR, moreover, immunocytochemical staining for liver proteins including albumin and alpha-fetoprotein. In addition, functional tests for glycogen storage, oil red examination, urea production and alpha-fetoprotein synthesis, as well as, cells differentiated with a hepatocyte-like morphology was also performed. RESULTS: Our results show that inactivated human adult bone marrow mesenchymal stem cell feeders could support the efficient differentiation of hiPSCs into HLCs. This process induced differentiation of iPSCs into definitive endocrine cells that expressed sox17, foxa2 and expression of the specific genes profiles in hepatic-like cells. In addition, immunocytochemical analysis confirmed albumin and alpha-fetoprotein protein expression, as well as, the hiPSCs-derived Hepatocyte-like Cells on human feeder exhibited a typical morphology. CONCLUSIONS: we suggested a successful and efficient culture for differentiation and maturation of hepatocytes on an alternative human feeders; this is an important step to generate safe and functional hepatocytes that is vital for regenerative medicine and transplantation on the cell-based therapies.

Momcilovic, O., et al. (2011). "Cell cycle adaptations and maintenance of genomic integrity in embryonic stem cells and induced pluripotent stem cells." Results Probl Cell Differ **53**: 415-458.

Pluripotent stem cells have the capability to undergo unlimited self-renewal and differentiation into

all somatic cell types. They have acquired specific adjustments in the cell cycle structure that allow them to rapidly proliferate, including cell cycle independent expression of cell cycle regulators and lax G (1) to S phase transition. However, due to the developmental role of embryonic stem cells (ES) it is essential to maintain genomic integrity and prevent acquisition of mutations that would be transmitted to multiple cell lineages. Several modifications in DNA damage response of ES cells accommodate dynamic cycling and preservation of genetic information. The absence of a G (1)/S cell cycle arrest promotes apoptotic response of damaged cells before DNA changes can be fixed in the form of mutation during the S phase, while G (2)/M cell cycle arrest allows repair of damaged DNA following replication. Furthermore, ES cells express higher level of DNA repair proteins, and exhibit enhanced repair of multiple types of DNA damage. Similarly to ES cells, induced pluripotent stem (iPS) cells are poised to proliferate and exhibit lack of G (1)/S cell cycle arrest, extreme sensitivity to DNA damage, and high level of expression of DNA repair genes. The fundamental mechanisms by which the cell cycle regulates genomic integrity in ES cells and iPS cells are similar, though not identical.

Moslem, M., et al. (2015). "Mesenchymal Stem/Stromal Cells Derived from Induced Pluripotent Stem Cells Support CD34(pos) Hematopoietic Stem Cell Propagation and Suppress Inflammatory Reaction." Stem Cells Int **2015**: 843058.

Mesenchymal stem/stromal cells (MSCs) represent a promising cell source for research and therapeutic applications, but their restricted ex vivo propagation capabilities limit putative applications. Substantial self-renewing of stem cells can be achieved by reprogramming cells into induced pluripotent stem cells (iPSCs) that can be easily expanded as undifferentiated cells even in mass culture. Here, we investigated a differentiation protocol enabling the generation and selection of human iPSC-derived MSCs exhibiting relevant surface marker expression profiles (CD105 and CD73) and functional characteristics. We generated such iPSC-MSCs from fibroblasts and bone marrow MSCs utilizing two different reprogramming constructs. All such iPSC-MSCs exhibited the characteristics of normal bone marrow-derived (BM) MSCs. In direct comparison to BM-MSCs our iPSC-MSCs exhibited a similar surface marker expression profile but shorter doubling times without reaching senescence within 20 passages. Considering functional capabilities, iPSC-MSCs provided supportive feeder layer for CD34(+) hematopoietic stem cells' self-renewal and colony forming capacities. Furthermore, iPSC-MSCs gained immunomodulatory function to suppress CD4(+) cell proliferation, reduce proinflammatory cytokines in mixed lymphocyte reaction, and increase regulatory CD4(+)/CD69(+)/CD25(+) T-lymphocyte population. In conclusion, we generated fully functional MSCs from various iPSC lines irrespective of their starting cell source or reprogramming factor composition and we suggest that such iPSC-MSCs allow repetitive cell applications for advanced therapeutic approaches.

Moslem, M., et al. (2013). "Therapeutic potential of human induced pluripotent stem cell-derived mesenchymal stem cells in mice with lethal fulminant hepatic failure." Cell Transplant **22**(10): 1785-1799.

Large-scale production and noninvasive methods for harvesting mesenchymal stem cells (MSCs), particularly in elderly individuals, has prompted researchers to find new patient-specific sources for MSCs in regenerative medicine. This study aims to produce MSCs from human induced pluripotent stem cells (hiPSCs) and to evaluate their therapeutic effects in a CCl4-induced mouse model of fulminant hepatic failure (FHF). hiPSC-MSCs have shown MSC morphology, antigen profile and differentiation capabilities, and improved hepatic function in our model. hiPSC-MSC-transplanted animals provide significant benefit in terms of survival, serum LDH, total bilirubin, and lipid peroxidation. hiPSC-MSC therapy resulted in a one-third reduction of histologic activity index and a threefold increase in the number of proliferating hepatocytes. This was accompanied by a significant decrease in the expression levels of collagen type I, Mmp13, Mmp2, and Mmp9 genes and increase in Timp1 and Timp2 genes in transplanted groups. hiPSC-MSCs secreted hepatocyte growth factor (HGF) in vitro and also expressed HGF in evaluated liver sections. Similar results were observed with human bone marrow (hBM)-derived MSCs. In conclusion, our results have demonstrated that hiPSC-MSCs might be valuable appropriate alternatives for hBM-MSCs in FHF liver repair and support liver function by cell therapy with a large-scale production capacity, patient-specific nature, and no invasive MSC harvesting.

Mosley, M. C., et al. (2017). "Neurite extension and neuronal differentiation of human induced pluripotent stem cell derived neural stem cells on polyethylene glycol hydrogels containing a continuous Young's Modulus gradient." J Biomed Mater Res A **105**(3): 824-833.

Mechanotransduction in neural cells involves multiple signaling pathways that are not fully understood. Differences in lineage and maturation state are suggested causes for conflicting reports on neural cell mechanosensitivity. To optimize matrices for use in stem cell therapy treatments transplanting

human induced pluripotent stem cell derived neural stem cells (hNSC) into lesions after spinal cord injury, the effects of Young's Modulus changes on hNSC behavior must be understood. The present study utilizes polyethylene glycol hydrogels containing a continuous gradient in Young's modulus to examine changes in the Young's Modulus of the culture substrate on hNSC neurite extension and neural differentiation. Changes in the Young's Modulus of the polyethylene glycol hydrogels was found to affect neurite extension and cellular organization on the matrices. hNSC cultured on 907 Pa hydrogels were found to extend longer neurites than hNSC cultured on other tested Young's Moduli hydrogels. The gene expression of beta tubulin III and microtubule-associated protein 2 in hNSC was affected by changes in the Young's Modulus of the hydrogel. The combinatory method approach used in the present study demonstrates that hNSC are mechanosensitive and the matrix Young's Modulus should be a design consideration for hNSC transplant applications. (c) 2016 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 105A: 824-833, 2017.

Moura, R., et al. (2010). "Induced pluripotent stem (iPS) cells and endothelial cell generation: SIRT-ainly a good idea!" Atherosclerosis **212**(1): 36-39.

Muffat, J., et al. (2018). "Human induced pluripotent stem cell-derived glial cells and neural progenitors display divergent responses to Zika and dengue infections." Proc Natl Acad Sci U S A **115**(27): 7117-7122.

Maternal Zika virus (ZIKV) infection during pregnancy is recognized as the cause of an epidemic of microcephaly and other neurological anomalies in human fetuses. It remains unclear how ZIKV accesses the highly vulnerable population of neural progenitors of the fetal central nervous system (CNS), and which cell types of the CNS may be viral reservoirs. In contrast, the related dengue virus (DENV) does not elicit teratogenicity. To model viral interaction with cells of the fetal CNS in vitro, we investigated the tropism of ZIKV and DENV for different induced pluripotent stem cell-derived human cells, with a particular focus on microglia-like cells. We show that ZIKV infected isogenic neural progenitors, astrocytes, and microglia-like cells (pMGLs), but was only cytotoxic to neural progenitors. Infected glial cells propagated ZIKV and maintained ZIKV load over time, leading to viral spread to susceptible cells. DENV triggered stronger immune responses and could be cleared by neural and glial cells more efficiently. pMGLs, when cocultured with neural spheroids, invaded the tissue and, when infected with ZIKV, initiated neural infection. Since microglia derive from primitive macrophages originating in proximity to the maternal vasculature, they may act as a viral reservoir for ZIKV and establish infection of the fetal brain. Infection of immature neural stem cells by invading microglia may occur in the early stages of pregnancy, before angiogenesis in the brain rudiments. Our data are also consistent with ZIKV and DENV affecting the integrity of the blood-brain barrier, thus allowing infection of the brain later in life.

Mulyasasmita, W., et al. (2014). "Avidity-controlled hydrogels for injectable co-delivery of induced pluripotent stem cell-derived endothelial cells and growth factors." J Control Release **191**: 71-81.

To translate recent advances in induced pluripotent stem cell biology to clinical regenerative medicine therapies, new strategies to control the co-delivery of cells and growth factors are needed. Building on our previous work designing Mixing-Induced Two-Component Hydrogels (MITCHs) from engineered proteins, here we develop protein-polyethylene glycol (PEG) hybrid hydrogels, MITCH-PEG, which form physical gels upon mixing for cell and growth factor co-delivery. MITCH-PEG is a mixture of C7, which is a linear, engineered protein containing seven repeats of the CC43 WW peptide domain (C), and 8-arm star-shaped PEG conjugated with either one or two repeats of a proline-rich peptide to each arm (P1 or P2, respectively). Both 20kDa and 40kDa star-shaped PEG variants were investigated, and all four PEG-peptide variants were able to undergo a sol-gel phase transition when mixed with the linear C7 protein at constant physiological conditions due to noncovalent hetero-dimerization between the C and P domains. Due to the dynamic nature of the C-P physical crosslinks, all four gels were observed to be reversibly shear-thinning and self-healing. The P2 variants exhibited higher storage moduli than the P1 variants, demonstrating the ability to tune the hydrogel bulk properties through a biomimetic peptide-avidity strategy. The 20kDa PEG variants exhibited slower release of encapsulated vascular endothelial growth factor (VEGF), due to a decrease in hydrogel mesh size relative to the 40kDa variants. Human induced pluripotent stem cell-derived endothelial cells (hiPSC-ECs) adopted a well-spread morphology within three-dimensional MITCH-PEG cultures, and MITCH-PEG provided significant protection from cell damage during ejection through a fine-gauge syringe needle. In a mouse hindlimb ischemia model of peripheral arterial disease, MITCH-PEG co-delivery of hiPSC-ECs and VEGF was found to reduce inflammation and promote muscle tissue regeneration compared to a saline control.

Nagamoto, Y., et al. (2015). "Efficient Engraftment of Human Induced Pluripotent Stem Cell-Derived Hepatocyte-Like Cells in uPA/SCID Mice by Overexpression of FNK, a Bcl-xL Mutant Gene." Cell Transplant **24**(6): 1127-1138.

Human liver chimeric mice are expected to be applied for drug toxicity tests and human hepatitis virus research. Human induced pluripotent stem cell-derived hepatocyte-like cells (iPSC-HLCs) are a highly attractive donor source for the generation of human liver chimeric mice because they can be produced on a large scale and established from an individual. Although these cells have been successfully used to generate human liver chimeric mice, there is still room for improvement in the repopulation efficiency. To enhance the repopulation efficacy, the human iPSC-HLCs were transduced with an adenovirus vector (Ad-FNK) expressing FNK, a hyperactive mutant gene from Bcl-xL, which was expected to inhibit apoptosis in the process of integration into liver parenchyma. We then transplanted Ad-FNK-transduced human iPSC-HLCs into urokinase-type plasminogen activator-transgenic severe combined immunodeficiency (uPA/SCID) mice (FNK mice) and evaluated the repopulation efficacy. The antiapoptotic effects of the human iPSC-HLCs were enhanced by FNK overexpression in vitro. Human albumin levels in the transplanted mice were significantly increased by transplantation of Ad-FNK-transduced human iPSC-HLCs (about 24,000 ng/ml). Immunohistochemical analysis with an anti-human alphaAT antibody revealed greater repopulation efficacy in the livers of FNK mice than control mice. Interestingly, the expression levels of human hepatocyte-related genes in the human iPSC-HLCs of FNK mice were much higher than those in the human iPSC-HLCs before transplantation. We succeeded in improving the repopulation efficacy of human liver chimeric mice generated by transplanting the Ad-FNK-transduced human iPSC-HLCs into uPA/SCID mice. Our method using ectopic expression of FNK was useful for generating human chimeric mice with high chimerism.

Nagasaka, R., et al. (2017). "Image-based cell quality evaluation to detect irregularities under same culture process of human induced pluripotent stem cells." J Biosci Bioeng **123**(5): 642-650.

To meet the growing demand for human induced pluripotent stem cells (iPSCs) for various applications, technologies that enable the manufacturing of iPSCs on a large scale should be developed. There are several technological challenges in iPSC manufacturing technology. Image-based cell quality evaluation technology for monitoring iPSC quality in culture enables the manufacture of intact cells for further applications. Although several studies have reported the effectiveness of image-based evaluation of iPSCs, it remains challenging to detect irregularities that may arise using the same processing operations during quality evaluation of automated processing. In this study, we investigated the evaluation performance of image-based cell quality analysis in detecting small differences that can result from human measurement, even when the same protocol is followed. To imitate such culture conditions, by image-analysis guided colony pickup, we changed the proportions of morphologically different subpopulations: "good morphology, regular morphology correlated with undifferentiation marker expression" and "bad morphology, irregular morphology correlated with loss of undifferentiation marker expression". In addition, comprehensive gene-expression and metabolomics analyses were carried out for the same samples to investigate performance differences. Our data shows an example of investigating the usefulness and sensitivity of quality evaluation methods for iPSC quality monitoring.

Nakamura, M. and H. Okano (2013). "Cell transplantation therapies for spinal cord injury focusing on induced pluripotent stem cells." Cell Res **23**(1): 70-80.

Stimulated by the 2012 Nobel Prize in Physiology or Medicine awarded for Shinya Yamanaka and Sir John Gurdon, there is an increasing interest in the induced pluripotent stem (iPS) cells and reprograming technologies in medical science. While iPS cells are expected to open a new era providing enormous opportunities in biomedical sciences in terms of cell therapies and regenerative medicine, safety-related concerns for iPS cell-based cell therapy should be resolved prior to the clinical application of iPS cells. In this review, the pre-clinical investigations of cell therapy for spinal cord injury (SCI) using neural stem/progenitor cells derived from iPS cells, and their safety issues in vivo, are outlined. We also wish to discuss the strategy for the first human trails of iPS cell-based cell therapy for SCI patients.

Nakamura, S., et al. (2014). "Expandable megakaryocyte cell lines enable clinically applicable generation of platelets from human induced pluripotent stem cells." Cell Stem Cell **14**(4): 535-548.

The donor-dependent supply of platelets is frequently insufficient to meet transfusion needs. To address this issue, we developed a clinically applicable strategy for the derivation of functional platelets from human pluripotent stem cells (PSCs). This approach involves the establishment of stable immortalized megakaryocyte progenitor cell lines (imMKCLs) from PSC-derived hematopoietic progenitors through the

overexpression of BMI1 and BCL-XL to respectively suppress senescence and apoptosis and the constrained overexpression of c-MYC to promote proliferation. The resulting imMKCLs can be expanded in culture over extended periods (4-5 months), even after cryopreservation. Halting the overexpression of c-MYC, BMI1, and BCL-XL in growing imMKCLs led to the production of CD42b (+) platelets with functionality comparable to that of native platelets on the basis of a range of assays in vitro and in vivo. The combination of robust expansion capacity and efficient platelet production means that appropriately selected imMKCL clones represent a potentially inexhaustible source of hPSC-derived platelets for clinical application.

Nakane, T., et al. (2017). "Impact of Cell Composition and Geometry on Human Induced Pluripotent Stem Cells-Derived Engineered Cardiac Tissue." Sci Rep **7**: 45641.

The current study describes a scalable, porous large-format engineered cardiac tissue (LF-ECT) composed of human induced pluripotent stem cells (hiPSCs) derived multiple lineage cardiac cells with varied 3D geometries and cell densities developed towards the goal of scale-up for large animal pre-clinical studies. We explored multiple 15 x 15 mm ECT geometries using molds with rectangular internal staggered posts (mesh, ME), without posts (plain sheet, PS), or long parallel posts (multiple linear bundles, ML) and a gel matrix containing hiPSC-derived cardiomyocytes, endothelial, and vascular mural cells matured in vitro for 14 days. ME-ECTs displayed the lowest dead cell ratio ($p < 0.001$) and matured into 0.5 mm diameter myofiber bundles with greater 3D cell alignment and higher active stress than PS-ECTs. Increased initial ECT cell number beyond 6 M per construct resulted in reduced cell survival and lower active stress. The 6M-ME-ECTs implanted onto 1 week post-infarct immune tolerant rat hearts engrafted, displayed evidence for host vascular coupling, and recovered myocardial structure and function with reduced scar area. We generated a larger (30 x 30 mm) ME-ECT to confirm scalability. Thus, large-format ECTs generated from hiPSC-derived cardiac cells may be feasible for large animal preclinical cardiac regeneration paradigms.

Nakayama, C., et al. (2018). "The development of induced pluripotent stem cell-derived mesenchymal stem/stromal cells from normal human and RDEB epidermal keratinocytes." J Dermatol Sci **91**(3): 301-310.

BACKGROUND: Epidermolysis bullosa (EB) is a group of hereditary disorders caused by mutations in the genes encoding structural molecules of the dermal-epidermal junction (DEJ). Cell-based therapies such as allogeneic mesenchymal stem/stromal cell (MSC) transplantation have recently been explored for severe EB types, such as recessive dystrophic EB (RDEB). However, hurdles exist in current MSC-based therapies, such as limited proliferation from a single cell source and limited cell survival due to potential allogenic rejection. OBJECTIVES: We aimed to develop MSCs from keratinocyte-derived induced pluripotent stem cells (iPSCs). METHODS: Keratinocyte-derived iPSCs (KC-iPSCs) of a healthy human and an RDEB patient were cultured with activin A, 6-bromoindirubin-3'-oxime and bone morphogenetic protein 4 to induce mesodermal lineage formation. These induced cells were subjected to immunohistochemical analysis, flow cytometric analysis and RNA microarray analysis in vitro, and were injected subcutaneously and intravenously to wounded immunodeficient mice to assess their wound-healing efficacy. RESULTS: After their induction, KC-iPSC-induced cells were found to be compatible with MSCs. Furthermore, with the subcutaneous and intravenous injection of the KC-iPSC-induced cells into wounded immunodeficient mice, human type VII collagen was detected at the DEJ of epithelized areas. CONCLUSIONS: We successfully established iPSC-derived MSCs from keratinocytes (KC-iPSC-MSCs) of a normal human and an RDEB patient. KC-iPSC-MSCs may have potential in therapies for RDEB.

Nakayama, M. (2009). "Cell Therapy Using Induced Pluripotent Stem (iPS) Cells Meets Next-Next Generation DNA Sequencing Technology." Curr Genomics **10**(5): 303-305.

The recent development of induced pluripotent stem (iPS) cell technology brings cell and gene therapies to patients one large step closer to reality. Technical improvements in various research fields sometimes come together fortuitously, leading to approaches to treating disease. If iPS cell technology continues to progress smoothly as expected and is actually applied to patients, the next logical step to ensuring the success of iPS cell therapy is to make use of next-next generation DNA sequencing technology and bioinformatics of recipient genomes. Before a patient-derived iPS cell colony is used for clinical therapy in a patient, the colony should undergo whole-genome DNA sequencing, thus avoiding risks associated with spontaneously mutagenized iPS cells. Researchers participating in the Human Genome Project need to take full advantage of both technologies-iPS cell technology and DNA sequencing-as doing so will help us achieve the original long-term goal of the project: developing therapies that will benefit human health.

Narsinh, K. H., et al. (2011). "Single cell transcriptional profiling reveals heterogeneity of human induced pluripotent stem cells." J Clin Invest **121**(3): 1217-1221.

Human induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs) are promising candidate cell sources for regenerative medicine. However, despite the common ability of hiPSCs and hESCs to differentiate into all 3 germ layers, their functional equivalence at the single cell level remains to be demonstrated. Moreover, single cell heterogeneity amongst stem cell populations may underlie important cell fate decisions. Here, we used single cell analysis to resolve the gene expression profiles of 362 hiPSCs and hESCs for an array of 42 genes that characterize the pluripotent and differentiated states. Comparison between single hESCs and single hiPSCs revealed markedly more heterogeneity in gene expression levels in the hiPSCs, suggesting that hiPSCs occupy an alternate, less stable pluripotent state. hiPSCs also displayed slower growth kinetics and impaired directed differentiation as compared with hESCs. Our results suggest that caution should be exercised before assuming that hiPSCs occupy a pluripotent state equivalent to that of hESCs, particularly when producing differentiated cells for regenerative medicine aims.

Navara, C. S., et al. (2013). "Derivation of induced pluripotent stem cells from the baboon: a nonhuman primate model for preclinical testing of stem cell therapies." Cell Reprogram **15**(6): 495-502.

Development of effective pluripotent stem cell-based therapies will require safety and efficacy testing in a clinically relevant preclinical model such as nonhuman primates (NHPs). Baboons and macaques are equally similar to humans genetically and both have been extensively used for biomedical research. Macaques are preferred for human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) research whereas baboons are preferred for transplantation studies because of the greater similarity of their anatomy and immunogenetic system to those of humans. We generated four induced pluripotent stem cell (iPSC) lines from skin cells of the olive baboon (Papio anubis). Each line shows the distinct morphology of primate pluripotent stem cells, including flat colonies with well-defined borders and a high nuclear/cytoplasm ratio. Each is positive for the pluripotency markers OCT4, SOX2, NANOG, and SSEA4. Pluripotency was confirmed in two lines by teratoma formation with representative tissues from each germ layer, whereas a third produced cells from all three germ layers following embryoid body differentiation. Three lines have a normal male karyotype and the fourth is missing the short arm of one copy of chromosome 18. This may serve as an in vitro model for the human developmental disorder 18p-, which impacts 1 in 50,000 births/year. These iPSC lines represent the first step toward establishing the baboon as a NHP model for developing stem cell-based therapies.

Ng, J., et al. (2016). "Immunomodulatory Properties of Induced Pluripotent Stem Cell-Derived Mesenchymal Cells." J Cell Biochem **117**(12): 2844-2853.

MSC-like populations derived from induced pluripotent stem cells (iPSC-MSC) serve as an alternative stem cell source due to their high proliferative capacity. In this study, we assessed the immunomodulatory potential of iPSC-MSC generated from periodontal ligament (PDL) and gingival (GF) tissue. The iPSC-MSC lines exhibited a similar level of suppression of mitogen-stimulated peripheral blood mononuclear cells (PBMNC) proliferation compared to their respective parental fibroblast populations in vitro. Moreover, iPSC-MSC demonstrated the ability to suppress T-cells effector cells, Th1/Th2/Th17 populations, and increase levels of Treg cells. In order to investigate the mechanisms involved, expression of common MSC-derived soluble factors known to supress lymphocyte proliferation were assessed in iPSC-MSC cultured with PBMNC with direct cell-cell contact or separated in transwells. Real-time PCR analysis of factors known to be involved in MSC mediated immune regulation, found a general trend of elevated IDO1 and IL6 transcript levels in iPSC-MSC lines and their respective primary cells co-cultured with activated PBMNC, with a wide range of gene expression levels between the different mesenchymal cell types. The results suggest that different iPSC-MSC may be useful as a potential alternative source of cells for future clinical use in therapeutic applications because of their potent immunosuppressive properties. J. Cell. Biochem. 117: 2844-2853, 2016. (c) 2016 Wiley Periodicals, Inc.

Nguyen, Q. H., et al. (2018). "Single-cell RNA-seq of human induced pluripotent stem cells reveals cellular heterogeneity and cell state transitions between subpopulations." Genome Res **28**(7): 1053-1066.

Heterogeneity of cell states represented in pluripotent cultures has not been described at the transcriptional level. Since gene expression is highly heterogeneous between cells, single-cell RNA sequencing can be used to identify how individual pluripotent cells function. Here, we present results from the analysis of single-cell RNA sequencing data from 18,787 individual WTC-CRISPRi human induced pluripotent stem cells. We developed an

unsupervised clustering method and, through this, identified four subpopulations distinguishable on the basis of their pluripotent state, including a core pluripotent population (48.3%), proliferative (47.8%), early primed for differentiation (2.8%), and late primed for differentiation (1.1%). For each subpopulation, we were able to identify the genes and pathways that define differences in pluripotent cell states. Our method identified four transcriptionally distinct predictor gene sets composed of 165 unique genes that denote the specific pluripotency states; using these sets, we developed a multigenic machine learning prediction method to accurately classify single cells into each of the subpopulations. Compared against a set of established pluripotency markers, our method increases prediction accuracy by 10%, specificity by 20%, and explains a substantially larger proportion of deviance (up to threefold) from the prediction model. Finally, we developed an innovative method to predict cells transitioning between subpopulations and support our conclusions with results from two orthogonal pseudotime trajectory methods.

Ninomiya, E., et al. (2014). "Glucocorticoids promote neural progenitor cell proliferation derived from human induced pluripotent stem cells." Springerplus **3**: 527.

Glucocorticoids (GCs) are frequently used for treating and preventing chronic lung disease and circulatory dysfunction in premature infants. However, there is growing concern about the detrimental effects of systemic GC administration on neurodevelopment. The first choice of GCs to minimize the adverse effects on the developing brain is still under debate. We investigated the effect of commonly used GCs such as dexamethasone (DEX), betamethasone (BET) and hydrocortisone (HDC) on the proliferation of human-induced pluripotent stem cell (iPSC)-derived neuronal progenitor cells (NPCs). In this study, NPCs were treated with various concentrations of GCs and subjected to cell proliferation assays. Furthermore, we quantified the number of microtubule-associated protein 2 (MAP2) positive neurons in NPCs by immunostaining. All GCs promoted NPC proliferation in a dose-dependent manner. We also confirmed that MAP2-positive neurons in NPCs increased upon GC treatment. However, differential effects of GCs on MAP2 positive neurons were observed when we treated NPCs with H2O2. The total numbers of NPCs increased upon any GC treatment even under oxidative conditions but the numbers of MAP2 positive neurons increased only by HDC treatment. GCs promoted human iPSCsaeuro"derived NPC proliferation and the differential effects of GCs became apparent under oxidative stress. Our results may support HDC as the preferred choice over DEX and BET to prevent adverse effects on the developing human brain.

Nishiyama, Y., et al. (2016). "Safe and efficient method for cryopreservation of human induced pluripotent stem cell-derived neural stem and progenitor cells by a programmed freezer with a magnetic field." Neurosci Res **107**: 20-29.

Stem cells represent a potential cellular resource in the development of regenerative medicine approaches to the treatment of pathologies in which specific cells are degenerated or damaged by genetic abnormality, disease, or injury. Securing sufficient supplies of cells suited to the demands of cell transplantation, however, remains challenging, and the establishment of safe and efficient cell banking procedures is an important goal. Cryopreservation allows the storage of stem cells for prolonged time periods while maintaining them in adequate condition for use in clinical settings. Conventional cryopreservation systems include slow-freezing and vitrification both have advantages and disadvantages in terms of cell viability and/or scalability. In the present study, we developed an advanced slow-freezing technique using a programmed freezer with a magnetic field called Cells Alive System (CAS) and examined its effectiveness on human induced pluripotent stem cell-derived neural stem/progenitor cells (hiPSC-NS/PCs). This system significantly increased cell viability after thawing and had less impact on cellular proliferation and differentiation. We further found that frozen-thawed hiPSC-NS/PCs were comparable with non-frozen ones at the transcriptome level. Given these findings, we suggest that the CAS is useful for hiPSC-NS/PCs banking for clinical uses involving neural disorders and may open new avenues for future regenerative medicine.

Nissenbaum, J., et al. (2013). "Global indiscriminate methylation in cell-specific gene promoters following reprogramming into human induced pluripotent stem cells." Stem Cell Reports **1**(6): 509-517.

Molecular reprogramming of somatic cells into human induced pluripotent stem cells (iPSCs) is accompanied by extensive changes in gene expression patterns and epigenetic marks. To better understand the link between gene expression and DNA methylation, we have profiled human somatic cells from different embryonic cell types (endoderm, mesoderm, and parthenogenetic germ cells) and the iPSCs generated from them. We show that reprogramming is accompanied by extensive DNA methylation in CpG-poor promoters, sparing CpG-rich promoters. Intriguingly, methylation in CpG-poor promoters occurred not only in downregulated genes,

but also in genes that are not expressed in the parental somatic cells or their respective iPSCs. These genes are predominantly tissue-specific genes of other cell types from different lineages. Our results suggest a role of DNA methylation in the silencing of the somatic cell identity by global nonspecific methylation of tissue-specific genes from all lineages, regardless of their expression in the parental somatic cells.

Niu, Z., et al. (2013). "Germ-like cell differentiation from induced pluripotent stem cells (iPSCs)." Cell Biochem Funct **31**(1): 12-19.

Historically, our understanding of molecular genetic aspects of germ cell development has been limited. Recently, results demonstrated that the derivation of pluripotent stem cells may provide the necessary genetic system to study germ cell development. Here, we characterized an induced pluripotent stem cell (iPSC) line, which can spontaneously differentiate into embryonic bodies (EBs) after 3 days of suspension culture, expressing specific markers of three germ layers. Then, we induced the iPSCs to differentiate into germ cells by culturing adherent EBs in retinoic acid (RA) and porcine follicular fluid (PFF) differentiation medium or seminiferous tubule transplantation. Our results indicated that RA and PFF were beneficial for the derivation of germ cells and oocyte-like cells from iPSCs, and iPSCs transplantation could make a contribution to repairing the testis of infertile mice. Our study offers an approach for further study on the development and the differentiation of germ cells derived from iPSCs.

No, J. G., et al. (2015). "Cell-free extract from porcine induced pluripotent stem cells can affect porcine somatic cell nuclear reprogramming." J Reprod Dev **61**(2): 90-98.

Pretreatment of somatic cells with undifferentiated cell extracts, such as embryonic stem cells and mammalian oocytes, is an attractive alternative method for reprogramming control. The properties of induced pluripotent stem cells (iPSCs) are similar to those of embryonic stem cells; however, no studies have reported somatic cell nuclear reprogramming using iPSC extracts. Therefore, this study aimed to evaluate the effects of porcine iPSC extracts treatment on porcine ear fibroblasts and early development of porcine cloned embryos produced from porcine ear skin fibroblasts pretreated with the porcine iPSC extracts. The Chariot (TM) reagent system was used to deliver the iPSC extracts into cultured porcine ear skin fibroblasts. The iPSC extracts-treated cells (iPSC-treated cells) were cultured for 3 days and used for analyzing histone modification and somatic cell nuclear transfer. Compared to the results for nontreated cells, the trimethylation status of histone H3 lysine residue 9 (H3K9) in the iPSC-treated cells significantly decreased. The expression of Jmjd2b, the H3K9 trimethylation-specific demethylase gene, significantly increased in the iPSC-treated cells; conversely, the expression of the proapoptotic genes, Bax and p53, significantly decreased. When the iPSC-treated cells were transferred into enucleated porcine oocytes, no differences were observed in blastocyst development and total cell number in blastocysts compared with the results for control cells. However, H3K9 trimethylation of pronuclear-stage-cloned embryos significantly decreased in the iPSC-treated cells. Additionally, Bax and p53 gene expression in the blastocysts was significantly lower in iPSC-treated cells than in control cells. To our knowledge, this study is the first to show that an extracts of porcine iPSCs can affect histone modification and gene expression in porcine ear skin fibroblasts and cloned embryos.

Nomura, Y., et al. (2012). "Human periodontal ligament fibroblasts are the optimal cell source for induced pluripotent stem cells." Histochem Cell Biol **137**(6): 719-732.

Among the various kinds of fibroblasts existing in the human body, the periodontal ligament (PDL) fibroblasts have been suggested as multipotent cells. Periodontal ligament fibroblasts are characterized by rapid turnover, a high remodeling capacity and remarkable capacity for renewal and repair. They also differentiate into osteoblasts and cementoblasts. We established iPS cells from human PDL fibroblasts by introducing the ES cell markers Oct3/4, Sox2, Nanog, Klf4 and Lin28 by retrovirus transduction, even without the oncogene c-Myc. The iPS cells established in this study expressed the ES cell markers and formed teratomas in SCID mice. The c-Myc expression level in the PDL fibroblasts was higher than that in the iPS cells by quantitative RT-PCR. Therefore, we have concluded that PDL fibroblasts could be an optimal cell source for iPS cells.

Nong, K., et al. (2016). "Hepatoprotective effect of exosomes from human-induced pluripotent stem cell-derived mesenchymal stromal cells against hepatic ischemia-reperfusion injury in rats." Cytotherapy **18**(12): 1548-1559.

BACKGROUND: This study aimed to evaluate the effect of exosomes produced by human-induced pluripotent stem cell-derived mesenchymal stromal cells (hiPSC-MSCs-Exo) on hepatic ischemia-reperfusion (I/R) injury. METHODS: Exosomes were isolated and concentrated from conditioned medium using ultracentrifugation and ultrafiltration. hiPSC-MSCs-Exo were injected systemically via the inferior

vena cava in a rat model of 70% warm hepatic I/R injury, and the therapeutic effect was evaluated. The serum levels of transaminases (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) were measured using an automatic analyzer. The expression of inflammatory factors was measured using enzyme-linked immunosorbent assay (ELISA). Histological changes indicated changes in pathology and inflammatory infiltration in liver tissue. Apoptosis of hepatic cells in liver tissue was measured using terminal-deoxynucleoitidyl transferase mediated nick end labeling (TUNEL) staining along with apoptotic markers. RESULTS: hiPSCs were efficiently induced into hiPSC-MSCs with typical MSC characteristics. hiPSC-MSCs-Exo had diameters ranging from 50 to 60 nm and expressed exosomal markers (CD9, CD63 and CD81). Hepatocyte necrosis and sinusoidal congestion were markedly suppressed with a lower Suzuki score after hiPSC-MSCs-Exo administration. The levels of the hepatocyte injury markers AST and ALT were significantly lower in the treated group than in the control group. Inflammatory markers, such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-6 and high mobility group box 1 (HMGB1), were significantly reduced after administration of hiPSC-MSCs-Exo, which suggests that the exosomes have a role in suppressing the inflammatory response. Additionally, in liver tissues from the experimental group, the levels of apoptotic markers, such as caspase-3 and bax, were significantly lower and the levels of oxidative markers, such as glutathione (GSH), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), were significantly higher than in the control group. These data point to an anti-apoptotic, anti-oxidative stress response role for hiPSC-MSCs-Exo. CONCLUSIONS: Our results demonstrated that hiPSC-MSCs-Exo alleviate hepatic I/R injury, possibly via suppression of inflammatory responses, attenuation of the oxidative stress response and inhibition of apoptosis.

Nsair, A., et al. (2012). "Characterization and therapeutic potential of induced pluripotent stem cell-derived cardiovascular progenitor cells." PLoS One **7**(10): e45603.

BACKGROUND: Cardiovascular progenitor cells (CPCs) have been identified within the developing mouse heart and differentiating pluripotent stem cells by intracellular transcription factors Nkx2.5 and Islet 1 (Isl1). Study of endogenous and induced pluripotent stem cell (iPSC)-derived CPCs has been limited due to the lack of specific cell surface markers to isolate them and conditions for their in vitro expansion that maintain their multipotency. METHODOLOGY/PRINCIPAL FINDINGS: We sought to identify specific cell surface markers that label endogenous embryonic CPCs and validated these markers in iPSC-derived Isl1(+)/Nkx2.5(+) CPCs. We developed conditions that allow propagation and characterization of endogenous and iPSC-derived Isl1(+)/Nkx2.5(+) CPCs and protocols for their clonal expansion in vitro and transplantation in vivo. Transcriptome analysis of CPCs from differentiating mouse embryonic stem cells identified a panel of surface markers. Comparison of these markers as well as previously described surface markers revealed the combination of Flt1(+)/Flt4(+) best identified and facilitated enrichment for Isl1(+)/Nkx2.5(+) CPCs from embryonic hearts and differentiating iPSCs. Endogenous mouse and iPSC-derived Flt1(+)/Flt4(+) CPCs differentiated into all three cardiovascular lineages in vitro. Flt1(+)/Flt4(+) CPCs transplanted into left ventricles demonstrated robust engraftment and differentiation into mature cardiomyocytes (CMs). CONCLUSION/SIGNIFICANCE: The cell surface marker combination of Flt1 and Flt4 specifically identify and enrich for an endogenous and iPSC-derived Isl1(+)/Nkx2.5(+) CPC with trilineage cardiovascular potential in vitro and robust ability for engraftment and differentiation into morphologically and electrophysiologically mature adult CMs in vivo post transplantation into adult hearts.

Nurnberg, E., et al. (2018). "Basal glucocorticoid receptor activation induces proliferation and inhibits neuronal differentiation of human induced pluripotent stem cell-derived neuronal precursor cells." J Steroid Biochem Mol Biol **182**: 119-126.

Glucocorticoids (GC) have first been shown to originate from the adrenal glands where synthesis and release is controlled by the hypothalamic-pituitary-adrenal (HPA) axis. Recently, it was shown that GC and other steroid hormones are also synthesized in the central nervous system, so-called neurosteroids. GC bind to specific GC receptors (GR) which function as ligand-activated transcription factors. GR are expressed in nearly all cell types in the brain, and therefore GC have a strong impact on neuronal development. Most knowledge of the influence of GC on neurodevelopment has been obtained from animal research. Recent advances in stem cell technology made it possible to generate neuronal precursor cells (NPCs) and neurons from human induced pluripotent stem cells (hiPSCs). To explore the cellular mechanism of GC affecting human neuronal development, we quantified the proliferation and differentiation of hiPSCs-derived NPCs in the absence and presence of the selective high-affinity GR agonist dexamethasone and the selective GR antagonist mifepristone, respectively. Our results show that inhibition of GR significantly reduced proliferation of NPCs and promoted differentiation whereas GR

activation suppressed neuronal differentiation. This implies that neuronal GC must be present in NPCs for proliferation. Consequently we identified the presence of 11-beta-hydroxylase CYP11B1, which hydroxylates the respective steroid precursors to bioactive GC, in NPCs. We propose that hiPSC technology offers an ideal system to get more insight into the synthesising and regulatory pathways in steroidogenesis in human neurons and to differentiate between the mechanism by which adrenal GC and neuronal GC impact on neurodevelopment.

Ohgushi, M., et al. (2010). "Molecular pathway and cell state responsible for dissociation-induced apoptosis in human pluripotent stem cells." Cell Stem Cell **7**(2): 225-239.

Human embryonic stem cells (hESCs), unlike mouse ones (mESCs), are vulnerable to apoptosis upon dissociation. Here, we show that the apoptosis, which is of a nonanoikis type, is caused by ROCK-dependent hyperactivation of actomyosin and efficiently suppressed by the myosin inhibitor Blebbistatin. The actomyosin hyperactivation is triggered by the loss of E-cadherin-dependent intercellular contact and also observed in dissociated mouse epiblast-derived pluripotent cells but not in mESCs. We reveal that Abr, a unique Rho-GEF family factor containing a functional Rac-GAP domain, is an indispensable upstream regulator of the apoptosis and ROCK/myosin hyperactivation. Rho activation coupled with Rac inhibition is induced in hESCs upon dissociation, but not in Abr-depleted hESCs or mESCs. Furthermore, artificial Rho or ROCK activation with Rac inhibition restores the vulnerability of Abr-depleted hESCs to dissociation-induced apoptosis. Thus, the Abr-dependent "Rho-high/Rac-low" state plays a decisive role in initiating the dissociation-induced actomyosin hyperactivation and apoptosis in hESCs.

Ohnishi, H., et al. (2015). "Limited hair cell induction from human induced pluripotent stem cells using a simple stepwise method." Neurosci Lett **599**: 49-54.

Disease-specific induced pluripotent stem cells (iPS) cells are expected to contribute to exploring useful tools for studying the pathophysiology of inner ear diseases and to drug discovery for treating inner ear diseases. For this purpose, stable induction methods for the differentiation of human iPS cells into inner ear hair cells are required. In the present study, we examined the efficacy of a simple induction method for inducing the differentiation of human iPS cells into hair cells. The induction of inner ear hair cell-like cells was performed using a stepwise method mimicking inner ear development. Human iPS cells were sequentially transformed into the preplacodal ectoderm, otic placode, and hair cell-like cells. As a first step, preplacodal ectoderm induction, human iPS cells were seeded on a Matrigel-coated plate and cultured in a serum free N2/B27 medium for 8 days according to a previous study that demonstrated spontaneous differentiation of human ES cells into the preplacodal ectoderm. As the second step, the cells after preplacodal ectoderm induction were treated with basic fibroblast growth factor (bFGF) for induction of differentiation into otic-placode-like cells for 15 days. As the final step, cultured cells were incubated in a serum free medium containing Matrigel for 48 days. After preplacodal ectoderm induction, over 90% of cultured cells expressed the genes that express in preplacodal ectoderm. By culture with bFGF, otic placode marker-positive cells were obtained, although their number was limited. Further 48-day culture in serum free media resulted in the induction of hair cell-like cells, which expressed a hair cell marker and had stereocilia bundle-like constructions on their apical surface. Our results indicate that hair cell-like cells are induced from human iPS cells using a simple stepwise method with only bFGF, without the use of xenogeneic cells.

Ohtani-Kaneko, R., et al. (2017). "Characterisation of human induced pluripotent stem cell-derived endothelial cells under shear stress using an easy-to-use microfluidic cell culture system." Biomed Microdevices **19**(4): 91.

Induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) can contribute to elucidating the pathogenesis of heart and vascular diseases and developing their treatments. Their precise characteristics in fluid flow however remain unclear. Therefore, the aim of the present study is to characterise these features. We cultured three types of ECs in a microfluidic culture system: commercially available human iPS-ECs, human umbilical vein endothelial cells (HUVECs) and human umbilical artery endothelial cells (HUAECs). We then examined the mRNA expression levels of endothelial marker gene cluster of differentiation 31 (CD31), fit-related receptor tyrosine kinase (Flk-1), and the smooth muscle marker gene smooth muscle alpha-actin, and investigated changes in plasminogen activator inhibitor-1 (PAI-1) secretion and intracellular F-actin arrangement following heat stress. We also compared expressions of the arterial and venous marker genes ephrinB2 and EphB4, and the endothelial gap junction genes connexin (Cx) 37, 40, and 43 under fluidic shear stress to determine their arterial or venous characteristics. We found that iPS-ECs had similar endothelial marker gene expressions and exhibited similar increases in PAI-1 secretion under heat stress

as HUVECs and HUAECs. In addition, F-actin arrangement in iPSC-ECs also responded to heat stress, as previously reported. However, they had different expression patterns of arterial and venous marker genes and Cx genes under different fluidic shear stress levels, showing that iPSC-ECs exhibit different characteristics from arterial and venous ECs. This microfluidic culture system equipped with variable shear stress control will provide an easy-to-use assay tool to examine characteristics of iPS-ECs generated by different protocols in various laboratories and contribute to basic and applied biomedical researches on iPS-ECs.

Okano, H., et al. (2013). "Steps toward safe cell therapy using induced pluripotent stem cells." Circ Res **112**(3): 523-533.

The enthusiasm for producing patient-specific human embryonic stem cells using somatic nuclear transfer has somewhat abated in recent years because of ethical, technical, and political concerns. However, the interest in generating induced pluripotent stem cells (iPSCs), in which pluripotency can be obtained by transcription factor transduction of various somatic cells, has rapidly increased. Human iPSCs are anticipated to open enormous opportunities in the biomedical sciences in terms of cell therapies for regenerative medicine and stem cell modeling of human disease. On the other hand, recent reports have emphasized the pitfalls of iPSC technology, including the potential for genetic and epigenetic abnormalities, tumorigenicity, and immunogenicity of transplanted cells. These constitute serious safety-related concerns for iPSC-based cell therapy. However, preclinical data supporting the safety and efficacy of iPSCs are also accumulating. In this Review, recent achievements and future tasks for safe iPSC-based cell therapy are summarized, using regenerative medicine for repair strategies in the damaged central nervous system (CNS) as a model. Insights on safety and preclinical use of iPSCs in cardiovascular repair model are also discussed.

Oshima, K., et al. (2010). "Mechanosensitive hair cell-like cells from embryonic and induced pluripotent stem cells." Cell **141**(4): 704-716.

Mechanosensitive sensory hair cells are the linchpin of our senses of hearing and balance. The inability of the mammalian inner ear to regenerate lost hair cells is the major reason for the permanence of hearing loss and certain balance disorders. Here, we present a stepwise guidance protocol starting with mouse embryonic stem and induced pluripotent stem cells, which were directed toward becoming ectoderm capable of responding to otic-inducing growth factors. The resulting otic progenitor cells were subjected to varying differentiation conditions, one of which promoted the organization of the cells into epithelial clusters displaying hair cell-like cells with stereociliary bundles. Bundle-bearing cells in these clusters responded to mechanical stimulation with currents that were reminiscent of immature hair cell transduction currents.

Ould-Brahim, F., et al. (2018). "Metformin Preconditioning of Human Induced Pluripotent Stem Cell-Derived Neural Stem Cells Promotes Their Engraftment and Improves Post-Stroke Regeneration and Recovery." Stem Cells Dev **27**(16): 1085-1096.

While transplantation of human induced pluripotent stem cell-derived neural stem cells (hiPSC-NSCs) shows therapeutic potential in animal stroke models, major concerns for translating hiPSC therapy to the clinic are efficacy and safety. Therefore, there is a demand to develop an optimal strategy to enhance the engraftment and regenerative capacity of transplanted hiPSC-NSCs to produce fully differentiated neural cells to replace lost brain tissues. Metformin, an FDA-approved drug, is an optimal neuroregenerative agent that not only promotes NSC proliferation but also drives NSCs toward differentiation. In this regard, we hypothesize that preconditioning of hiPSC-NSCs with metformin before transplantation into the stroke-damaged brain will improve engraftment and regenerative capabilities of hiPSC-NSCs, ultimately enhancing functional recovery. In this study, we show that pretreatment of hiPSC-NSCs with metformin enhances the proliferation and differentiation of hiPSC-NSCs in culture. Furthermore, metformin-preconditioned hiPSC-NSCs show increased engraftment 1 week post-transplantation in a rat endothelin-1 focal ischemic stroke model. In addition, metformin-preconditioned cell grafts exhibit increased survival compared to naive cell grafts at 7 weeks post-transplantation. Analysis of the grafts demonstrates that metformin preconditioning enhances the differentiation of hiPSC-NSCs at the expense of their proliferation. As an outcome, rats receiving metformin-preconditioned cells display accelerated gross motor recovery and reduced infarct volume. These studies represent a vital step forward in the optimization of hiPSC-NSC-based transplantation to promote post-stroke recovery.

Ozeki, N., et al. (2017). "Gelatin scaffold combined with bone morphogenetic protein-4 induces odontoblast-like cell differentiation involving integrin profile changes, autophagy-related gene 10, and Wnt5 sequentially in human induced pluripotent stem cells." Differentiation **93**: 1-14.

While human induced pluripotent stem (hiPS) cells have potential use in regenerative medicine, there

are no reports on odontoblastic differentiation of hiPS cells. In the current study, to examine integrin profiles and explore the early signaling cascade of odontoblastic differentiation in hiPS cells, we investigated the regulation of autophagy-related gene (Atg) and wingless/int1 (Wnt) signaling in gelatin scaffold (GS) combined with bone morphogenetic protein (BMP)-4 (GS/BMP-4)-mediated odontoblastic differentiation. Following GS/BMP-4 treatment, there was a dramatic loss of alpha3 and alpha6 integrins, and reciprocal strong induction of alpha1 integrin expression in the differentiated cells. GS/BMP-4 increased the mRNA and protein levels of Atg10, Lrp5/Fzd9 (an Atg10 receptor), and Wnt5 together with the amount of autophagosomes and autophagic fluxes. Treatment with siRNAs against Atg10 and Wnt5a individually suppressed the GS/BMP-4-induced increase in odontoblastic differentiation. The odontoblastic phenotype was inhibited by chloroquine, but increased after treatment with rapamycin (an autophagy enhancer). Taken together with our previous findings, we have replicated our results from the rodent system in a novel human system. We have revealed a unique sequential cascade involving Atg10, Wnt5a, alpha1 integrin, and matrix metalloproteinase-3 in GS/BMP-4-induced differentiation of hiPS cells into odontoblast-like cells at a relatively early stage.

Ozeki, N., et al. (2017). "MicroRNA-211 and autophagy-related gene 14 signaling regulate osteoblast-like cell differentiation of human induced pluripotent stem cells." Exp Cell Res **352**(1): 63-74.

MicroRNAs (miRNAs) have been the subject of recent attention as key regulatory factors in cell differentiation. In the current study, to explore the early signaling cascade of osteogenic differentiation of human induced pluripotent stem (hiPS) cells, we investigated miR-211 regulation and autophagy-related gene (Atg) signaling in osteogenic differentiation. In addition to reciprocal strong induction of miR-211 expression in differentiated cells following osteogenic differentiation, we found abundant Argonaute 3 bound to miR-211. There were also dramatic increases in the mRNA and protein levels of Atg14 together with increases in the amount of autophagosomes as well as autophagic fluxes. While transfection of a miR-211 inhibitor abrogated the induction of Atg14, autophagy events, osteoblast differentiation markers, and induction of calcification were suppressed markedly. Treatment with small interfering RNAs against Atg14 also suppressed the osteogenic differentiation medium (ODM)-induced increase in osteogenic differentiation. The osteogenic phenotype was inhibited by chloroquine (an autophagy inhibitor), but increased after treatment with rapamycin (an autophagy inducer). Taken together with our previous findings, we have revealed a unique sequential cascade involving miR-211 and Atg14 in ODM-induced differentiation of hiPS cells into osteoblast-like cells at a relatively early stage.

Paik, D. T., et al. (2018). "Large-Scale Single-Cell RNA-Seq Reveals Molecular Signatures of Heterogeneous Populations of Human Induced Pluripotent Stem Cell-Derived Endothelial Cells." Circ Res.

Rationale: Human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) have risen as a useful tool in cardiovascular research, offering a wide gamut of translational and clinical applications. However, inefficiency of the currently available iPSC-EC differentiation protocol and underlying heterogeneity of derived iPSC-ECs remain as major limitations of iPSC-EC technology. Objective: Here we performed droplet-based single-cell RNA-sequencing (scRNA-seq) of the human iPSCs following iPSC-EC differentiation. Droplet-based scRNA-seq enables analysis of thousands of cells in parallel, allowing comprehensive analysis of transcriptional heterogeneity. Methods and Results: Bona fide iPSC-EC cluster was identified by scRNA-seq, which expressed high levels of endothelial-specific genes. iPSC-ECs, sorted by CD144 antibody-conjugated magnetic sorting, exhibited standard endothelial morphology and function including tube formation, response to inflammatory signals, and production of nitric oxide. Non-endothelial cell populations resulting from the differentiation protocol were identified, which included immature and atrial-like cardiomyocytes, hepatic-like cells, and vascular smooth muscle cells. Furthermore, scRNA-seq analysis of purified iPSC-ECs revealed transcriptional heterogeneity with four major subpopulations, marked by robust enrichment of CLDN5, APLNR, GJA5, and ESM1 genes respectively. Conclusions: Massively parallel, droplet-based scRNA-seq allowed meticulous analysis of thousands of human iPSCs subjected to iPSC-EC differentiation. Results showed inefficiency of the differentiation technique, which can be improved with further studies based on identification of molecular signatures that inhibit expansion of non-endothelial cell types. Subtypes of bona fide human iPSC-ECs were also identified, allowing us to sort for iPSC-ECs with specific biological function and identity.

Panula, S., et al. (2011). "Human germ cell differentiation from fetal- and adult-derived induced pluripotent stem cells." Hum Mol Genet **20**(4): 752-762.

Historically, our understanding of molecular genetic aspects of human germ cell development has

been limited, at least in part due to inaccessibility of early stages of human development to experimentation. However, the derivation of pluripotent stem cells may provide the necessary human genetic system to study germ cell development. In this study, we compared the potential of human induced pluripotent stem cells (iPSCs), derived from adult and fetal somatic cells to form primordial and meiotic germ cells, relative to human embryonic stem cells. We found that approximately 5% of human iPSCs differentiated to primordial germ cells (PGCs) following induction with bone morphogenetic proteins. Furthermore, we observed that PGCs expressed green fluorescent protein from a germ cell-specific reporter and were enriched for the expression of endogenous germ cell-specific proteins and mRNAs. In response to the overexpression of intrinsic regulators, we also observed that iPSCs formed meiotic cells with extensive synaptonemal complexes and post-meiotic haploid cells with a similar pattern of ACROSIN staining as observed in human spermatids. These results indicate that human iPSCs derived from reprogramming of adult somatic cells can form germline cells. This system may provide a useful model for molecular genetic studies of human germline formation and pathology and a novel platform for clinical studies and potential therapeutical applications.

Papapetrou, E. P. (2017). "Gene and Cell Therapy for beta-Thalassemia and Sickle Cell Disease with Induced Pluripotent Stem Cells (iPSCs): The Next Frontier." Adv Exp Med Biol **1013**: 219-240.

In recent years, breakthroughs in human pluripotent stem cell (hPSC) research, namely cellular reprogramming and the emergence of sophisticated genetic engineering technologies, have opened new frontiers for cell and gene therapy. The prospect of using hPSCs, either autologous or histocompatible, as targets of genetic modification and their differentiated progeny as cell products for transplantation, presents a new paradigm of regenerative medicine of potential tremendous value for the treatment of blood disorders, including beta-thalassemia (BT) and sickle cell disease (SCD). Despite advances at a remarkable pace and great promise, many roadblocks remain before clinical translation can be realistically considered. Here we discuss the theoretical advantages of cell therapies utilizing hPSC derivatives, recent proof-of-principle studies and the main challenges towards realizing the potential of hPSC therapies in the clinic.

Park, S., et al. (2017). "A Comprehensive, Ethnically Diverse Library of Sickle Cell Disease-Specific Induced Pluripotent Stem Cells." Stem Cell Reports **8**(4): 1076-1085.

Sickle cell anemia affects millions of people worldwide and is an emerging global health burden. As part of a large NIH-funded NextGen Consortium, we generated a diverse, comprehensive, and fully characterized library of sickle-cell-disease-specific induced pluripotent stem cells (iPSCs) from patients of different ethnicities, beta-globin gene (HBB) haplotypes, and fetal hemoglobin (HbF) levels. iPSCs stand to revolutionize the way we study human development, model disease, and perhaps eventually, treat patients. Here, we describe this unique resource for the study of sickle cell disease, including novel haplotype-specific polymorphisms that affect disease severity, as well as for the development of patient-specific therapeutics for this phenotypically diverse disorder. As a complement to this library, and as proof of principle for future cell- and gene-based therapies, we also designed and employed CRISPR/Cas gene editing tools to correct the sickle hemoglobin (HbS) mutation.

Parmar, V. M., et al. (2018). "A2E-associated cell death and inflammation in retinal pigmented epithelial cells from human induced pluripotent stem cells." Stem Cell Res **27**: 95-104.

Accumulation of lipofuscin in the retinal pigmented epithelium (RPE) is observed in retinal degenerative diseases including Stargardt disease and age-related macular degeneration. Bis-retinoid N-retinyl-N-retinylidene ethanolamine (A2E) is a major component of lipofuscin. A2E has been implicated in RPE atrophy and retinal inflammation; however, mice with A2E accumulation display only a mild retinal phenotype. In the current study, human iPSC-RPE (hiPSC-RPE) cells were generated from healthy individuals to examine effects of A2E in human RPE cells. hiPSC-RPE cells displayed RPE-specific features, which include expression of RPE-specific genes, tight junction formation and ability to carry out phagocytosis. hiPSC-RPE cells demonstrated cell death and increased VEGF-A production in a time-dependent manner when they were cocultured with 10muM of A2E. PCR array analyses revealed upregulation of 26 and 12 pro-inflammatory cytokines upon A2E and H2O2 exposure respectively, indicating that A2E and H2O2 can cause inflammation in human retinas. Notably, identified gene profiles were different between A2E- and H2O2- treated hiPSC-RPE cells. A2E caused inflammatory changes observed in retinal degenerative diseases more closely as compared to H2O2. Collectively, these data obtained with hiPSC-RPE cells provide evidence that A2E plays an important role in pathogenesis of retinal degenerative diseases in humans.

Pellett, S., et al. (2015). "Human Induced Pluripotent Stem Cell Derived Neuronal Cells Cultured on Chemically-Defined Hydrogels for Sensitive In Vitro Detection of Botulinum Neurotoxin." Sci Rep **5**: 14566.

Botulinum neurotoxin (BoNT) detection provides a useful model for validating cell-based neurotoxicity screening approaches, as sensitivity is dependent on functionally competent neurons and clear quantitative endpoints are available for correlating results to approved animal testing protocols. Here, human induced pluripotent stem cell (iPSC)-derived neuronal cells were cultured on chemically-defined poly (ethylene glycol) (PEG) hydrogels formed by "thiol-ene" photopolymerization and tested as a cell-based neurotoxicity assay by determining sensitivity to active BoNT/A1. BoNT/A1 sensitivity was comparable to the approved in vivo mouse bioassay for human iPSC-derived neurons and neural stem cells (iPSC-NSCs) cultured on PEG hydrogels or treated tissue culture polystyrene (TCP) surfaces. However, maximum sensitivity for BoNT detection was achieved two weeks earlier for iPSC-NSCs that were differentiated and matured on PEG hydrogels compared to TCP. Therefore, chemically-defined synthetic hydrogels offer benefits over standard platforms when optimizing culture conditions for cell-based screening and achieve sensitivities comparable to an approved animal testing protocol.

Pettinato, G., et al. (2014). "Formation of well-defined embryoid bodies from dissociated human induced pluripotent stem cells using microfabricated cell-repellent microwell arrays." Sci Rep **4**: 7402.

A simple, scalable, and reproducible technology that allows direct formation of large numbers of homogeneous and synchronized embryoid bodies (EBs) of defined sizes from dissociated human induced pluripotent stem cells (hiPSCs) was developed. Non-cell-adhesive hydrogels were used to create round-bottom microwells to host dissociated hiPSCs. No Rho-associated kinase inhibitor (ROCK-i), or centrifugation was needed and the side effects of ROCK-i can be avoided. The key requirement for the successful EB formation in addition to the non-cell-adhesive round-bottom microwells is the input cell density per microwell. Too few or too many cells loaded into the microwells will compromise the EB formation process. In parallel, we have tested our microwell-based system for homogeneous hEB formation from dissociated human embryonic stem cells (hESCs). Successful production of homogeneous hEBs from dissociated hESCs in the absence of ROCK-i and centrifugation was achieved within an optimal range of input cell density per microwell. Both the hiPSC- and hESC-derived hEBs expressed key proteins characteristic of all the three developmental germ layers, confirming their EB identity. This novel EB production technology may represent a versatile platform for the production of homogeneous EBs from dissociated human pluripotent stem cells (hPSCs).

Phanthong, P., et al. (2017). "Enhancement of beta-Globin Gene Expression in Thalassemic IVS2-654 Induced Pluripotent Stem Cell-Derived Erythroid Cells by Modified U7 snRNA." Stem Cells Transl Med **6**(4): 1059-1069.

The therapeutic use of patient-specific induced pluripotent stem cells (iPSCs) is emerging as a potential treatment of beta-thalassemia. Ideally, patient-specific iPSCs would be genetically corrected by various approaches to treat beta-thalassemia including lentiviral gene transfer, lentivirus-delivered shRNA, and gene editing. These corrected iPSCs would be subsequently differentiated into hematopoietic stem cells and transplanted back into the same patient. In this article, we present a proof of principle study for disease modeling and screening using iPSCs to test the potential use of the modified U7 small nuclear (sn) RNA to correct a splice defect in IVS2-654 beta-thalassemia. In this case, the aberration results from a mutation in the human beta-globin intron 2 causing an aberrant splicing of beta-globin pre-mRNA and preventing synthesis of functional beta-globin protein. The iPSCs (derived from mesenchymal stromal cells from a patient with IVS2-654 beta-thalassemia/hemoglobin (Hb) E) were transduced with a lentivirus carrying a modified U7 snRNA targeting an IVS2-654 beta-globin pre-mRNA in order to restore the correct splicing. Erythroblasts differentiated from the transduced iPSCs expressed high level of correctly spliced beta-globin mRNA suggesting that the modified U7 snRNA was expressed and mediated splicing correction of IVS2-654 beta-globin pre-mRNA in these cells. Moreover, a less active apoptosis cascade process was observed in the corrected cells at transcription level. This study demonstrated the potential use of a genetically modified U7 snRNA with patient-specific iPSCs for the partial restoration of the aberrant splicing process of beta-thalassemia. Stem Cells Translational Medicine 2017;6:1059-1069.

Polinati, P. P., et al. (2015). "Patient-Specific Induced Pluripotent Stem Cell-Derived RPE Cells: Understanding the Pathogenesis of Retinopathy in Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency." Invest Ophthalmol Vis Sci **56**(5): 3371-3382.

PURPOSE: Retinopathy is an important manifestation of trifunctional protein (TFP) deficiencies but not of other defects of fatty acid

oxidation. The common homozygous mutation in the TFP alpha-subunit gene HADHA (hydroxyacyl-CoA dehydrogenase), c.1528G>C, affects the long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) activity of TFP and blindness in infancy. The pathogenesis of the retinopathy is unknown. This study aimed to utilize human induced pluripotent stem cell (hiPSC) technology to create a disease model for the disorder, and to derive clues for retinopathy pathogenesis. METHODS: We implemented hiPSC technology to generate LCHAD deficiency (LCHADD) patient-specific retinal pigment epithelial (RPE) monolayers. These patient and control RPEs were extensively characterized for function and structure, as well as for lipid composition by mass spectrometry. RESULTS: The hiPSC-derived RPE monolayers of patients and controls were functional, as they both were able to phagocytose the photoreceptor outer segments in vitro. Interestingly, the patient RPEs had intense cytoplasmic neutral lipid accumulation, and lipidomic analysis revealed an increased triglyceride accumulation. Further, patient RPEs were small and irregular in shape, and their tight junctions were disorganized. Their ultrastructure showed decreased pigmentation, few melanosomes, and more melanolysosomes. CONCLUSIONS: We demonstrate that the RPE cell model reveals novel early pathogenic changes in LCHADD retinopathy, with robust lipid accumulation, inefficient pigmentation that is evident soon after differentiation, and a defect in forming tight junctions inducing apoptosis. We propose that LCHADD-RPEs are an important model for mitochondrial TFP retinopathy, and that their early pathogenic changes contribute to infantile blindness of LCHADD.

Polo, J. M., et al. (2010). "Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells." Nat Biotechnol **28**(8): 848-855.

Induced pluripotent stem cells (iPSCs) have been derived from various somatic cell populations through ectopic expression of defined factors. It remains unclear whether iPSCs generated from different cell types are molecularly and functionally similar. Here we show that iPSCs obtained from mouse fibroblasts, hematopoietic and myogenic cells exhibit distinct transcriptional and epigenetic patterns. Moreover, we demonstrate that cellular origin influences the in vitro differentiation potentials of iPSCs into embryoid bodies and different hematopoietic cell types. Notably, continuous passaging of iPSCs largely attenuates these differences. Our results suggest that early-passage iPSCs retain a transient epigenetic memory of their somatic cells of origin, which manifests as differential gene expression and altered differentiation capacity. These observations may influence ongoing attempts to use iPSCs for disease modeling and could also be exploited in potential therapeutic applications to enhance differentiation into desired cell lineages.

Pomeshchik, Y., et al. (2015). "Transplanted Human Induced Pluripotent Stem Cell-Derived Neural Progenitor Cells Do Not Promote Functional Recovery of Pharmacologically Immunosuppressed Mice With Contusion Spinal Cord Injury." Cell Transplant **24**(9): 1799-1812.

Improved functional recovery after spinal cord injury by transplantation of induced pluripotent stem cell-derived neural stem/progenitor cells (iPSC-NPCs) has been reported. However, beneficial effects of iPSC-based therapy have so far been produced mostly using genetically immunodeficient rodents. Because of the long time required for generation and characterization of iPSCs, the use of autologous iPSCs for treating patients with acute spinal cord injury (SCI) is not feasible. Therefore, it is of utmost importance to investigate the effect of iPSC-based therapy on functional recovery after SCI using pharmacologically immunosuppressed, immunocompetent animal models. Here we studied the functional outcome following subacute transplantation of human iPSC-derived NPCs into contused mouse spinal cord when tacrolimus was used as an immunosuppressive agent. We show that human iPSC-derived NPCs transplanted into pharmacologically immunosuppressed C57BL/6J mice exhibited poor long-term survival and failed to improve functional recovery after SCI as measured by Basso Mouse Scale (BMS) for locomotion and CatWalk gait analysis when compared to vehicle-treated animals. The scarce effect of iPSC-based therapy observed in the current study may be attributable to insufficient immunosuppressive effect, provided by monotherapy with tacrolimus in combination with immunogenicity of transplanted cells and complex microenvironment of the injured spinal cord. Our results highlight the importance of extensive preclinical studies of transplanted cells before the clinical application of iPSC-based cell therapy is achieved.

Prigione, A., et al. (2011). "Human induced pluripotent stem cells harbor homoplasmic and heteroplasmic mitochondrial DNA mutations while maintaining human embryonic stem cell-like metabolic reprogramming." Stem Cells **29**(9): 1338-1348.

Human induced pluripotent stem cells (iPSCs) have been recently found to harbor genomic alterations. However, the integrity of mitochondrial DNA (mtDNA) within reprogrammed cells has yet to be investigated. mtDNA mutations occur at a high rate and contribute to the pathology of a number of human

disorders. Furthermore, the lack of mtDNA integrity may alter cellular bioenergetics and limit efficient differentiation. We demonstrated previously that the derivation of iPSCs is associated with mitochondrial remodeling and a metabolic switch towards glycolysis. Here, we have discovered that alterations of mtDNA can occur upon the induction of pluripotency. Massively parallel pyrosequencing of mtDNA revealed that human iPSCs derived from young healthy donors harbored single base mtDNA mutations (substitutions, insertions, and deletions), both homoplasmic (in all mtDNA molecules) and heteroplasmic (in a fraction of mtDNAs), not present in the parental cells. mtDNA modifications were mostly common variants and not disease related. Moreover, iPSC lines bearing different mtDNA mutational loads maintained a consistent human embryonic stem cell-like reprogramming of energy metabolism. This involved the upregulation of glycolytic enzymes, increased glucose-6-phosphate levels, and the over-expression of pyruvate dehydrogenase kinase 1 protein, which reroutes the bioenergetic flux toward glycolysis. Hence, mtDNA mutations within iPSCs may not necessarily impair the correct establishment of pluripotency and the associated metabolic reprogramming. Nonetheless, the occurrence of pathogenic mtDNA modifications might be an important aspect to monitor when characterizing iPSC lines. Finally, we speculate that this random rearrangement of mtDNA molecules might prove beneficial for the derivation of mutation-free iPSCs from patients with mtDNA disorders.

Qi, X., et al. (2016). "Exosomes Secreted by Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells Repair Critical-Sized Bone Defects through Enhanced Angiogenesis and Osteogenesis in Osteoporotic Rats." Int J Biol Sci **12**(7): 836-849.

Bone defects caused by trauma, severe infection, tumor resection and skeletal abnormalities are common osteoporotic conditions and major challenges in orthopedic surgery, and there is still no effective solution to this problem. Consequently, new treatments are needed to develop regeneration procedures without side effects. Exosomes secreted by mesenchymal stem cells (MSCs) derived from human induced pluripotent stem cells (hiPSCs, hiPSC-MSC-Exos) incorporate the advantages of both MSCs and iPSCs with no immunogenicity. However, there are no reports on the application of hiPSC-MSC-Exos to enhance angiogenesis and osteogenesis under osteoporotic conditions. HiPSC-MSC-Exos were isolated and identified before use. The effect of hiPSC-MSC-Exos on the proliferation and osteogenic differentiation of bone marrow MSCs derived from ovariectomized (OVX) rats (rBMSCs-OVX) in vitro were investigated. In vivo, hiPSC-MSC-Exos were implanted into critical size bone defects in ovariectomized rats, and bone regeneration and angiogenesis were examined by microcomputed tomography (micro-CT), sequential fluorescent labeling analysis, microfil perfusion and histological and immunohistochemical analysis. The results in vitro showed that hiPSC-MSC-Exos enhanced cell proliferation and alkaline phosphatase (ALP) activity, and up-regulated mRNA and protein expression of osteoblast-related genes in rBMSCs-OVX. In vivo experiments revealed that hiPSC-MSC-Exos dramatically stimulated bone regeneration and angiogenesis in critical-sized calvarial defects in ovariectomized rats. The effect of hiPSC-MSC-Exos increased with increasing concentration. In this study, we showed that hiPSC-MSC-Exos effectively stimulate the proliferation and osteogenic differentiation of rBMSCs-OVX, with the effect increasing with increasing exosome concentration. Further analysis demonstrated that the application of hiPSC-MSC-Exos+beta-TCP scaffolds promoted bone regeneration in critical-sized calvarial defects by enhancing angiogenesis and osteogenesis in an ovariectomized rat model.

Qin, J., et al. (2014). "Cell fusion enhances mesendodermal differentiation of human induced pluripotent stem cells." Stem Cells Dev **23**(23): 2875-2882.

Human induced pluripotent stem cells (iPS cells) resemble embryonic stem cells and can differentiate into cell derivatives of all three germ layers. However, frequently the differentiation efficiency of iPS cells into some lineages is rather poor. Here, we found that fusion of iPS cells with human hematopoietic stem cells (HSCs) enhances iPS cell differentiation. Such iPS hybrids showed a prominent differentiation bias toward hematopoietic lineages but also toward other mesendodermal lineages. Additionally, during differentiation of iPS hybrids, expression of early mesendodermal markers-Brachyury (T), MIX1 Homeobox-Like Protein 1 (MIXL1), and Goosecoid (GSC)-appeared with faster kinetics than in parental iPS cells. Following iPS hybrid differentiation there was a prominent induction of NODAL and inhibition of NODAL signaling blunted mesendodermal differentiation. This indicates that NODAL signaling is critically involved in mesendodermal bias of iPS hybrid differentiation. In summary, we demonstrate that iPS cell fusion with HSCs prominently enhances iPS cell differentiation.

Qin, Y., et al. (2016). "Potential Cancer Prevention and Treatment by Silencing the Killer Cell

Immunoglobulin-like Receptor Gene in Natural Killer Cells Derived from Induced Pluripotent Stem Cells." Enliven J Stem Cell Res Regen Med **3**(1).

Cancer immunosurveillance is an important host protection process, monitoring the presence of irregular cells that could potentially transform into tumor cells, effectively clearing the body of transformed tumor cells at their earliest stages, and thus maintaining regular cellular homeostasis. Natural killer (NK) cells are effector lymphocytes of the innate immune system, playing a critical role in surveillance for tumor cells, while also eliminating virally infected cells. The significance of the anti-tumor role of NK cells was recently further verified by findings that immunosuppression in most cancer patients is not perceptible until late stages. NK cells express the low-affinity Fc-activating receptor, CD16, and the inhibitory receptor, killer cell immunoglobulin-like receptor (KIR). Consequently, activation of NK cells is determined by the balance of inhibitory and activating receptor stimulation. Here, we propose establishing an induced pluripotent stem cell (iPSC)-derived NK cell line with KIR gene knockout or knockdown as a possible regimen to treat and prevent cancer. We further postulate that an optimal mixture of NK iPSCs with and without KIR gene knockout, would reach a maximum antitumor activity, with minimal side effects. We also discuss the possible advantages of KIR-knockout NK iPSCs for adoptive immunotherapy in patients with cancer.

Quintana-Bustamante, O. and J. C. Segovia (2016). "Generation of Patient-Specific induced Pluripotent Stem Cell from Peripheral Blood Mononuclear Cells by Sendai Reprogramming Vectors." Methods Mol Biol **1353**: 1-11.

Induced pluripotent stem cells (iPSC) technology has changed preclinical research since their generation was described by Shinya Yamanaka in 2006. iPSCs are derived from somatic cells after being reprogrammed back to an embryonic state by specific combination of reprogramming factors. These reprogrammed cells resemble all the characteristic of embryonic stem cells (ESC). The reprogramming technology is even more valuable to research diseases biology and treatment by opening gene and cell therapies in own patient's iPSC. Patient-specific iPSC can be generated from a large variety of patient cells by any of the myriad of reprogramming platforms described. Here, we describe the generation of patient-specific iPSC from patient peripheral blood mononuclear cells by Sendai Reprogramming vectors.

Rajaei, B., et al. (2017). "Pancreatic Endoderm-Derived From Diabetic Patient-Specific Induced Pluripotent Stem Cell Generates Glucose-Responsive Insulin-Secreting Cells." J Cell Physiol **232**(10): 2616-2625.

Human-induced pluripotent stem cells (hiPSCs) can potentially serve as an invaluable source for cell replacement therapy and allow the creation of patient- and disease-specific stem cells without the controversial use of embryos and avoids any immunological incompatibility. The generation of insulin-producing pancreatic beta-cells from pluripotent stem cells in vitro provides an unprecedented cell source for personal drug discovery and cell transplantation therapy in diabetes. A new five-step protocol was introduced in this study, effectively induced hiPSCs to differentiate into glucose-responsive insulin-producing cells. This process mimics in vivo pancreatic organogenesis by directing cells through stages resembling definitive endoderm, primitive gut-tube endoderm, posterior foregut, pancreatic endoderm, and endocrine precursor. Each stage of differentiation were characterized by stage-specific markers. The produced cells exhibited many properties of functional beta-cells, including expression of critical beta-cells transcription factors, the potency to secrete C-peptide in response to high levels of glucose and the presence of mature endocrine secretory granules. This high efficient differentiation protocol, established in this study, yielded 79.18% insulin-secreting cells which were responsive to glucose five times higher than the basal level. These hiPSCs-derived glucose-responsive insulin-secreting cells might provide a promising approach for the treatment of type I diabetes mellitus. J. Cell. Physiol. 232: 2616-2625, 2017. (c) 2016 Wiley Periodicals, Inc.

Rajaei, B., et al. (2017). "In Vitro Generation of Glucose-Responsive Insulin-Secreting Cells from Pancreatic and Duodenal Homeobox 1-Overexpressing Human-Induced Pluripotent Stem Cell Derived from Diabetic Patient." ASAIO J.

Pancreatic and duodenal homeobox 1 (PDX1), a member of the homeodomain-containing transcription factor family, is a key transcription factor for pancreas development and mature beta-cell function. In this study, induced overexpression of PDX1 resulted in producing susceptible cells for pancreatic differentiation and was well beneficial to enhance beta-cell production, maturation, function, and survival. Induced PDX1 overexpression in harmony with a set of signaling molecules involves in guiding the signaling pathways toward pancreas development, leaded to high-efficient in vitro generation of ectopic insulin-producing cells (IPCs) with the effectively reduced number of polyhormonal cells and increased number of INS single-positive cells. This strategy yielded 85.61% glucose-responsive insulin-positive cells in vitro, which was seven times higher than the

basal level, and electron microscopy images revealed the presence of mature beta-cell secretory granules. The generation of glucose-responsive insulin-secreting beta-like cells from human-induced pluripotent stem cells (hiPSCs) in vitro would provide a promising approach to produce an unprecedented cell source for cell transplantation therapy in diabetes without the ethical obstacle of embryonic stem cells and would bypass immune rejection. These cells are an invaluable source for disease modeling, drug discovery, and pharmacogenomics studies as well.

Rajesh, D., et al. (2011). "Human lymphoblastoid B-cell lines reprogrammed to EBV-free induced pluripotent stem cells." Blood **118**(7): 1797-1800.

Generation of patient-specific induced pluripotent cells (iPSCs) holds great promise for regenerative medicine. Epstein-Barr virus immortalized lymphoblastoid B-cell lines (LCLs) can be generated from a minimal amount of blood and are banked worldwide as cellular reference material for immunologic or genetic analysis of pedigreed study populations. We report the generation of iPSCs from 2 LCLs (LCL-iPSCs) via a feeder-free episomal method using a cocktail of transcription factors and small molecules. LCL-derived iPSCs exhibited normal karyotype, expressed pluripotency markers, lost oriP/EBNA-1 episomal vectors, generated teratomas, retained donor identity, and differentiated in vitro into hematopoietic, cardiac, neural, and hepatocyte-like lineages. Significantly, although the parental LCLs express viral EBNA-1 and other Epstein-Barr virus latency-related elements for their survival, their presence was not detectable in LCL-iPSCs. Thus, reprogramming LCLs could offer an unlimited source for patient-specific iPSCs.

Ratajczak, M. Z., et al. (2016). "Stem cells and clinical practice: new advances and challenges at the time of emerging problems with induced pluripotent stem cell therapies." Pol Arch Med Wewn **126**(11): 879-890.

Humans, like other species that reproduce sexually, originate from a fertilized oocyte (zygote), which is a totipotent stem cell giving rise to an adult organism. During the process of embryogenesis, stem cells at different levels of the developmental hierarchy establish all 3 germ layers and give rise to tissuecommitted stem cells, which are responsible for rejuvenation of a given tissue or organ. The robustness of the stem cell compartment is one of the major factors that directly impact life quality as well as lifespan. Stem cells continuously replace cells and tissues that are used up during life; however, this replacement occurs at a different pace in various organs. The rapidly developing field of regenerative medicine is taking advantage of these physiological properties of stem cells and is attempting to employ them in clinical settings to regenerate damaged organs (eg, the heart, liver or bone). For this purpose, the stem cells most successfully employed so far are adult tissue-derived stem cells isolated mainly from bone marrow, mobilized peripheral blood, umbilical cord blood, fat tissue, and even myocardial biopsies. At the same time, attempts to employ embryonic stem cells and induced pluripotent stem cells in the clinic have failed due to their genomic instability and the risk of tumor formation. In this review, we will discuss the various potential sources of stem cells that are currently employed in regenerative medicine and the mechanisms that explain their beneficial effects. We will also highlight the preliminary results of clinical trials as well as the emerging problems relating to stem cell therapies in cardiology.

Reboun, M., et al. (2016). "X-Chromosome Inactivation Analysis in Different Cell Types and Induced Pluripotent Stem Cells Elucidates the Disease Mechanism in a Rare Case of Mucopolysaccharidosis Type II in a Female." Folia Biol (Praha) **62**(2): 82-89.

Mucopolysaccharidosis type II (MPS II) is an X-linked lysosomal storage disorder resulting from deficiency of iduronate-2-sulphatase activity. The disease manifests almost exclusively in males; only 16 symptomatic heterozygote girls have been reported so far. We describe the results of X-chromosome inactivation analysis in a 5-year-old girl with clinically severe disease and heterozygous mutation p.Arg468Gln in the IDS gene. X inactivation analysed at three X-chromosome loci showed extreme skewing (96/4 to 99/1) in two patient's cell types. This finding correlated with exclusive expression of the mutated allele. Induced pluripotent stem cells (iPSC) generated from the patient's peripheral blood demonstrated characteristic pluripotency markers, deficiency of enzyme activity, and mutation in the IDS gene. These cells were capable of differentiation into other cell types (cardiomyocytes, neurons). In MPS II iPSC clones, the X inactivation ratio remained highly skewed in culture conditions that led to partial X inactivation reset in Fabry disease iPSC clones. Our data, in accordance with the literature, suggest that extremely skewed X inactivation favouring the mutated allele is a crucial condition for manifestation of MPS II in females. This suggests that the X inactivation status and enzyme activity have a prognostic value and should be used to evaluate MPS II in females. For the first time, we show generation of iPSC from a symptomatic MPS II female patient that can serve as a cellular model for further research of the pathogenesis and treatment of this disease.

Ronaghi, M., et al. (2010). "Challenges of stem cell therapy for spinal cord injury: human embryonic stem cells, endogenous neural stem cells, or induced pluripotent stem cells?" Stem Cells **28**(1): 93-99.

Spinal cord injury (SCI) causes myelopathy, damage to white matter, and myelinated fiber tracts that carry sensation and motor signals to and from the brain. The gray matter damage causes segmental losses of interneurons and motoneurons and restricts therapeutic options. Recent advances in stem cell biology, neural injury, and repair, and the progress toward development of neuroprotective and regenerative interventions are the basis for increased optimism. This review summarizes the pathophysiological mechanisms following SCI and compares human embryonic, adult neural, and the induced pluripotent stem cell-based therapeutic strategies for SCI.

Rouhani, F. J., et al. (2016). "Mutational History of a Human Cell Lineage from Somatic to Induced Pluripotent Stem Cells." PLoS Genet **12**(4): e1005932.

The accuracy of replicating the genetic code is fundamental. DNA repair mechanisms protect the fidelity of the genome ensuring a low error rate between generations. This sustains the similarity of individuals whilst providing a repertoire of variants for evolution. The mutation rate in the human genome has recently been measured to be 50-70 de novo single nucleotide variants (SNVs) between generations. During development mutations accumulate in somatic cells so that an organism is a mosaic. However, variation within a tissue and between tissues has not been analysed. By reprogramming somatic cells into induced pluripotent stem cells (iPSCs), their genomes and the associated mutational history are captured. By sequencing the genomes of polyclonal and monoclonal somatic cells and derived iPSCs we have determined the mutation rates and show how the patterns change from a somatic lineage in vivo through to iPSCs. Somatic cells have a mutation rate of 14 SNVs per cell per generation while iPSCs exhibited a ten-fold lower rate. Analyses of mutational signatures suggested that deamination of methylated cytosine may be the major mutagenic source in vivo, whilst oxidative DNA damage becomes dominant in vitro. Our results provide insights for better understanding of mutational processes and lineage relationships between human somatic cells. Furthermore it provides a foundation for interpretation of elevated mutation rates and patterns in cancer.

Rubach, M., et al. (2014). "Mesenchymal stem cells and their conditioned medium improve integration of purified induced pluripotent stem cell-derived cardiomyocyte clusters into myocardial tissue." Stem Cells Dev **23**(6): 643-653.

Induced pluripotent stem cell-derived cardiomyocytes (iPS-CMs) might become therapeutically relevant to regenerate myocardial damage. Purified iPS-CMs exhibit poor functional integration into myocardial tissue. The aim of this study was to investigate whether murine mesenchymal stem cells (MSCs) or their conditioned medium (MScond) improves the integration of murine iPS-CMs into myocardial tissue. Vital or nonvital embryonic murine ventricular tissue slices were cocultured with purified clusters of iPS-CMs in combination with murine embryonic fibroblasts (MEFs), MSCs, or MScond. Morphological integration was assessed by visual scoring and functional integration by isometric force and field potential measurements. We observed a moderate morphological integration of iPS-CM clusters into vital, but a poor integration into nonvital, slices. MEFs and MSCs but not MScond improved morphological integration of CMs into nonvital slices and enabled purified iPS-CMs to confer force. Coculture of vital slices with iPS-CMs and MEFs or MSCs resulted in an improved electrical integration. A comparable improvement of electrical coupling was achieved with the cell-free MScond, indicating that soluble factors secreted by MSCs were involved in electrical coupling. We conclude that cells such as MSCs support the engraftment and adhesion of CMs, and confer force to noncontractile tissue. Furthermore, soluble factors secreted by MSCs mediate electrical coupling of purified iPS-CM clusters to myocardial tissue. These data suggest that MSCs may increase the functional engraftment and therapeutic efficacy of transplanted iPS-CMs into infarcted myocardium.

Rufaihah, A. J., et al. (2013). "Human induced pluripotent stem cell-derived endothelial cells exhibit functional heterogeneity." Am J Transl Res **5**(1): 21-35.

Human induced pluripotent stem cell-derived endothelial cells (hiPSC-ECs) are promising for treatment of vascular diseases. However, hiPSC-ECs purified based on CD31 expression are comprised of arterial, venous, and lymphatic subtypes. It is unclear whether hiPSC-ECs are heterogeneous in nature, and whether there may be functional benefits of enriching for specific subtypes. Therefore, we sought to characterize the hiPSC-ECs and enrich for each subtype, and demonstrate whether such enrichment would have functional significance. The hiPSC-ECs were generated from differentiation of hiPSCs using vascular endothelial growth factor (VEGF)-A and bone morphogenetic protein-4. The hiPSC-ECs were purified based on positive expression of CD31.

Subsequently, we sought to enrich for each subtype. Arterial hiPSC-ECs were induced using higher concentrations of VEGF-A and 8-bromoadenosine-3':5'-cyclic monophosphate in the media, whereas lower concentrations of VEGF-A favored venous subtype. VEGF-C and angiopoietin-1 promoted the expression of lymphatic phenotype. Upon FACS purification based on CD31+ expression, the hiPSC-EC population was observed to display typical endothelial surface markers and functions. However, the hiPSC-EC population was heterogeneous in that they displayed arterial, venous, and to a lesser degree, lymphatic lineage markers. Upon comparing vascular formation in matrigel plugs in vivo, we observed that arterial enriched hiPSC-ECs formed a more extensive capillary network in this model, by comparison to a heterogeneous population of hiPSC-ECs. This study demonstrates that FACS purification of CD31+ hiPSC-ECs produces a diverse population of ECs. Refining the differentiation methods can enrich for subtype-specific hiPSC-ECs with functional benefits of enhancing neovascularization.

Ruiz, S., et al. (2015). "Limiting replication stress during somatic cell reprogramming reduces genomic instability in induced pluripotent stem cells." Nat Commun **6**: 8036.

The generation of induced pluripotent stem cells (iPSC) from adult somatic cells is one of the most remarkable discoveries in recent decades. However, several works have reported evidence of genomic instability in iPSC, raising concerns on their biomedical use. The reasons behind the genomic instability observed in iPSC remain mostly unknown. Here we show that, similar to the phenomenon of oncogene-induced replication stress, the expression of reprogramming factors induces replication stress. Increasing the levels of the checkpoint kinase 1 (CHK1) reduces reprogramming-induced replication stress and increases the efficiency of iPSC generation. Similarly, nucleoside supplementation during reprogramming reduces the load of DNA damage and genomic rearrangements on iPSC. Our data reveal that lowering replication stress during reprogramming, genetically or chemically, provides a simple strategy to reduce genomic instability on mouse and human iPSC.

Saadai, P., et al. (2013). "Human induced pluripotent stem cell-derived neural crest stem cells integrate into the injured spinal cord in the fetal lamb model of myelomeningocele." J Pediatr Surg **48**(1): 158-163.

BACKGROUND/PURPOSE: Neurological function in patients with myelomeningocele (MMC) is limited even after prenatal repair. Neural crest stem cells (NCSCs) can improve neurological function in models of spinal cord injury. We aimed to evaluate the survival, integration, and differentiation of human NCSCs derived from induced pluripotent stem cells (iPSC-NCSCs) in the fetal lamb model of MMC. METHODS: Human iPSCs derived from skin fibroblasts were differentiated into NCSCs in vitro, mixed with hydrogel, and seeded on nanofibrous scaffolds for surgical transplantation. Fetal lambs (n=2) underwent surgical MMC creation and repair with iPSC-NCSC seeded scaffolds. Gross necropsy and immunohistochemistry were performed at term. RESULTS: IPSC-NCSCs expressed NCSC markers, maintained > 95% viability, and demonstrated neuronal differentiation in vitro. Immunohistochemical analysis of repaired spinal cords thirty days after transplantation demonstrated the co-localization of human nuclear mitotic apparatus protein (NuMA) and Neurofilament M subunit (NFM) in the area of spinal cord injury. No gross tumors were identified. CONCLUSIONS: Human iPSC-NCSCs survived, integrated, and differentiated into neuronal lineage in the fetal lamb model of MMC. This is the first description of human stem cell engraftment in a model of fetal MMC and supports the concept of using NCSCs to address spinal cord damage in MMC.

Sakurai, F., et al. (2017). "Human induced-pluripotent stem cell-derived hepatocyte-like cells as an in vitro model of human hepatitis B virus infection." Sci Rep **7**: 45698.

In order to understand the life cycle of hepatitis B virus (HBV) and to develop efficient anti-HBV drugs, a useful in vitro cell culture system which allows HBV infection and recapitulates virus-host interactions is essential; however, pre-existing in vitro HBV infection models are often problematic. Here, we examined the potential of human induced-pluripotent stem (iPS) cell-derived hepatocyte-like cells (iPS-HLCs) as an in vitro HBV infection model. Expression levels of several genes involved in HBV infection, including the sodium taurocholate cotransporting polypeptide (NTCP) gene, were gradually elevated as the differentiation status of human iPS cells proceeded to iPS-HLCs. The mRNA levels of these genes were comparable between primary human hepatocytes (PHHs) and iPS-HLCs. Following inoculation with HBV, we found significant production of HBV proteins and viral RNAs in iPS-HLCs. The three major forms of the HBV genome were detected in iPS-HLCs by Southern blotting analysis. Anti-HBV agents entecavir and Myrcludex-B, which are a nucleoside analogue reverse transcriptase inhibitor and a synthetic pre-S1 peptide, respectively, significantly inhibited HBV infection in iPS-HLCs. These data demonstrate

that iPS-HLCs can be used as a promising in vitro HBV infection model.

Salewski, R. P., et al. (2010). "Are induced pluripotent stem cells the future of cell-based regenerative therapies for spinal cord injury?" J Cell Physiol **222**(3): 515-521.

Despite advances in medical and surgical care, current clinical therapies for spinal cord injury (SCI) are limited. During the last two decades, the search for new therapies has been revolutionized by the discovery of stem cells, inspiring scientists and clinicians to search for stem cell-based reparative approaches for many disorders, including neurotrauma. Cell-based therapies using embryonic and adult stem cells in animal models of these disorders have provided positive outcome results. However, the availability of clinically suitable cell sources for human application has been hindered by both technical and ethical issues. The recent discovery of induced pluripotent stem (iPS) cells holds the potential to revolutionize the field of regenerative medicine by offering the option of autologous transplantation, thus eliminating the issue of host rejection. Herein, we will provide the rationale for the use of iPS cells in SCI therapies. In this review, we will evaluate the recent advancements in the field of iPS cells including their capacity for differentiation toward neural lineages that may allow iPS cells transplantation in cell-based therapy for spinal cord repair.

Salewski, R. P., et al. (2015). "Transplantation of Induced Pluripotent Stem Cell-Derived Neural Stem Cells Mediate Functional Recovery Following Thoracic Spinal Cord Injury Through Remyelination of Axons." Stem Cells Transl Med **4**(7): 743-754.

UNLABELLED:: Neural stem cells (NSCs) from embryonic or fetal/adult tissue sources have shown considerable promise in regenerative strategies for traumatic spinal cord injury (SCI). However, there are limitations with their use related to the availability, immunogenicity, and uncertainty of the mechanisms involved. To address these issues, definitive NSCs derived from induced pluripotent stem (iPS) cells generated using a nonviral, piggyBac transposon approach, were investigated. Committed NSCs were generated from iPS cells using a free-floating neurosphere methodology previously described by our laboratory. To delineate the mechanism of action, specifically the role of exogenous myelination, NSCs derived from wildtype (wt) and nonmyelinating Shiverer (shi) iPS cell lines were used following thoracic SCI with subacute intraspinal transplantation. Behavioral, histological, and electrophysiological outcomes were analyzed to assess the effectiveness of this treatment. The wt- and shi-iPS-NSCs were validated and shown to be equivalent except in myelination capacity. Both iPS-NSC lines successfully integrated into the injured spinal cord and predominantly differentiated to oligodendrocytes, but only the wt-iPS-NSC treatment resulted in a functional benefit. The wt-iPS-dNSCs, which exhibited the capacity for remyelination, significantly improved neurobehavioral function (Basso Mouse Scale and CatWalk), histological outcomes, and electrophysiological measures of axonal function (sucrose gap analysis) compared with the nonmyelinating iPS-dNSCs and cell-free controls. In summary, we demonstrated that iPS cells can generate translationally relevant NSCs for applications in SCI. Although NSCs have a diverse range of functions in the injured spinal cord, remyelination is the predominant mechanism of recovery following thoracic SCI. SIGNIFICANCE: Gain-of-function/loss-of-function techniques were used to examine the mechanistic importance of graft-derived remyelination following thoracic spinal cord injury (SCI). The novel findings of this study include the first use of neural stem cells (NSCs) from induced pluripotent stem cells (iPSCs) derived using the clonal neurosphere expansion conditions, for the treatment of SCI, the first characterization and in vivo application of iPSCs from Shiverer mouse fibroblasts, and the first evidence of the importance of remyelination by pluripotent-sourced NSCs for SCI repair and regeneration.

Salomonis, N., et al. (2016). "Integrated Genomic Analysis of Diverse Induced Pluripotent Stem Cells from the Progenitor Cell Biology Consortium." Stem Cell Reports **7**(1): 110-125.

The rigorous characterization of distinct induced pluripotent stem cells (iPSC) derived from multiple reprogramming technologies, somatic sources, and donors is required to understand potential sources of variability and downstream potential. To achieve this goal, the Progenitor Cell Biology Consortium performed comprehensive experimental and genomic analyses of 58 iPSC from ten laboratories generated using a variety of reprogramming genes, vectors, and cells. Associated global molecular characterization studies identified functionally informative correlations in gene expression, DNA methylation, and/or copy-number variation among key developmental and oncogenic regulators as a result of donor, sex, line stability, reprogramming technology, and cell of origin. Furthermore, X-chromosome inactivation in PSC produced highly correlated differences in teratoma-lineage staining and regulator expression upon differentiation. All experimental results, and raw, processed, and metadata from these analyses, including powerful tools, are interactively accessible from a new online portal at https://www.synapse.org to

serve as a reusable resource for the stem cell community.

Sanchez-Freire, V., et al. (2014). "Effect of human donor cell source on differentiation and function of cardiac induced pluripotent stem cells." J Am Coll Cardiol **64**(5): 436-448.

BACKGROUND: Human-induced pluripotent stem cells (iPSCs) are a potentially unlimited source for generation of cardiomyocytes (iPSC-CMs). However, current protocols for iPSC-CM derivation face several challenges, including variability in somatic cell sources and inconsistencies in cardiac differentiation efficiency. OBJECTIVES: This study aimed to assess the effect of epigenetic memory on differentiation and function of iPSC-CMs generated from somatic cell sources of cardiac versus noncardiac origins. METHODS: Cardiac progenitor cells (CPCs) and skin fibroblasts from the same donors were reprogrammed into iPSCs and differentiated into iPSC-CMs via embryoid body and monolayer-based differentiation protocols. RESULTS: Differentiation efficiency was found to be higher in CPC-derived iPSC-CMs (CPC-iPSC-CMs) than in fibroblast-derived iPSC-CMs (Fib-iPSC-CMs). Gene expression analysis during cardiac differentiation demonstrated up-regulation of cardiac transcription factors in CPC-iPSC-CMs, including NKX2-5, MESP1, ISL1, HAND2, MYOCD, MEF2C, and GATA4. Epigenetic assessment revealed higher methylation in the promoter region of NKX2-5 in Fib-iPSC-CMs compared with CPC-iPSC-CMs. Epigenetic differences were found to dissipate with increased cell passaging, and a battery of in vitro assays revealed no significant differences in their morphological and electrophysiological properties at early passage. Finally, cell delivery into a small animal myocardial infarction model indicated that CPC-iPSC-CMs and Fib-iPSC-CMs possess comparable therapeutic capabilities in improving functional recovery in vivo. CONCLUSIONS: This is the first study to compare differentiation of iPSC-CMs from human CPCs versus human fibroblasts from the same donors. The authors demonstrate that although epigenetic memory improves differentiation efficiency of cardiac versus noncardiac somatic cell sources in vitro, it does not contribute to improved functional outcome in vivo.

Sarafian, R., et al. (2018). "Monitoring cell line identity in collections of human induced pluripotent stem cells." Stem Cell Res **28**: 66-70.

The ability to reprogram somatic cells into induced pluripotent stem cells (hiPSCs) has led to the generation of large collections of cell lines from thousands of individuals with specific phenotypes, many of which will be shared among different research groups as invaluable tools for biomedical research. As hiPSC-based research involves extensive culture of many cell lines, the issue periodic cell line identification is particularly important to ensure that cell line identity remains accurate. Here we analyzed the different commercially available genotyping methods considering ease of in-house genotyping, cost and informativeness, and applied one of them in our workflow for hiPSC generation. We show that the chosen STR method was able to establish a unique DNA profile for each of the 35 individuals/hiPSC lines at the examined sites, as well as identify two discrepancies resulting from inadvertently exchanged samples. Our results highlight the importance of hiPSC line genotyping by an in-house method that allows periodic cell line identification and demonstrate that STR is a useful approach to supplement less frequent karyotyping and epigenetic evaluations.

Sato, M., et al. (2017). "Regeneration of cervical reserve cell-like cells from human induced pluripotent stem cells (iPSCs): A new approach to finding targets for cervical cancer stem cell treatment." Oncotarget **8**(25): 40935-40945.

Cervical reserve cells are epithelial progenitor cells that are pathologically evident as the origin of cervical cancer. Thus, investigating the characteristics of cervical reserve cells could yield insight into the features of cervical cancer stem cells (CSCs). In this study, we established a method for the regeneration of cervical reserve cell-like properties from human induced pluripotent stem cells (iPSCs) and named these cells induced reserve cell-like cells (iRCs). Approximately 70% of iRCs were positive for the reserve cell markers p63, CK5 and CK8. iRCs also expressed the SC junction markers CK7, AGR2, CD63, MMP7 and GDA. While iRCs expressed neither ERalpha nor ERbeta, they expressed CA125. These data indicated that iRCs possessed characteristics of cervical epithelial progenitor cells. iRCs secreted higher levels of several inflammatory cytokines such as macrophage migration inhibitory factor (MIF), soluble intercellular adhesion molecule 1 (sICAM-1) and C-X-C motif ligand 10 (CXCL-10) compared with normal cervical epithelial cells. iRCs also expressed human leukocyte antigen-G (HLA-G), which is an important cell-surface antigen for immune tolerance and carcinogenesis. Together with the fact that cervical CSCs can originate from reserve cells, our data suggested that iRCs were potent immune modulators that might favor cervical cancer cell survival. In conclusion, by generating reserve cell-like properties from iPSCs, we provide a new approach that may yield new insight into cervical cancer stem cells and help find new oncogenic targets.

Sebastiano, V., et al. (2011). "In situ genetic correction of the sickle cell anemia mutation in human induced pluripotent stem cells using engineered zinc finger nucleases." Stem Cells **29**(11): 1717-1726.

The combination of induced pluripotent stem cell (iPSC) technology and targeted gene modification by homologous recombination (HR) represents a promising new approach to generate genetically corrected, patient-derived cells that could be used for autologous transplantation therapies. This strategy has several potential advantages over conventional gene therapy including eliminating the need for immunosuppression, avoiding the risk of insertional mutagenesis by therapeutic vectors, and maintaining expression of the corrected gene by endogenous control elements rather than a constitutive promoter. However, gene targeting in human pluripotent cells has remained challenging and inefficient. Recently, engineered zinc finger nucleases (ZFNs) have been shown to substantially increase HR frequencies in human iPSCs, raising the prospect of using this technology to correct disease causing mutations. Here, we describe the generation of iPSC lines from sickle cell anemia patients and in situ correction of the disease causing mutation using three ZFN pairs made by the publicly available oligomerized pool engineering method (OPEN). Gene-corrected cells retained full pluripotency and a normal karyotype following removal of reprogramming factor and drug-resistance genes. By testing various conditions, we also demonstrated that HR events in human iPSCs can occur as far as 82 bps from a ZFN-induced break. Our approach delineates a roadmap for using ZFNs made by an open-source method to achieve efficient, transgene-free correction of monogenic disease mutations in patient-derived iPSCs. Our results provide an important proof of principle that ZFNs can be used to produce gene-corrected human iPSCs that could be used for therapeutic applications.

Seki, T., et al. (2014). "Generation and characterization of functional cardiomyocytes derived from human T cell-derived induced pluripotent stem cells." PLoS One **9**(1): e85645.

Induced pluripotent stem cells (iPSCs) have been proposed as novel cell sources for genetic disease models and revolutionary clinical therapies. Accordingly, human iPSC-derived cardiomyocytes are potential cell sources for cardiomyocyte transplantation therapy. We previously developed a novel generation method for human peripheral T cell-derived iPSCs (TiPSCs) that uses a minimally invasive approach to obtain patient cells. However, it remained unknown whether TiPSCs with genomic rearrangements in the T cell receptor (TCR) gene could differentiate into functional cardiomyocyte in vitro. To address this issue, we investigated the morphology, gene expression pattern, and electrophysiological properties of TiPSC-derived cardiomyocytes differentiated by floating culture. RT-PCR analysis and immunohistochemistry showed that the TiPSC-derived cardiomyocytes properly express cardiomyocyte markers and ion channels, and show the typical cardiomyocyte morphology. Multiple electrode arrays with application of ion channel inhibitors also revealed normal electrophysiological responses in the TiPSC-derived cardiomyocytes in terms of beating rate and the field potential waveform. In this report, we showed that TiPSCs successfully differentiated into cardiomyocytes with morphology, gene expression patterns, and electrophysiological features typical of native cardiomyocytes. TiPSCs-derived cardiomyocytes obtained from patients by a minimally invasive technique could therefore become disease models for understanding the mechanisms of cardiac disease and cell sources for revolutionary cardiomyocyte therapies.

Selvaraj, V., et al. (2010). "Switching cell fate: the remarkable rise of induced pluripotent stem cells and lineage reprogramming technologies." Trends Biotechnol **28**(4): 214-223.

Cell reprogramming, in which a differentiated cell is made to switch its fate, is an emerging field with revolutionary prospects in biotechnology and medicine. The recent discovery of induced pluripotency by means of in vitro reprogramming has made way for unprecedented approaches for regenerative medicine, understanding human disease and drug discovery. Moreover, recent studies on regeneration and repair by direct lineage reprogramming in vivo offer an attractive novel alternative to cell therapy. Although we continue to push the limits of current knowledge in the field of cell reprogramming, the mechanistic elements that underlie these processes remain largely elusive. This article reviews landmark developments in cell reprogramming, current knowledge, and technological developments now on the horizon with significant promise for biomedical applications.

Senju, S., et al. (2011). "Generation of dendritic cells and macrophages from human induced pluripotent stem cells aiming at cell therapy." Gene Ther **18**(9): 874-883.

This report describes generation of dendritic cells (DCs) and macrophages from human induced pluripotent stem (iPS) cells. iPS cell-derived DC (iPS-DC) exhibited the morphology of typical DC and function of T-cell stimulation and antigen presentation. iPS-DC loaded with cytomegalovirus (CMV) peptide induced vigorous expansion of CMV-specific

autologous CD8+ T cells. Macrophages (iPS-MP) with activity of zymosan phagocytosis and C5a-induced chemotaxis were also generated from iPS cells. Genetically modified iPS-MPs were generated by the introduction of expression vectors into undifferentiated iPS cells, isolation of transfectant iPS cell clone and subsequent differentiation. By this procedure, we generated iPS-MP expressing a membrane-bound form of single chain antibody (scFv) specific to amyloid beta (Abeta), the causal protein of Alzheimer's disease. The scFv-transfectant iPS-MP exhibited efficient Abeta-specific phagocytosis activity. iPS-MP expressing CD20-specific scFv engulfed and killed BALL-1 B-cell leukemia cells. Anti-BALL-1 effect of iPS-MP in vivo was demonstrated in a xeno-transplantation model using severe combined immunodeficient mice. In addition, we established a xeno-free culture protocol to generate iPS-DC and iPS-MP. Collectively, we demonstrated the possibility of application of iPS-DC and macrophages to cell therapy.

Sequiera, G. L., et al. (2017). "Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells as an Individual-Specific and Renewable Source of Adult Stem Cells." Methods Mol Biol **1553**: 183-190.

This chapter deals with the employment of human-induced pluripotent stem cells (hiPSCs) as a candidate to differentiate into mesenchymal stem cells (MSCs). This would enable to help establish a regular source of human MSCs with the aim of avoiding the problems associated with procuring the MSCs either from different healthy individuals or patients, limited extraction potentials, batch-to-batch variations or from diverse sources such as bone marrow or adipose tissue. The procedures described herein allow for a guided and ensured approach for the regular maintenance of hiPSCs and their subsequent differentiation into MSCs using the prescribed medium. Subsequently, an easy protocol for the successive isolation and purification of the hiPSC-differentiated MSCs is outlined, which is carried out through passaging and can be further sorted through flow cytometry. Further, the maintenance and expansion of the resultant hiPSC-differentiated MSCs using appropriate characterization techniques, i.e., Reverse-transcription PCR and immunostaining is also elaborated. The course of action has been deliberated keeping in mind the awareness and the requisites available to even beginner researchers who mostly have access to regular consumables and medium components found in the general laboratory.

Shaer, A., et al. (2014). "Differentiation of human induced pluripotent stem cells into insulin-like cell clusters with miR-186 and miR-375 by using chemical transfection." Appl Biochem Biotechnol **174**(1): 242-258.

Diabetes mellitus is characterized by either the inability to produce insulin or insensitivity to insulin secreted by the body. Islet cell replacement is an effective approach for diabetes treatment; however, it is not sufficient for all the diabetic patients. MicroRNAs (miRNAs) are a class of small noncoding RNAs that play an important role in mediating a broad and expanding range of biological activities, such as pancreas development. The present study aimed to develop a protocol to efficiently differentiate human induced pluripotent stem (iPS) cells into islet-like cell clusters (ILCs) in vitro by using miR-186 and miR-375. The human iPS colonies were transfected with hsa-miR-186 and hsa-miR-375 by using siPORT NeoFX Transfection Agent, and the differentiation was compared to controls. Total RNA was extracted 24 and 48 h after transfection. The gene expressions of insulin, NGN3, GLUT2, PAX4, PAX6, KIR6.2, NKX6.1, PDX1, Glucagon, and OCT4 were then evaluated through real-time qPCR. On the third day, the potency of the clusters was assessed in response to high glucose levels. Dithizone (DTZ) was used to identify the existence of the beta-cells. Besides, the presence of insulin and NGN3 proteins was investigated by immunocytochemistry. Morphological changes were observed on the first day after the chemical transfection, and cell clusters were formed on the third day. The expression of pancreatic specific transcription factors was increased on the first day and significantly increased on the second day. The ILCs were positive for insulin and NGN3 proteins in the immunocytochemistry. Besides, the clusters were stained with DTZ and secreted insulin in glucose challenge test. Overexpression of miR-186 and miR-375 can be an alternative strategy for producing ILCs from the iPS cells in a short time. This work provides a new approach by using patient-specific iPSCs for beta-cell replacement therapy in diabetic patients.

Shafa, M., et al. (2018). "Human induced pluripotent stem cell-derived lung progenitor and alveolar epithelial cells attenuate hyperoxia-induced lung injury." Cytotherapy **20**(1): 108-125.

BACKGROUND AIMS: Bronchopulmonary dysplasia (BPD), a chronic lung disease characterized by disrupted lung growth, is the most common complication in extreme premature infants. BPD leads to persistent pulmonary disease later in life. Alveolar epithelial type 2 cells (AEC2s), a subset of which represent distal lung progenitor cells (LPCs), promote normal lung growth and repair. AEC2 depletion may contribute to persistent lung injury in BPD. We hypothesized that induced pluripotent stem cell (iPSC)-derived AECs prevent lung damage in

experimental oxygen-induced BPD. METHODS: Mouse AECs (mAECs), miPSCs/mouse embryonic stem sells, human umbilical cord mesenchymal stromal cells (hUCMSCs), human (h)iPSCs, hiPSC-derived LPCs and hiPSC-derived AECs were delivered intratracheally to hyperoxia-exposed newborn mice. Cells were pre-labeled with a red fluorescent dye for in vivo tracking. RESULTS: Airway delivery of primary mAECs and undifferentiated murine pluripotent cells prevented hyperoxia-induced impairment in lung function and alveolar growth in neonatal mice. Similar to hUCMSC therapy, undifferentiated hiPSCs also preserved lung function and alveolar growth in hyperoxia-exposed neonatal NOD/SCID mice. Long-term assessment of hiPSC administration revealed local teratoma formation and cellular infiltration in various organs. To develop a clinically relevant cell therapy, we used a highly efficient method to differentiate hiPSCs into a homogenous population of AEC2s. Airway delivery of hiPSC-derived AEC2s and hiPSC-derived LPCs, improved lung function and structure and resulted in long-term engraftment without evidence of tumor formation. CONCLUSIONS: hiPSC-derived AEC2 therapy appears effective and safe in this model and warrants further exploration as a therapeutic option for BPD and other lung diseases characterized by AEC injury.

Shi, Y. (2009). "Induced pluripotent stem cells, new tools for drug discovery and new hope for stem cell therapies." Curr Mol Pharmacol **2**(1): 15-18.

Somatic cell nuclear transfer or therapeutic cloning has provided great hope for stem cell-based therapies. However, therapeutic cloning has been experiencing both ethical and technical difficulties. Recent breakthrough studies using a combination of four factors to reprogram human somatic cells into pluripotent stem cells without using embryos or eggs have led to an important revolution in stem cell research. Comparative analysis of human induced pluripotent stem cells and human embryonic stem cells using assays for morphology, cell surface marker expression, gene expression profiling, epigenetic status, and differentiation potential have revealed a remarkable degree of similarity between these two pluripotent stem cell types. This mini-review summarizes these ground-breaking studies. These advances in reprogramming will enable the creation of patient-specific stem cell lines to study various disease mechanisms. The cellular models created will provide valuable tools for drug discovery. Furthermore, this reprogramming system provides great potential to design customized patient-specific stem cell therapies with economic feasibility.

Shi, Z., et al. (2012). "Induced pluripotent stem cell-related genes influence biological behavior and 5-fluorouracil sensitivity of colorectal cancer cells." J Zhejiang Univ Sci B **13**(1): 11-19.

OBJECTIVE: We aimed to perform a preliminary study of the association between induced pluripotent stem cell (iPS)-related genes and biological behavior of human colorectal cancer (CRC) cells, and the potential for developing anti-cancer drugs targeting these genes. METHODS: We used real-time reverse transcriptase polymerase chain reaction (RT-PCR) to evaluate the transcript levels of iPS-related genes NANOG, OCT4, SOX2, C-MYC and KLF4 in CRC cell lines and cancer stem cells (CSCs)-enriched tumor spheres. NANOG was knockdowned in CRC cell line SW620 by lentiviral transduction. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays, plate colony formation, and a mouse xenograft model were used to evaluate alterations in biological behavior in NANOG-knockdown SW620 cells. Also, mock-knockdown and NANOG-knockdown cells were treated with 5-fluorouracil (5-FU) and survival rate was measured by MTT assay to evaluate drug sensitivity. RESULTS: A significant difference in the transcript levels of iPS-related genes between tumor spheres and their parental bulky cells was observed. NANOG knockdown suppressed proliferation, colony formation, and in vivo tumorigenicity but increased the sensitivity to 5-FU of SW620 cells. 5-FU treatment greatly inhibited the expression of the major stemness-associated genes NANOG, OCT4, and SOX2. CONCLUSIONS: These results collectively suggest an overlap between iPS-related genes and CSCs in CRC. Quenching a certain gene NANOG may truncate the aggressiveness of CRC cells.

Shimba, K., et al. (2016). "Cell-cycle-dependent Ca (2+) transients in human induced pluripotent stem cells revealed by a simultaneous imaging of cell nuclei and intracellular Ca (2+) level." Integr Biol (Camb) **8**(9): 985-990.

Cell cycle phase and [Ca (2+)]i are key determinants of self-renewal and differentiation in pluripotent stem cells. However, little is known about their relationship in human pluripotent stem cells owing to the lack of an effective method. Here, we applied an imaging-based approach for evaluating the relationship between the cell cycle and Ca (2+) transients in human induced pluripotent stem (iPS) cells. Ca imaging and DNA staining was simultaneously performed at the same site. Then, individual cells were recognized and the cell cycle phase was estimated from the image of nuclei. We found that 18 +/- 4% of human iPS cells exhibited spontaneous Ca (2+) transients and their inter-transient

interval was 119 +/- 19 s. Ca wave events were observed in 64% of the sample and the [Ca (2+)]i elevation propagated among 47 +/- 30 cells with a duration of 57 +/- 22 s. With the imaging-based approach, we demonstrated that the ratio of cells exhibiting Ca (2+) transients significantly decreased during cell cycle progression, suggesting that the relationship previously described in mouse cells holds true in the human context as well. These results suggest that our method is suitable for evaluating Ca (2+) transients, the cell cycle phase, and their relationship with densely cultured cells.

Shofuda, T., et al. (2013). "Human Decidua-Derived Mesenchymal Cells Are a Promising Source for the Generation and Cell Banking of Human Induced Pluripotent Stem Cells." Cell Med **4**(3): 125-147.

Placental tissue is a biomaterial with remarkable potential for use in regenerative medicine. It has a three-layer structure derived from the fetus (amnion and chorion) and the mother (decidua), and it contains huge numbers of cells. Moreover, placental tissue can be collected without any physical danger to the donor and can be matched with a variety of HLA types. The decidua-derived mesenchymal cells (DMCs) are highly proliferative fibroblast-like cells that express a similar pattern of CD antigens as bone marrow-derived mesenchymal cells (BM-MSCs). Here we demonstrated that induced pluripotent stem (iPS) cells could be efficiently generated from DMCs by retroviral transfer of reprogramming factor genes. DMC-hiPS cells showed equivalent characteristics to human embryonic stem cells (hESCs) in colony morphology, global gene expression profile (including human pluripotent stem cell markers), DNA methylation status of the OCT3/4 and NANOG promoters, and ability to differentiate into components of the three germ layers in vitro and in vivo. The RNA expression of XIST and the methylation status of its promoter region suggested that DMC-iPSCs, when maintained undifferentiated and pluripotent, had three distinct states: (1) complete X-chromosome reactivation, (2) one inactive X-chromosome, or (3) an epigenetic aberration. Because DMCs are derived from the maternal portion of the placenta, they can be collected with the full consent of the adult donor and have considerable ethical advantages for cell banking and the subsequent generation of human iPS cells for regenerative applications.

Shtrichman, R., et al. (2013). "Induced pluripotent stem cells (iPSCs) derived from different cell sources and their potential for regenerative and personalized medicine." Curr Mol Med **13**(5): 792-805.

Human induced pluripotent stem cells (hiPSCs) have great potential as a robust source of progenitors for regenerative medicine. The novel technology also enables the derivation of patient-specific cells for applications to personalized medicine, such as for personal drug screening and toxicology. However, the biological characteristics of iPSCs are not yet fully understood and their similarity to human embryonic stem cells (hESCs) is still unresolved. Variations among iPSCs, resulting from their original tissue or cell source, and from the experimental protocols used for their derivation, significantly affect epigenetic properties and differentiation potential. Here we review the potential of iPSCs for regenerative and personalized medicine, and assess their expression pattern, epigenetic memory and differentiation capabilities in relation to their parental tissue source. We also summarize the patient-specific iPSCs that have been derived for applications in biological research and drug discovery; and review risks that must be overcome in order to use iPSC technology for clinical applications.

Shu, T., et al. (2015). "Protective Effects and Mechanisms of Salvianolic Acid B Against H (2)O (2)-Induced Injury in Induced Pluripotent Stem Cell-Derived Neural Stem Cells." Neurochem Res **40**(6): 1133-1143.

Induced pluripotent stem cells (iPSCs) have the potential to differentiate into neural lineages. Salvianolic acid B (Sal B) is a commonly used, traditional Chinese medicine for enhancing neuroprotective effects, and has antioxidant, anti-inflammatory, and antiapoptotic properties. Here, we explore the potential mechanism of Sal B in protecting iPSC-derived neural stem cells (NSCs) against H2O2-induced injury. iPSCs were induced into NSCs, iPSC-derived NSCs were treated with 50 muM Sal B for 24.5 h and 500 muM H2O2 for 24 h. The resulting effects were examined by flow cytometry analysis, quantitative reverse-transcription polymerase chain reaction, and western blotting. Upon H2O2 exposure, Sal B significantly promoted cell viability and stabilization of the mitochondrial membrane potential. Sal B also visibly decreased the cell apoptotic ratio. In addition, Sal B markedly reduced expression of matrix metalloproteinase (MMP)-2 and -9, and phosphospecific signal transducer and activator of transcription 3 (p-STAT3), and increased the level of tissue inhibitor of metalloproteinase (TIMP)-2 in iPSC-derived NSCs induced by H2O2. These results suggest that Sal B protects iPSC-derived NSCs against H2O2-induced oxidative stress. The mechanisms of this stress tolerance may be attributed to modulation of the MMP/TIMP system and inhibition of the STAT3 signaling pathway.

Silk, K. M., et al. (2012). "Cross-presentation of tumour antigens by human induced pluripotent stem cell-derived CD141(+)XCR1+ dendritic cells." Gene Ther **19**(10): 1035-1040.

Monocyte-derived dendritic cells (moDC) have been widely used in cancer immunotherapy but show significant donor-to-donor variability and low capacity for the cross-presentation of tumour-associated antigens (TAA) to CD8(+) T cells, greatly limiting the success of this approach. Given recent developments in induced pluripotency and the relative ease with which induced pluripotent stem (iPS) cell lines may be generated from individuals, we have succeeded in differentiating dendritic cells (DC) from human leukocyte antigen (HLA)-A (*)0201(+) iPS cells (iPS cell-derived DC (ipDC)), using protocols compliant with their subsequent clinical application. Unlike moDC, a subset of ipDC was found to coexpress CD141 and XCR1 that have been shown previously to define the human equivalent of mouse CD8alpha (+) DC, in which the capacity for cross-presentation has been shown to reside. Accordingly, ipDC were able to cross-present the TAA, Melan A, to a CD8(+) T-cell clone and stimulate primary Melan A-specific responses among naive T cells from an HLA-A (*)0201(+) donor. Given that CD141(+)XCR1(+) DC are present in peripheral blood in trace numbers that preclude their clinical application, the ability to generate a potentially unlimited source from iPS cells offers the possibility of harnessing their capacity for cross-priming of cytotoxic T lymphocytes for the induction of tumour-specific immune responses.

Sirenko, O., et al. (2014). "High-content assays for hepatotoxicity using induced pluripotent stem cell-derived cells." Assay Drug Dev Technol **12**(1): 43-54.

Development of predictive in vitro assays for early toxicity evaluation is extremely important for improving the drug development process and reducing drug attrition rates during clinical development. High-content imaging-based in vitro toxicity assays are emerging as efficient tools for safety and efficacy testing to improve drug development efficiency. In this report we have used an induced pluripotent stem cell (iPSC)-derived hepatocyte cell model having a primary tissue-like phenotype, unlimited availability, and the potential to compare cells from different individuals. We examined a number of assays and phenotypic markers and developed automated screening methods for assessing multiparameter readouts of general and mechanism-specific hepatotoxicity. Endpoints assessed were cell viability, nuclear shape, average and integrated cell area, mitochondrial membrane potential, phospholipid accumulation, cytoskeleton integrity, and apoptosis. We assayed compounds with known mechanisms of toxicity and also evaluated a diverse hepatotoxicity library of 240 compounds. We conclude that high-content automated screening assays using iPSC-derived hepatocytes are feasible, provide information about mechanisms of toxicity, and can facilitate the safety assessment of drugs and chemicals.

Smith, M. J., et al. (2015). "In Vitro T-Cell Generation From Adult, Embryonic, and Induced Pluripotent Stem Cells: Many Roads to One Destination." Stem Cells **33**(11): 3174-3180.

T lymphocytes are critical mediators of the adaptive immune system and have the capacity to serve as therapeutic agents in the areas of transplant and cancer immunotherapy. While T cells can be isolated and expanded from patients, T cells derived in vitro from both hematopoietic stem/progenitor cells (HSPCs) and human pluripotent stem cells (hPSCs) offer great potential advantages in generating a self-renewing source of T cells that can be readily genetically modified. T-cell differentiation in vivo is a complex process requiring tightly regulated signals; providing the correct signals in vitro to induce T-cell lineage commitment followed by their development into mature, functional, single positive T cells, is similarly complex. In this review, we discuss current methods for the in vitro derivation of T cells from murine and human HSPCs and hPSCs that use feeder-cell and feeder-cell-free systems. Furthermore, we explore their potential for adoption for use in T-cell-based therapies.

Solari, C., et al. (2011). "Induced pluripotent stem cells' self-renewal and pluripotency is maintained by a bovine granulosa cell line-conditioned medium." Biochem Biophys Res Commun **410**(2): 252-257.

Induced pluripotent stem cells (iPSCs) are a promising type of stem cells, comparable to embryonic stem cells (ESCs) in terms of self-renew and pluripotency, generated by reprogramming somatic cells. These cells are an attractive approach to supply patient-specific pluripotent cells, for producing in vitro models of disease, drug discovery, toxicology and potentially treating degenerative disease circumventing immune rejection. In spite of the great advance since iPSCs' establishment, their obtention and propagation is an increasing area of great interest. In a recent work, we have shown that the conditioned medium from a bovine granulosa cell line (BGC-CM) is able to preserve the basic properties of mESCs. Therefore, based on our previous results and the reported resemblance between iPSCs and ESCs, we hypothesized that BGC-CM could provide a favorable context to culturing iPSCs. In this work, we have reprogrammed mouse embryonic fibroblasts obtaining

iPSC lines, and showed that they can be propagated in BGC-CM while maintaining self-renewal and pluripotency, evidenced by expression of specific gene markers and capability of in vitro and in vivo differentiation to cell types from the three germ layers. We believe that these findings may provide a novel context to propagate iPSCs to study the molecular mechanisms involved in self-renewal and pluripotency.

Somers, A., et al. (2010). "Generation of transgene-free lung disease-specific human induced pluripotent stem cells using a single excisable lentiviral stem cell cassette." Stem Cells **28**(10): 1728-1740.

The development of methods to achieve efficient reprogramming of human cells while avoiding the permanent presence of reprogramming transgenes represents a critical step toward the use of induced pluripotent stem cells (iPSC) for clinical purposes, such as disease modeling or reconstituting therapies. Although several methods exist for generating iPSC free of reprogramming transgenes from mouse cells or neonatal normal human tissues, a sufficiently efficient reprogramming system is still needed to achieve the widespread derivation of disease-specific iPSC from humans with inherited or degenerative diseases. Here, we report the use of a humanized version of a single lentiviral "stem cell cassette" vector to accomplish efficient reprogramming of normal or diseased skin fibroblasts obtained from humans of virtually any age. Simultaneous transfer of either three or four reprogramming factors into human target cells using this single vector allows derivation of human iPSC containing a single excisable viral integration that on removal generates human iPSC free of integrated transgenes. As a proof of principle, here we apply this strategy to generate >100 lung disease-specific iPSC lines from individuals with a variety of diseases affecting the epithelial, endothelial, or interstitial compartments of the lung, including cystic fibrosis, alpha-1 antitrypsin deficiency-related emphysema, scleroderma, and sickle-cell disease. Moreover, we demonstrate that human iPSC generated with this approach have the ability to robustly differentiate into definitive endoderm in vitro, the developmental precursor tissue of lung epithelia.

Somoza, R. A. and F. J. Rubio (2012). "Cell therapy using induced pluripotent stem cells or somatic stem cells: this is the question." Curr Stem Cell Res Ther **7**(3): 191-196.

A lot of effort has been developed to bypass the use of embryonic stem cells (ES) in human therapies, because of several concerns and ethical issues. Some unsolved problems of using stem cells for human therapies, excluding the human embryonic origin, are: how to regulate cell plasticity and proliferation, immunological compatibility, potential adverse side-effects when stem cells are systemically administrated, and the in vivo signals to rule out a specific cell fate after transplantation. Currently, it is known that almost all tissues of an adult organism have somatic stem cells (SSC). Whereas ES are primary involved in the genesis of new tissues and organs, SSC are involved in regeneration processes, immuno-regulatory and homeostasis mechanisms. Although the differentiating potential of ES is higher than SSC, several studies suggest that some types of SSC, such as mesenchymal stem cells (MSC), can be induced epigenetically to differentiate into tissue-specific cells of different lineages. This unexpected pluripotency and the variety of sources that they come from, can make MSC-like cells suitable for the treatment of diverse pathologies and injuries. New hopes for cell therapy came from somatic/mature cells and the discovery that could be reprogrammed to a pluripotent stage similar to ES, thus generating induced pluripotent stem cells (iPS). For this, it is necessary to overexpress four main reprogramming factors, Sox2, Oct4, Klf4 and c-Myc. The aim of this review is to analyze the potential and requirements of cellular based tools in human therapy strategies, focusing on the advantage of using MSC over iPS.

Sotthibundhu, A., et al. (2016). "Rapamycin regulates autophagy and cell adhesion in induced pluripotent stem cells." Stem Cell Res Ther **7**(1): 166.

BACKGROUND: Cellular reprogramming is a stressful process, which requires cells to engulf somatic features and produce and maintain stemness machineries. Autophagy is a process to degrade unwanted proteins and is required for the derivation of induced pluripotent stem cells (iPSCs). However, the role of autophagy during iPSC maintenance remains undefined. METHODS: Human iPSCs were investigated by microscopy, immunofluorescence, and immunoblotting to detect autophagy machinery. Cells were treated with rapamycin to activate autophagy and with bafilomycin to block autophagy during iPSC maintenance. High concentrations of rapamycin treatment unexpectedly resulted in spontaneous formation of round floating spheres of uniform size, which were analyzed for differentiation into three germ layers. Mass spectrometry was deployed to reveal altered protein expression and pathways associated with rapamycin treatment. RESULTS: We demonstrate that human iPSCs express high basal levels of autophagy, including key components of APMKalpha, ULK1/2, BECLIN-1, ATG13, ATG101, ATG12, ATG3, ATG5, and LC3B. Block of autophagy by bafilomycin induces iPSC death and rapamycin attenuates the bafilomycin effect.

Rapamycin treatment upregulates autophagy in iPSCs in a dose/time-dependent manner. High concentration of rapamycin reduces NANOG expression and induces spontaneous formation of round and uniformly sized embryoid bodies (EBs) with accelerated differentiation into three germ layers. Mass spectrometry analysis identifies actin cytoskeleton and adherens junctions as the major targets of rapamycin in mediating iPSC detachment and differentiation. CONCLUSIONS: High levels of basal autophagy activity are present during iPSC derivation and maintenance. Rapamycin alters expression of actin cytoskeleton and adherens junctions, induces uniform EB formation, and accelerates differentiation. IPSCs are sensitive to enzyme dissociation and require a lengthy differentiation time. The shape and size of EBs also play a role in the heterogeneity of end cell products. This research therefore highlights the potential of rapamycin in producing uniform EBs and in shortening iPSC differentiation duration.

Stebbins, M. J., et al. (2018). "Activation of RARalpha, RARgamma, or RXRalpha Increases Barrier Tightness in Human Induced Pluripotent Stem Cell-Derived Brain Endothelial Cells." Biotechnol J **13**(2).

The blood-brain barrier (BBB) is critical to central nervous system (CNS) health. Brain microvascular endothelial cells (BMECs) are often used as in vitro BBB models for studying BBB dysfunction and therapeutic screening applications. Human pluripotent stem cells (hPSCs) can be differentiated to cells having key BMEC barrier and transporter properties, offering a renewable, scalable source of human BMECs. hPSC-derived BMECs have previously been shown to respond to all-trans retinoic acid (RA), and the goal of this study was to identify the stages at which differentiating human induced pluripotent stem cells (iPSCs) respond to activation of RA receptors (RARs) to impart BBB phenotypes. Here the authors identified that RA application to iPSC-derived BMECs at days 6-8 of differentiation led to a substantial elevation in transendothelial electrical resistance and induction of VE-cadherin expression. Specific RAR agonists identified RARalpha, RARgamma, and RXRalpha as receptors capable of inducing barrier phenotypes. Moreover, RAR/RXRalpha costimulation elevated VE-cadherin expression and improved barrier fidelity to levels that recapitulated the effects of RA. This study elucidates the roles of RA signaling in iPSC-derived BMEC differentiation, and identifies directed agonist approaches that can improve BMEC fidelity for drug screening studies while also distinguishing potential nuclear receptor targets to explore in BBB dysfunction and therapy.

Sugawara, T., et al. (2012). "Investigating cellular identity and manipulating cell fate using induced pluripotent stem cells." Stem Cell Res Ther **3**(2): 8.

Induced pluripotent stem (iPS) cells, obtained from reprogramming somatic cells by ectopic expression of a defined set of transcription factors or chemicals, are expected to be used as differentiated cells for drug screening or evaluations of drug toxicity and cell replacement therapies. As pluripotent stem cells, iPS cells are similar to embryonic stem (ES) cells in morphology and marker expression. Several types of iPS cells have been generated using combinations of reprogramming molecules and/or small chemical compounds from different types of tissues. A comprehensive approach, such as global gene or microRNA expression analysis and whole genomic DNA methylation profiling, has demonstrated that iPS cells are similar to their embryonic counterparts. Considering the substantial variation among iPS cell lines reported to date, the safety and therapeutic implications of these differences should be thoroughly evaluated before they are used in cell therapies. Here, we review recent research defining the concept of standardization for iPS cells, their ability to differentiate and the identity of the differentiated cells.

Sugita, S., et al. (2015). "Inhibition of T-cell activation by retinal pigment epithelial cells derived from induced pluripotent stem cells." Invest Ophthalmol Vis Sci **56**(2): 1051-1062.

PURPOSE: The purpose of this study was to determine whether human retinal pigment epithelial (RPE) cells from induced pluripotent stem (iPS) cells could inhibit T-cell activation in vitro. METHODS: Cultured iPS-derived RPE (iPS-RPE) cells were established from fresh skin tissues or dental pulp cells obtained from healthy donors or a retinal patient after informed consent was obtained. To confirm expression of the specific markers on iPS and iPS-RPE cells, immunohistochemistry, quantitative RT-PCR (qRT-PCR), and flow cytometry were performed. Target T cells were obtained from peripheral blood mononuclear cells of healthy donors. Target T cells were assessed for proliferation by incorporation of bromodeoxyuridine or carboxyfluorescein succinimidyl ester for production of cytokines such as IFN-gamma. Expression of TGFbeta and other candidate molecules by iPS-RPE cells was evaluated with flow cytometry, ELISA, multiplex cytokine array, immunohistochemistry, and qRT-PCR. RESULTS: The RPE cells we established from iPS cells had many characteristics of mature RPE cells but no characteristics of pluripotent stem cells. Cultured iPS-

RPE cells inhibited cell proliferation and production of IFN-gamma by activated CD4(+) T cells. In some bystander T cells, iPS-derived RPE cells induced CD25(+)Foxp3(+) regulatory T cells in vitro. Induced pluripotent stem-RPE cells constitutively expressed TGFbeta and suppressed activation of T cells via soluble TGFbeta, because TGFbeta-downregulated iPS-RPE cells did not inhibit this T-cell activation. CONCLUSIONS: Cultured iPS-derived retinal cells fully suppress T-cell activation. Transplantation of iPS-RPE cells into the eye might be a therapy for retinal disorders.

Sugita, S., et al. (2018). "Natural Killer Cell Inhibition by HLA-E Molecules on Induced Pluripotent Stem Cell-Derived Retinal Pigment Epithelial Cells." Invest Ophthalmol Vis Sci **59**(5): 1719-1731.

Purpose: To determine whether human induced pluripotent stem (iPS) cell-derived retinal pigment epithelial (RPE) cells (iPS-RPE) can suppress natural killer (NK) cell activation. Methods: iPS-RPE cells were cocultured with peripheral blood mononuclear cells (PBMCs) or purified NK cells from healthy donors after stimulation with cytokines. To confirm expression of NK cell-specific markers, flow cytometry and quantitative RT-PCR (qRT-PCR) were performed. NK cells (or PBMCs) cocultured with iPS-RPE cells were assessed for proliferation by Ki-67 expression with flow cytometry, and NK suppression by RPE cells was assessed for granzyme B production with ELISA. Human leukocyte antigen (HLA) expression including HLA-E on iPS-RPE cells was evaluated with flow cytometry and qRT-PCR. The effect of HLA-E downregulation was also investigated using small interfering RNA (siRNA) systems. Following iPS-RPE cell transplantation in vivo, we evaluated NK cell invasion in the retina with immunohistochemistry. Results: Activated NK cells expressed NK-related markers such as CD16, CD56, and CD11b, and NK cells produced cytotoxic agents such as granzyme B, perforin, and TNF-alpha. Human iPS-RPE cells inhibited cell proliferation and production of these cytotoxic agents by activated NK cells in vitro. iPS-RPE cells constitutively expressed HLA-E and suppressed NK cell activation through an interaction between HLA-E and CD94/NKG2A. Moreover, immunohistochemical evaluation of monkey RPE transplantation into in vivo immune rejection models showed no NK cell invasion in the retina in allografts or xenografts except for one xenografted eye. Conclusions: Cultured iPS cell-derived RPE cells greatly suppress NK cell activation. Thus, NK cells might be inactivated when exposed to this type of retinal cell.

Sugiura, M., et al. (2014). "Induced pluripotent stem cell generation-associated point mutations arise during the initial stages of the conversion of these cells." Stem Cell Reports **2**(1): 52-63.

A large number of point mutations have been identified in induced pluripotent stem cell (iPSC) genomes to date. Whether these mutations are associated with iPSC generation is an important and controversial issue. In this study, we approached this critical issue in different ways, including an assessment of iPSCs versus embryonic stem cells (ESCs), and an investigation of variant allele frequencies and the heterogeneity of point mutations within a single iPSC clone. Through these analyses, we obtained strong evidence that iPSC-generation-associated point mutations occur frequently in a transversion-predominant manner just after the onset of cell lineage conversion. The heterogeneity of the point mutation profiles within an iPSC clone was also revealed and reflects the history of the emergence of each mutation. Further, our results suggest a possible approach for establishing iPSCs with fewer point mutations.

Sun, N. and H. Zhao (2014). "Seamless correction of the sickle cell disease mutation of the HBB gene in human induced pluripotent stem cells using TALENs." Biotechnol Bioeng **111**(5): 1048-1053.

Sickle cell disease (SCD) is the most common human genetic disease which is caused by a single mutation of human beta-globin (HBB) gene. The lack of long-term treatment makes the development of reliable cell and gene therapies highly desirable. Disease-specific patient-derived human induced pluripotent stem cells (hiPSCs) have great potential for developing novel cell and gene therapies. With the disease-causing mutations corrected in situ, patient-derived hiPSCs can restore normal cell functions and serve as a renewable autologous cell source for the treatment of genetic disorders. Here we successfully utilized transcription activator-like effector nucleases (TALENs), a recently emerged novel genome editing tool, to correct the SCD mutation in patient-derived hiPSCs. The TALENs we have engineered are highly specific and generate minimal off-target effects. In combination with piggyBac transposon, TALEN-mediated gene targeting leaves no residual ectopic sequences at the site of correction and the corrected hiPSCs retain full pluripotency and a normal karyotype. Our study demonstrates an important first step of using TALENs for the treatment of genetic diseases such as SCD, which represents a significant advance toward hiPSC-based cell and gene therapies.

Suzuki, H., et al. (2010). "Therapeutic angiogenesis by transplantation of induced pluripotent stem cell-derived Flk-1 positive cells." BMC Cell Biol **11**: 72.

BACKGROUND: Induced pluripotent stem (iPS) cells are the novel stem cell population induced from somatic cells. It is anticipated that iPS will be used in the expanding field of regenerative medicine. Here, we investigated whether implantation of fetal liver kinase-1 positive (Flk-1+) cells derived from iPS cells could improve angiogenesis in a mouse hind limb model of ischemia. RESULTS: Flk-1+ cells were induced from iPS cells after four to five days of culture. Hind limb ischemia was surgically induced and sorted Flk-1+ cells were directly injected into ischemic hind limbs of athymic nude mice. Revascularization of the ischemic hind limb was accelerated in mice that were transplanted with Flk-1+ cells compared with control mice, which were transplanted with vehicle, as evaluated by laser Doppler blood flowmetry. Transplantation of Flk-1+ cells also increased expression of VEGF mRNA in ischemic tissue compared to controls. CONCLUSIONS: Direct local implantation of iPS cell-derived Flk-1+ cells would salvage tissues from ischemia. These data indicate that iPS cells could be valuable in the therapeutic induction of angiogenesis.

Suzuki, N., et al. (2012). "Establishment of retinal progenitor cell clones by transfection with Pax6 gene of mouse induced pluripotent stem (iPS) cells." Neurosci Lett **509**(2): 116-120.

We previously reported that transfection of Pax6 gene which regulated early events in eye development into mouse ES cells brought about their differentiation into retinal progenitors. Here, we attempted to establish cloned retinal progenitors which had ability to further differentiate into photoreceptor like cells by transfecting mouse induced pluripotent stem (iPS) cells with Pax6 gene. Undifferentiated iPS cells were transfected with Pax6 cDNA, followed by selection with G418. After limiting dilution culture, we selected cloned Pax6-transfected cells, which simultaneously expressed mRNAs of Nestin, Musashi1, Six3 and Chx10 for further characterization. We obtained totally 8 clonally expanding Pax6-transfected cells. They started to express mRNAs of Brn3b, Cone-rod homeobox (Crx), pkc, CD73, rhodopsin and the gamma-subunit of rod cGMP phosphodiesterase (PDEgamma). Flow cytometric analysis revealed that almost half of the cells were CD73+, a marker of photoreceptor precursors. Western blotting confirmed cytoplasmic protein expression of rhodopsin. High KCl stimulation increased free Ca influx into the cells on Ca (2+) imaging. iPS cells transfected with Pax6 gene, followed by subsequent limiting dilution culture became retinal progenitors including photoreceptor like cells. The cloned cell lines may be useful for analyzing differentiation requirement of retinal progenitors.

Takayama, K., et al. (2017). "Generation of safe and therapeutically effective human induced pluripotent stem cell-derived hepatocyte-like cells for regenerative medicine." Hepatol Commun **1**(10): 1058-1069.

Hepatocyte-like cells (HLCs) differentiated from human induced pluripotent stem (iPS) cells are expected to be applied for regenerative medicine. In this study, we attempted to generate safe and therapeutically effective human iPS-HLCs for hepatocyte transplantation. First, human iPS-HLCs were generated from a human leukocyte antigen-homozygous donor on the assumption that the allogenic transplantation might be carried out. Highly efficient hepatocyte differentiation was performed under a feeder-free condition using human recombinant laminin 111, laminin 511, and type IV collagen. The percentage of asialoglycoprotein receptor 1-positive cells was greater than 80%, while the percentage of residual undifferentiated cells was approximately 0.003%. In addition, no teratoma formation was observed even at 16 weeks after human iPS-HLC transplantation. Furthermore, harmful genetic somatic single-nucleotide substitutions were not observed during the hepatocyte differentiation process. We also developed a cryopreservation protocol for hepatoblast-like cells without negatively affecting their hepatocyte differentiation potential by programming the freezing temperature. To evaluate the therapeutic potential of human iPS-HLCs, these cells (1 x 10(6) cells/mouse) were intrasplenically transplanted into acute liver injury mice treated with 3 mL/kg CCl4 only once and chronic liver injury mice treated with 0.6 mL/kg CCl4 twice weekly for 8 weeks. By human iPS-HLC transplantation, the survival rate of the acute liver injury mice was significantly increased and the liver fibrosis level of chronic liver injury mice was significantly decreased. Conclusion: We were able to generate safe and therapeutically effective human iPS-HLCs for hepatocyte transplantation. (Hepatology Communications 2017;1:1058-1069).

Takei, H., et al. (2018). "Skewed megakaryopoiesis in human induced pluripotent stem cell-derived haematopoietic progenitor cells harbouring calreticulin mutations." Br J Haematol **181**(6): 791-802.

Somatic mutations in the calreticulin (CALR) gene have been found in most patients with JAK2- and MPL-unmutated Philadelphia chromosome-negative

myeloproliferative neoplasms (MPNs). It has recently been shown that mutant CALR constitutively activates the thrombopoietin receptor MPL and, thus, plays a causal role in the development of MPNs. However, the roles of mutant CALR in human haematopoietic cell differentiation remain predominantly elusive. To examine the impact of the 5-base insertion mutant CALR gene (Ins5) on haematopoietic cell differentiation, we generated induced pluripotent stem cells from an essential thrombocythaemia (ET) patient harbouring a CALR-Ins5 mutation and from a healthy individual (WT). Megakaryopoiesis was more prominent in Ins5-haematopoietic progenitor cells (Ins5-HPCs) than in WT-HPCs, implying that the system recapitulates megakaryocytosis observed in the bone marrow of CALR-mutant ET patients. Ins5-HPCs exhibited elevated expression levels of GATA1 and GATA2, suggesting a premature commitment to megakaryocytic differentiation in progenitor cells. We also demonstrated that 3-hydroxy anagrelide markedly perturbed megakaryopoiesis, but not erythropoiesis. Collectively, we established an in vitro model system that recapitulates megakaryopoiesis caused by mutant CALR. This system can be used to validate therapeutic compounds for MPN patients harbouring CALR mutations and in detailed studies on mutant CALR in human haematological cell differentiation.

Talbot, N. C., et al. (2017). "Bovine trophectoderm cells induced from bovine fibroblasts with induced pluripotent stem cell reprogramming factors." Mol Reprod Dev **84**(6): 468-485.

Thirteen independent induced bovine trophectroderm (iBT) cell lines were established by reprogramming bovine fetal liver-derived fibroblasts after viral-vector transduction with either six or eight factors, including POU5F1 (OCT4), KLF4, SOX2, MYC, NANOG, LIN28, SV40 large T antigen, and hTERT. Light- and electron-microscopy analysis showed that the iBT cells had epithelial cell morphology typical of bovine trophectoderm cells. Reverse-transcription-PCR assays indicated that all of the cell lines expressed interferon-tau (IFNT) at passages 1 or 2. At later passages (>/= passage 8), however, immunoblot and antiviral activity assays revealed that more than half of the iBT cell lines had stopped expressing IFNT. Messenger RNAs specific to trophectoderm differentiation and function were found in the iBT cell lines, and 2-dimensional-gel analysis for cellular proteins showed an expression pattern similar to that of trophectoderm cell lines derived from bovine blastocysts. Integration of some of the human reprogramming factors, including POU5F1, KLF4, SOX2, MYC, NANOG, and LIN28, were detected by PCR, but their transcription was mostly absent in the iBT cell lines. Gene expression assessment of endogenous bovine reprogramming factor orthologs revealed endogenous bLIN28 and bMYC transcripts in all; bSOX2 and bNANOG in none; and bKLF4 and bPOU5F1 in less than half of the iBT cell lines. These results demonstrate that bovine trophectoderm can be induced via reprogramming factor expression from bovine liver-derived fibroblasts, although other fibroblast populations-e.g., derived from fetal thigh tissue-may produce similar results, albeit at lower frequencies.

Tamaoki, N., et al. (2010). "Dental pulp cells for induced pluripotent stem cell banking." J Dent Res **89**(8): 773-778.

Defined sets of transcriptional factors can reprogram human somatic cells to induced pluripotent stem (iPS) cells. However, many types of human cells are not easily accessible to minimally invasive procedures. Here we evaluated dental pulp cells (DPCs) as an optimal source of iPS cells, since they are easily obtained from extracted teeth and can be expanded under simple culture conditions. From all 6 DPC lines tested with the conventional 3 or 4 reprogramming factors, iPS cells were effectively established from 5 DPC lines. Furthermore, determination of the HLA types of 107 DPC lines revealed 2 lines homozygous for all 3 HLA loci and showed that if an iPS bank is established from these initial pools, the bank will cover approximately 20% of the Japanese population with a perfect match. Analysis of these data demonstrates the promising potential of DPC collections as a source of iPS cell banks for use in regenerative medicine.

Tamo, L., et al. (2018). "Generation of an alveolar epithelial type II cell line from induced pluripotent stem cells." Am J Physiol Lung Cell Mol Physiol.

Differentiation of primary alveolar type II epithelial cells (AEC type II) to AEC type I in culture is a major barrier in the study of the alveolar epithelium in vitro. The establishment of an AEC type II cell line derived from induced pluripotent stem cells (iPSC) represents a novel opportunity to study alveolar epithelial cell biology, for instance in the context of lung injury, fibrosis and repair. In the present study, we generated long lasting AECII from iPSC (iPSC-AEC). Long lasting iPSC-AECII (LL)-iPSC-AECII) displayed morphological characteristics of AECII including growth in a cobble stone monolayer, the presence of lamellar bodies and microvilli as shown by electron microscopy. Also, (LL)-iPSC-AECII expressed AEC type II proteins such as cytokeratin, surfactant protein C and lysotracker DND 26 (a marker for lamellar bodies). Furthermore, the (LL)-iPSC-AECII exhibited functional properties of AECII

by an increase of transepithelial electrical resistance (TERR) over time, secretion of inflammatory mediators in biologically relevant quantities (interleukin-6, interleukin-8) and efficient in vitro alveolar epithelial wound repair. Consistent with the AECII phenotype, the cell line showed the ability to uptake and release surfactant protein B, to secrete phospholipids and to differentiate into AEC type I. In summary, we established a long lasting, but finite AEC type II cell line derived from iPSC as a novel cellular model to study alveolar epithelial cell biology in lung health and disease.

Tan, H. K., et al. (2014). "Human finger-prick induced pluripotent stem cells facilitate the development of stem cell banking." Stem Cells Transl Med **3**(5): 586-598.

Induced pluripotent stem cells (iPSCs) derived from somatic cells of patients can be a good model for studying human diseases and for future therapeutic regenerative medicine. Current initiatives to establish human iPSC (hiPSC) banking face challenges in recruiting large numbers of donors with diverse diseased, genetic, and phenotypic representations. In this study, we describe the efficient derivation of transgene-free hiPSCs from human finger-prick blood. Finger-prick sample collection can be performed on a "do-it-yourself" basis by donors and sent to the hiPSC facility for reprogramming. We show that single-drop volumes of finger-prick samples are sufficient for performing cellular reprogramming, DNA sequencing, and blood serotyping in parallel. Our novel strategy has the potential to facilitate the development of large-scale hiPSC banking worldwide.

Tan, R. P., et al. (2018). "Integration of induced pluripotent stem cell-derived endothelial cells with polycaprolactone/gelatin-based electrospun scaffolds for enhanced therapeutic angiogenesis." Stem Cell Res Ther **9**(1): 70.

BACKGROUND: Induced pluripotent stem-cell derived endothelial cells (iPSC-ECs) can be generated from any somatic cell and their iPSC sources possess unlimited self-renewal. Previous demonstration of their proangiogenic activity makes them a promising cell type for treatment of ischemic injury. As with many other stem cell approaches, the low rate of in-vivo survival has been a major limitation to the efficacy of iPSC-ECs to date. In this study, we aimed to increase the in-vivo lifetime of iPSC-ECs by culturing them on electrospun polycaprolactone (PCL)/gelatin scaffolds, before quantifying the subsequent impact on their proangiogenic function. METHODS: iPSC-ECs were isolated and stably transfected with a luciferase reporter to facilitate quantification of cell numbers and non-invasive imaging in-vivo PCL/gelatin scaffolds were engineered using electrospinning to obtain woven meshes of nanofibers. iPSC-ECs were cultured on scaffolds for 7 days. Subsequently, cell growth and function were assessed in vitro followed by implantation in a mouseback subcutaneous model for 7 days. RESULTS: Using a matrix of conditions, we found that scaffold blends with ratios of PCL:gelatin of 70:30 (PG73) spun at high flow rates supported the greatest levels of iPSC-EC growth, retention of phenotype, and function in vitro. Implanting iPSC-ECs seeded on PG73 scaffolds in vivo improved their survival up to 3 days, compared to cells directly injected into control wounds, which were no longer observable within 1 h. Enhanced engraftment improved blood perfusion, observed through non-invasive laser Doppler imaging. Immunohistochemistry revealed a corresponding increase in host angiogenic mechanisms characterized by the enhanced recruitment of macrophages and the elevated expression of proangiogenic cytokines vascular endothelial growth factor and placental growth factor. CONCLUSIONS: Knowledge of these mechanisms combined with a deeper understanding of the scaffold parameters influencing this function provides the groundwork for optimizing future iPSC-EC therapies utilizing engraftment platforms. The development of combined scaffold and iPSC-EC therapies could ultimately improve therapeutic angiogenesis and the treatment of ischemic injury.

Tang, L., et al. (2017). "Modeling cadmium-induced endothelial toxicity using human pluripotent stem cell-derived endothelial cells." Sci Rep **7**(1): 14811.

Cadmium (Cd) is a harmful heavy metal that results in vascular diseases such as atherosclerosis. Prior evidence revealed that Cd induced endothelial cell (EC) death and dysfunction, supporting that ECs are a primary target of Cd-induced toxicity, and can cause severe pathologies of vascular diseases. However, the underlying mechanisms remain unclear. In this study, we investigated the mechanisms of Cd-induced endothelial toxicity in a human model system of H9 human pluripotent stem cell-derived endothelial cells (H9-ECs). We showed that H9-ECs were susceptible to CdCl2 induction, leading to detrimental changes of cell structure and significantly elevated level of apoptosis. We demonstrated that CdCl2-treated H9-ECs gave rise to a clear EC dysfunction phenotype and significantly differential transcriptomic profile. Signaling pathway analysis revealed that P38 or ERK signaling pathway is critical to cadmium-induced EC apoptosis and dysfunction, and inhibition of P38 or ERK effectively rescued CdCl2-induced endothelial toxicity in H9-ECs. Conclusively, hPSC-

ECs can be a reliable model to recapitulate the EC pathological features and transcriptomic profile, which may provide a unique platform for understanding the cellular and molecular mechanisms of Cd-induced endothelial toxicity and for identifying therapeutic drugs for Cd-induced vascular diseases.

Tang, Z. H., et al. (2016). "Genetic Correction of Induced Pluripotent Stem Cells From a Deaf Patient With MYO7A Mutation Results in Morphologic and Functional Recovery of the Derived Hair Cell-Like Cells." Stem Cells Transl Med **5**(5): 561-571.

UNLABELLED: The genetic correction of induced pluripotent stem cells (iPSCs) induced from somatic cells of patients with sensorineural hearing loss (caused by hereditary factors) is a promising method for its treatment. The correction of gene mutations in iPSCs could restore the normal function of cells and provide a rich source of cells for transplantation. In the present study, iPSCs were generated from a deaf patient with compound heterozygous MYO7A mutations (c.1184G>A and c.4118C>T; P-iPSCs), the asymptomatic father of the patient (MYO7A c.1184G>A mutation; CF-iPSCs), and a normal donor (MYO7A (WT/WT); C-iPSCs). One of MYO7A mutation sites (c.4118C>T) in the P-iPSCs was corrected using CRISPR/Cas9. The corrected iPSCs (CP-iPSCs) retained cell pluripotency and normal karyotypes. Hair cell-like cells induced from CP-iPSCs showed restored organization of stereocilia-like protrusions; moreover, the electrophysiological function of these cells was similar to that of cells induced from C-iPSCs and CF-iPSCs. These results might facilitate the development of iPSC-based gene therapy for genetic disorders. SIGNIFICANCE: Induced pluripotent stem cells (iPSCs) were generated from a deaf patient with compound heterozygous MYO7A mutations (c.1184G>A and c.4118C>T). One of the MYO7A mutation sites (c.4118C>T) in the iPSCs was corrected using CRISPR/Cas9. The genetic correction of MYO7A mutation resulted in morphologic and functional recovery of hair cell-like cells derived from iPSCs. These findings confirm the hypothesis that MYO7A plays an important role in the assembly of stereocilia into stereociliary bundles. Thus, the present study might provide further insight into the pathogenesis of sensorineural hearing loss and facilitate the development of therapeutic strategies against monogenic disease through the genetic repair of patient-specific iPSCs.

Tashiro, S., et al. (2018). "High cell density suppresses BMP4-induced differentiation of human pluripotent stem cells to produce macroscopic spatial patterning in a unidirectional perfusion culture chamber." J Biosci Bioeng **126**(3): 379-388.

Spatial pattern formation is a critical step in embryogenesis. Bone morphogenetic protein 4 (BMP4) and its inhibitors are major factors for the formation of spatial patterns during embryogenesis. However, spatial patterning of the human embryo is unclear because of ethical issues and isotropic culture environments resulting from conventional culture dishes. Here, we utilized human pluripotent stem cells (hiPSCs) and a simple anisotropic (unidirectional perfusion) culture chamber, which creates unidirectional conditions, to measure the cell community effect. The influence of cell density on BMP4-induced differentiation was explored during static culture using a conventional culture dish. Immunostaining of the early differentiation marker SSEA-1 and the mesendoderm marker BRACHYURY revealed that high cell density suppressed differentiation, with small clusters of differentiated and undifferentiated cells formed. Addition of five-fold higher concentration of BMP4 showed similar results, suggesting that suppression was not caused by depletion of BMP4 but rather by high cell density. Quantitative RT-PCR array analysis showed that BMP4 induced multi-lineage differentiation, which was also suppressed under high-density conditions. We fabricated an elongated perfusion culture chamber, in which proteins were transported unidirectionally, and hiPSCs were cultured with BMP4. At low density, the expression was the same throughout the chamber. However, at high density, SSEA-1 and BRACHYURY were expressed only in upstream cells, suggesting that some autocrine/paracrine factors inhibited the action of BMP4 in downstream cells to form the spatial pattern. Human iPSCs cultured in a perfusion culture chamber might be useful for studying in vitro macroscopic pattern formation in human embryogenesis.

Taura, A., et al. (2014). "Effects of mouse utricle stromal tissues on hair cell induction from induced pluripotent stem cells." BMC Neurosci **15**: 121.

BACKGROUND: Hair cells are important for maintaining our sense of hearing and balance. However, they are difficult to regenerate in mammals once they are lost. Clarification of the molecular mechanisms underlying inner ear disorders is also impeded by the anatomical limitation of experimental access to the human inner ear. Therefore, the generation of hair cells, possibly from induced pluripotent stem (iPS) cells, is important for regenerative therapy and studies of inner ear diseases. RESULTS: We generated hair cells from mouse iPS cells using an established stepwise induction protocol. First, iPS cells were differentiated into the ectodermal

lineage by floating culture. Next, they were treated with basic fibroblast growth factor to induce otic progenitor cells. Finally, the cells were co-cultured with three kinds of mouse utricle tissues: stromal tissue, stromal tissue + sensory epithelium, and the extracellular matrix of stromal tissue. Hair cell-like cells were successfully generated from iPS cells using mouse utricle stromal tissues. However, no hair cell-like cells with hair bundle-like structures were formed using other tissues. CONCLUSIONS: Hair cell-like cells were induced from mouse iPS cells using mouse utricle stromal tissues. Certain soluble factors from mouse utricle stromal cells might be important for induction of hair cells from iPS cells.

Tchao, J., et al. (2013). "Engineered Human Muscle Tissue from Skeletal Muscle Derived Stem Cells and Induced Pluripotent Stem Cell Derived Cardiac Cells." Int J Tissue Eng **2013**: 198762.

During development, cardiac and skeletal muscle share major transcription factors and sarcomere proteins which were generally regarded as specific to either cardiac or skeletal muscle but not both in terminally differentiated adult cardiac or skeletal muscle. Here, we investigated whether artificial muscle constructed from human skeletal muscle derived stem cells (MDSCs) recapitulates developmental similarities between cardiac and skeletal muscle. We constructed 3-dimensional collagen-based engineered muscle tissue (EMT) using MDSCs (MDSC-EMT) and compared the biochemical and contractile properties with EMT using induced pluripotent stem (iPS) cell-derived cardiac cells (iPS-EMT). Both MDSC-EMT and iPS-EMT expressed cardiac specific troponins, fast skeletal muscle myosin heavy chain, and connexin-43 mimicking developing cardiac or skeletal muscle. At the transcriptional level, MDSC-EMT and iPS-EMT upregulated both cardiac and skeletal muscle-specific genes and expressed Nkx2.5 and Myo-D proteins. MDSC-EMT displayed intracellular calcium ion transients and responses to isoproterenol. Contractile force measurements of MDSC-EMT demonstrated functional properties of immature cardiac and skeletal muscle in both tissues. Results suggest that the EMT from MDSCs mimics developing cardiac and skeletal muscle and can serve as a useful in vitro functioning striated muscle model for investigation of stem cell differentiation and therapeutic options of MDSCs for cardiac repair.

Templin, C., et al. (2012). "Transplantation and tracking of human-induced pluripotent stem cells in a pig model of myocardial infarction: assessment of cell survival, engraftment, and distribution by hybrid single photon emission computed tomography/computed tomography of sodium iodide symporter transgene expression." Circulation **126**(4): 430-439.

BACKGROUND: Evaluation of novel cellular therapies in large-animal models and patients is currently hampered by the lack of imaging approaches that allow for long-term monitoring of viable transplanted cells. In this study, sodium iodide symporter (NIS) transgene imaging was evaluated as an approach to follow in vivo survival, engraftment, and distribution of human-induced pluripotent stem cell (hiPSC) derivatives in a pig model of myocardial infarction. METHODS AND RESULTS: Transgenic hiPSC lines stably expressing a fluorescent reporter and NIS (NIS (pos)-hiPSCs) were established. Iodide uptake, efflux, and viability of NIS (pos)-hiPSCs were assessed in vitro. Ten (+/-2) days after induction of myocardial infarction by transient occlusion of the left anterior descending artery, catheter-based intramyocardial injection of NIS (pos)-hiPSCs guided by 3-dimensional NOGA mapping was performed. Dual-isotope single photon emission computed tomographic/computed tomographic imaging was applied with the use of (123)I to follow donor cell survival and distribution and with the use of (99m)TC-tetrofosmin for perfusion imaging. In vitro, iodide uptake in NIS (pos)-hiPSCs was increased 100-fold above that of nontransgenic controls. In vivo, viable NIS (pos)-hiPSCs could be visualized for up to 15 weeks. Immunohistochemistry demonstrated that hiPSC-derived endothelial cells contributed to vascularization. Up to 12 to 15 weeks after transplantation, no teratomas were detected. CONCLUSIONS: This study describes for the first time the feasibility of repeated long-term in vivo imaging of viability and tissue distribution of cellular grafts in large animals. Moreover, this is the first report demonstrating vascular differentiation and long-term engraftment of hiPSCs in a large-animal model of myocardial infarction. NIS (pos)-hiPSCs represent a valuable tool to monitor and improve current cellular treatment strategies in clinically relevant animal models.

Theka, I., et al. (2013). "Rapid generation of functional dopaminergic neurons from human induced pluripotent stem cells through a single-step procedure using cell lineage transcription factors." Stem Cells Transl Med **2**(6): 473-479.

Current protocols for in vitro differentiation of human induced pluripotent stem cells (hiPSCs) to generate dopamine (DA) neurons are laborious and time-expensive. In order to accelerate the overall process, we have established a fast protocol by expressing the developmental transcription factors ASCL1, NURR1, and LMX1A. With this method, we were able to generate mature and functional

dopaminergic neurons in as few as 21 days, skipping all the intermediate steps for inducting and selecting embryoid bodies and rosette-neural precursors. Strikingly, the resulting neuronal conversion process was very proficient, with an overall efficiency that was more than 93% of all the coinfected cells. hiPSC-derived DA neurons expressed all the critical molecular markers of the DA molecular machinery and exhibited sophisticated functional features including spontaneous electrical activity and dopamine release. This one-step protocol holds important implications for in vitro disease modeling and is particularly amenable for exploitation in high-throughput screening protocols.

Thoma, E. C., et al. (2016). "Establishment of a translational endothelial cell model using directed differentiation of induced pluripotent stem cells from Cynomolgus monkey." Sci Rep **6**: 35830.

Due to their broad differentiation potential, pluripotent stem cells (PSCs) offer a promising approach for generating relevant cellular models for various applications. While human PSC-based cellular models are already advanced, similar systems for non-human primates (NHPs) are still lacking. However, as NHPs are the most appropriate animals for evaluating the safety of many novel pharmaceuticals, the availability of in vitro systems would be extremely useful to bridge the gap between cellular and animal models. Here, we present a NHP in vitro endothelial cell system using induced pluripotent stem cells (IPSCs) from Cynomolgus monkey (Macaca fascicularis). Based on an adapted protocol for human IPSCs, we directly differentiated macaque IPSCs into endothelial cells under chemically defined conditions. The resulting endothelial cells can be enriched using immuno-magnetic cell sorting and display endothelial marker expression and function. RNA sequencing revealed that the differentiation process closely resembled vasculogenesis.

Tomokiyo, A., et al. (2017). "Generation of Neural Crest-Like Cells From Human Periodontal Ligament Cell-Derived Induced Pluripotent Stem Cells." J Cell Physiol **232**(2): 402-416.

Neural crest cells (NCC) hold great promise for tissue engineering, however the inability to easily obtain large numbers of NCC is a major factor limiting their use in studies of regenerative medicine. Induced pluripotent stem cells (iPSC) are emerging as a novel candidate that could provide an unlimited source of NCC. In the present study, we examined the potential of neural crest tissue-derived periodontal ligament (PDL) iPSC to differentiate into neural crest-like cells (NCLC) relative to iPSC generated from a non-neural crest derived tissue, foreskin fibroblasts (FF). We detected high HNK1 expression during the differentiation of PDL and FF iPSC into NCLC as a marker for enriching for a population of cells with NCC characteristics. We isolated PDL iPSC- and FF iPSC-derived NCLC, which highly expressed HNK1. A high proportion of the HNK1-positive cell populations generated, expressed the MSC markers, whilst very few cells expressed the pluripotency markers or the hematopoietic markers. The PDL and FF HNK1-positive populations gave rise to smooth muscle, neural, glial, osteoblastic and adipocytic like cells and exhibited higher expression of smooth muscle, neural, and glial cell-associated markers than the PDL and FF HNK1-negative populations. Interestingly, the HNK1-positive cells derived from the PDL-iPSC exhibited a greater ability to differentiate into smooth muscle, neural, glial cells and adipocytes, than the HNK1-positive cells derived from the FF-iPSC. Our work suggests that HNK1-enriched NCLC from neural crest tissue-derived iPSC more closely resemble the phenotypic and functional hallmarks of NCC compared to the HNK1-low population and non-neural crest iPSC-derived NCLC. J. Cell. Physiol. 232: 402-416, 2017. (c) 2016 Wiley Periodicals, Inc.

Tsuruya, K., et al. (2015). "A Paracrine Mechanism Accelerating Expansion of Human Induced Pluripotent Stem Cell-Derived Hepatic Progenitor-Like Cells." Stem Cells Dev **24**(14): 1691-1702.

Hepatic stem/progenitor cells in liver development have a high proliferative potential and the ability to differentiate into both hepatocytes and cholangiocytes. In this study, we focused on the cell surface molecules of human induced pluripotent stem (iPS) cell-derived hepatic progenitor-like cells (HPCs) and analyzed how these molecules modulate expansion of these cells. Human iPS cells were differentiated into immature hepatic lineage cells by cytokines. In addition to hepatic progenitor markers (CD13 and CD133), the cells were coimmunostained for various cell surface markers (116 types). The cells were analyzed by flow cytometry and in vitro colony formation culture with feeder cells. Twenty types of cell surface molecules were highly expressed in CD13(+)CD133(+) cells derived from human iPS cells. Of these molecules, CD221 (insulin-like growth factor receptor), which was expressed in CD13(+)CD133(+) cells, was quickly downregulated after in vitro expansion. The proliferative ability was suppressed by a neutralizing antibody and specific inhibitor of CD221. Overexpression of CD221 increased colony-forming ability. We also found that inhibition of CD340 (erbB2) and CD266 (fibroblast growth factor-inducible 14) signals suppressed proliferation. In

addition, both insulin-like growth factor (a ligand of CD221) and tumor necrosis factor-like weak inducer of apoptosis (a ligand of CD266) were provided by feeder cells in our culture system. This study revealed the expression profiles of cell surface molecules in human iPS cell-derived HPCs and that the paracrine interactions between HPCs and other cells through specific receptors are important for proliferation.

Tucker, B. A., et al. (2011). "Exome sequencing and analysis of induced pluripotent stem cells identify the cilia-related gene male germ cell-associated kinase (MAK) as a cause of retinitis pigmentosa." Proc Natl Acad Sci U S A **108**(34): E569-576.

Retinitis pigmentosa (RP) is a genetically heterogeneous heritable disease characterized by apoptotic death of photoreceptor cells. We used exome sequencing to identify a homozygous Alu insertion in exon 9 of male germ cell-associated kinase (MAK) as the cause of disease in an isolated individual with RP. Screening of 1,798 unrelated RP patients identified 20 additional probands homozygous for this insertion (1.2%). All 21 affected probands are of Jewish ancestry. MAK encodes a kinase involved in the regulation of photoreceptor-connecting cilium length. Immunohistochemistry of human donor tissue revealed that MAK is expressed in the inner segments, cell bodies, and axons of rod and cone photoreceptors. Several isoforms of MAK that result from alternative splicing were identified. Induced pluripotent stem cells were derived from the skin of the proband and a patient with non-MAK-associated RP (RP control). In the RP control individual, we found that a transcript lacking exon 9 was predominant in undifferentiated cells, whereas a transcript bearing exon 9 and a previously unrecognized exon 12 predominated in cells that were differentiated into retinal precursors. However, in the proband with the Alu insertion, the developmental switch to the MAK transcript bearing exons 9 and 12 did not occur.

Uchida, N., et al. (2017). "Efficient Generation of beta-Globin-Expressing Erythroid Cells Using Stromal Cell-Derived Induced Pluripotent Stem Cells from Patients with Sickle Cell Disease." Stem Cells **35**(3): 586-596.

Human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells represent an ideal source for in vitro modeling of erythropoiesis and a potential alternative source for red blood cell transfusions. However, iPS cell-derived erythroid cells predominantly produce epsilon- and gamma-globin without beta-globin production. We recently demonstrated that ES cell-derived sacs (ES sacs), known to express hemangioblast markers, allow for efficient erythroid cell generation with beta-globin production. In this study, we generated several iPS cell lines derived from bone marrow stromal cells (MSCs) and peripheral blood erythroid progenitors (EPs) from sickle cell disease patients, and evaluated hematopoietic stem/progenitor cell (HSPC) generation after iPS sac induction as well as subsequent erythroid differentiation. MSC-derived iPS sacs yielded greater amounts of immature hematopoietic progenitors (VEGFR2 + GPA-), definitive HSPCs (CD34 + CD45+), and megakaryoerythroid progenitors (GPA + CD41a+), as compared to EP-derived iPS sacs. Erythroid differentiation from MSC-derived iPS sacs resulted in greater amounts of erythroid cells (GPA+) and higher beta-globin (and betaS-globin) expression, comparable to ES sac-derived cells.

Vallier, L., et al. (2009). "Signaling pathways controlling pluripotency and early cell fate decisions of human induced pluripotent stem cells." Stem Cells **27**(11): 2655-2666.

Human pluripotent stem cells from embryonic origins and those generated from reprogrammed somatic cells share many characteristics, including indefinite proliferation and a sustained capacity to differentiate into a wide variety of cell types. However, it remains to be demonstrated whether both cell types rely on similar mechanisms to maintain their pluripotent status and to control their differentiation. Any differences in such mechanisms would suggest that reprogramming of fibroblasts to generate induced pluripotent stem cells (iPSCs) results in novel states of pluripotency. In that event, current methods for expanding and differentiating human embryonic stem cells (ESCs) might not be directly applicable to human iPSCs. However, we show here that human iPSCs rely on activin/nodal signaling to control Nanog expression and thereby maintain pluripotency, thus revealing their mechanistic similarity to human ESCs. We also show that growth factors necessary and sufficient for achieving specification of human ESCs into extraembryonic tissues, neuroectoderm, and mesendoderm also drive differentiation of human iPSCs into the same tissues. Importantly, these experiments were performed in fully chemically defined medium devoid of factors that could obscure analysis of developmental mechanisms or render the resulting tissues incompatible with future clinical applications. Together these data reveal that human iPSCs rely on mechanisms similar to human ESCs to maintain their pluripotency and to control their differentiation, showing that these pluripotent cell types are functionally equivalent.

Veraitch, O., et al. (2013). "Human induced pluripotent stem cell-derived ectodermal precursor

cells contribute to hair follicle morphogenesis in vivo." J Invest Dermatol **133**(6): 1479-1488.

Well-orchestrated epithelial-mesenchymal interactions are crucial for hair follicle (HF) morphogenesis. In this study, ectodermal precursor cells (EPCs) with the capacity to cross talk with hair-inductive dermal cells were generated from human induced pluripotent stem cells (hiPSCs) and assessed for HF-forming ability in vivo. EPCs derived from three hiPSC lines generated with 4 or 3 factors (POU5F1, SOX2, KLF4 +/- MYC) mostly expressed keratin 18, a marker of epithelial progenitors. When cocultured with human dermal papilla (DP) cells, a 4 factor 201B7 hiPSC-EPC line upregulated follicular keratinocyte (KC) markers more significantly than normal human adult KCs (NHKCs) and other hiPSC-EPC lines. DP cells preferentially increased DP biomarker expression in response to this line. Interestingly, 201B7 hiPSCs were shown to be ectodermal/epithelial prone, and the derived EPCs were putatively in a wingless-type MMTV integration site family (WNT)-activated state. Importantly, co-transplantation of 201B7 hiPSC-EPCs, but not NHKCs, with trichogenic mice dermal cells into immunodeficient mice resulted in HF formation. Human HF stem cell markers were detected in reconstituted HFs; however, a low frequency of human-derived cells implied that hiPSC-EPCs contributed to HF morphogenesis via direct repopulation and non-cell autonomous activities. The current study suggests a, to our knowledge, previously unrecognized advantage of using hiPSCs to enhance epithelial-mesenchymal interactions in HF bioengineering.

Veraitch, O., et al. (2017). "Induction of hair follicle dermal papilla cell properties in human induced pluripotent stem cell-derived multipotent LNGFR (+)THY-1(+) mesenchymal cells." Sci Rep **7**: 42777.

The dermal papilla (DP) is a specialised mesenchymal component of the hair follicle (HF) that plays key roles in HF morphogenesis and regeneration. Current technical difficulties in preparing trichogenic human DP cells could be overcome by the use of highly proliferative and plastic human induced pluripotent stem cells (hiPSCs). In this study, hiPSCs were differentiated into induced mesenchymal cells (iMCs) with a bone marrow stromal cell phenotype. A highly proliferative and plastic LNGFR (+)THY-1(+) subset of iMCs was subsequently programmed using retinoic acid and DP cell activating culture medium to acquire DP properties. The resultant cells (induced DP-substituting cells [iDPSCs]) exhibited up-regulated DP markers, interacted with human keratinocytes to up-regulate HF related genes, and when co-grafted with human keratinocytes in vivo gave rise to fibre structures with a hair cuticle-like coat resembling the hair shaft, as confirmed by scanning electron microscope analysis.

Verpelli, C., et al. (2013). "Comparative neuronal differentiation of self-renewing neural progenitor cell lines obtained from human induced pluripotent stem cells." Front Cell Neurosci **7**: 175.

Most human neuronal disorders are associated with genetic alterations that cause defects in neuronal development and induce precocious neurodegeneration. In order to fully characterize the molecular mechanisms underlying the onset of these devastating diseases, it is important to establish in vitro models able to recapitulate the human pathology as closely as possible. Here we compared three different differentiation protocols for obtaining functional neurons from human induced pluripotent stem cells (hiPSCs): human neural progenitors (hNPs) obtained from hiPSCs were differentiated by co-culturing them with rat primary neurons, glial cells or simply by culturing them on matrigel in neuronal differentiation medium, and the differentiation level was compared using immunofluorescence, biochemical and electrophysiological methods. We show that the differentiated neurons displayed distinct maturation properties depending on the protocol used and the faster morphological and functional maturation was obtained when hNPs were co-cultured with rat primary neurons.

Vignesh, S., et al. (2018). "Fabrication of micropatterned alginate-gelatin and k-carrageenan hydrogels of defined shapes using simple wax mould method as a platform for stem cell/induced Pluripotent Stem Cells (iPSC) culture." Int J Biol Macromol **112**: 737-744.

Micropatterning techniques involve soft lithography, which is laborious, expensive and restricted to a narrow spectrum of biomaterials. In this work we report, first time employment of patterned wax moulds for generation of micropatterned alginate-gelatin and kappa-carrageenan (kappa-CRG) hydrogel systems by a novel, simple and cost effective method. We generated and characterized uniform and reproducible micropatterned hydrogels of varying sizes and shapes such as square projections, square grooves, and circular grids and crisscrossed hillocks. The rheological analysis showed that kappa-carrageenan hydrogels had higher gel strength when compared to alginate-gelatin hydrogels. Human Mesenchymal stem cells (hMSCs) and Human Induced Pluripotent Stem Cells (hiPSCs) were found to be cytocompatible with these hydrogels. This micropatterned hydrogel system may have potential

application in tissue engineering and also in understanding the basic biology behind the stem cell/iPSC fate.

Warlich, E., et al. (2014). "FAS-based cell depletion facilitates the selective isolation of mouse induced pluripotent stem cells." PLoS One **9**(7): e102171.

Cellular reprogramming of somatic cells into induced pluripotent stem cells (iPSC) opens up new avenues for basic research and regenerative medicine. However, the low efficiency of the procedure remains a major limitation. To identify iPSC, many studies to date relied on the activation of pluripotency-associated transcription factors. Such strategies are either retrospective or depend on genetically modified reporter cells. We aimed at identifying naturally occurring surface proteins in a systematic approach, focusing on antibody-targeted markers to enable live-cell identification and selective isolation. We tested 170 antibodies for differential expression between mouse embryonic fibroblasts (MEF) and mouse pluripotent stem cells (PSC). Differentially expressed markers were evaluated for their ability to identify and isolate iPSC in reprogramming cultures. Epithelial cell adhesion molecule (EPCAM) and stage-specific embryonic antigen 1 (SSEA1) were upregulated early during reprogramming and enabled enrichment of OCT4 expressing cells by magnetic cell sorting. Downregulation of somatic marker FAS was equally suitable to enrich OCT4 expressing cells, which has not been described so far. Furthermore, FAS downregulation correlated with viral transgene silencing. Finally, using the marker SSEA-1 we exemplified that magnetic separation enables the establishment of bona fide iPSC and propose strategies to enrich iPSC from a variety of human source tissues.

Watanabe, D., et al. (2018). "The Generation of Human gammadeltaT Cell-Derived Induced Pluripotent Stem Cells from Whole Peripheral Blood Mononuclear Cell Culture." Stem Cells Transl Med **7**(1): 34-44.

gammadeltaT cells constitute a small proportion of lymphocytes in peripheral blood. Unlike alphabetaT cells, the anti-tumor activities are exerted through several different pathways in a MHC-unrestricted manner. Thus, immunotherapy using gammadeltaT cells is considered to be effective for various types of cancer. Occasionally, however, ex vivo expanded cells are not as effective as expected due to cell exhaustion. To overcome the issue of T-cell exhaustion, researchers have generated induced pluripotent stem cells (iPSCs) that harbor the same T-cell receptor (TCR) genes as their original T-cells, which provide nearly limitless sources for antigen-specific cytotoxic T lymphocytes (CTLs). However, these technologies have focused on alphabetaT cells and require a population of antigen-specific CTLs, which are purified by cell sorting with HLA-peptide multimer, as the origin of iPS cells. In the present study, we aimed to develop an efficient and convenient system for generating iPSCs that harbor rearrangements of the TCRG and TCRD gene regions (gammadeltaT-iPSCs) without cell-sorting. We stimulated human whole peripheral blood mononuclear cell (PBMC) culture using Interleukin-2 and Zoledronate to activate gammadeltaT cells. Gene transfer into those cells with the Sendai virus vector resulted in gammadeltaT cell-dominant expression of exogenous genes. The introduction of reprogramming factors into the stimulated PBMC culture allowed us to establish iPSC lines.

Weist, R., et al. (2018). "Differential Expression of Cholinergic System Components in Human Induced Pluripotent Stem Cells, Bone Marrow-Derived Multipotent Stromal Cells, and Induced Pluripotent Stem Cell-Derived Multipotent Stromal Cells." Stem Cells Dev **27**(3): 166-183.

The components of the cholinergic system are evolutionary very old and conserved molecules that are expressed in typical spatiotemporal patterns. They are involved in signaling in the nervous system, whereas their functions in nonneuronal tissues are hardly understood. Stem cells present an attractive cellular system to address functional issues. This study therefore compared human induced pluripotent stem cells (iPSCs; from cord blood endothelial cells), mesenchymal stromal cells derived from iPSCs (iPSC-MSCs), and bone marrow-derived MSCs (BM-MSCs) from up to 33 different human donors with respect to gene expressions of components of the cholinergic system. The status of cells was identified and characterized by the detection of cell surface antigens using flow cytometry. Acetylcholinesterase expression in iPSCs declined during their differentiation into MSCs and was comparably low in BM-MSCs. Butyrylcholinesterase was present in iPSCs, increased upon transition from the three-dimensional embryoid body phase into monolayer culture, and declined upon further differentiation into iPSC-MSCs. In BM-MSCs a notable butyrylcholinesterase expression could be detected in only four donors, but was elusive in other patient-derived samples.

West, J. D. and E. J. Carrier (2018). "Precision Modeling of Pulmonary Hypertension Pathology with Induced Pluripotent Stem Cell-derived Cells." Am J Respir Crit Care Med **198**(2): 154-155.

Wheeler, H. E., et al. (2015). "Modeling chemotherapeutic neurotoxicity with human induced pluripotent stem cell-derived neuronal cells." PLoS One **10**(2): e0118020.

There are no effective agents to prevent or treat chemotherapy-induced peripheral neuropathy (CIPN), the most common non-hematologic toxicity of chemotherapy. Therefore, we sought to evaluate the utility of human neuron-like cells derived from induced pluripotent stem cells (iPSCs) as a means to study CIPN. We used high content imaging measurements of neurite outgrowth phenotypes to compare the changes that occur to iPSC-derived neuronal cells among drugs and among individuals in response to several classes of chemotherapeutics. Upon treatment of these neuronal cells with the neurotoxic drug paclitaxel, vincristine or cisplatin, we identified significant differences in five morphological phenotypes among drugs, including total outgrowth, mean/median/maximum process length, and mean outgrowth intensity ($P < 0.05$). The differences in damage among drugs reflect differences in their mechanisms of action and clinical CIPN manifestations. We show the potential of the model for gene perturbation studies by demonstrating decreased expression of TUBB2A results in significantly increased sensitivity of neurons to paclitaxel (0.23 +/- 0.06 decrease in total neurite outgrowth, $P = 0.011$). The variance in several neurite outgrowth and apoptotic phenotypes upon treatment with one of the neurotoxic drugs is significantly greater between than within neurons derived from four different individuals ($P < 0.05$), demonstrating the potential of iPSC-derived neurons as a genetically diverse model for CIPN. The human neuron model will allow both for mechanistic studies of specific genes and genetic variants discovered in clinical studies and for screening of new drugs to prevent or treat CIPN.

White, M. P., et al. (2013). "Limited gene expression variation in human embryonic stem cell and induced pluripotent stem cell-derived endothelial cells." Stem Cells **31**(1): 92-103.

Recent evidence suggests human embryonic stem cell (hESC) and induced pluripotent stem (iPS) cell lines have differences in their epigenetic marks and transcriptomes, yet the impact of these differences on subsequent terminally differentiated cells is less well understood. Comparison of purified, homogeneous populations of somatic cells derived from multiple independent human iPS and ES lines will be required to address this critical question. Here, we report a differentiation protocol based on embryonic development that consistently yields large numbers of endothelial cells (ECs) derived from multiple hESCs or iPS cells. Mesoderm differentiation of embryoid bodies was maximized, and defined growth factors were used to generate KDR (+) EC progenitors. Magnetic purification of a KDR (+) progenitor subpopulation resulted in an expanding, homogeneous pool of ECs that expressed EC markers and had functional properties of ECs. Comparison of the transcriptomes revealed limited gene expression variability between multiple lines of human iPS-derived ECs or between lines of ES- and iPS-derived ECs. These results demonstrate a method to generate large numbers of pure human EC progenitors and differentiated ECs from pluripotent stem cells and suggest individual lineages derived from human iPS cells may have significantly less variance than their pluripotent founders.

Whitt, J., et al. (2016). "Induced pluripotent stem cell-derived mesenchymal stem cells: A leap toward personalized therapies." Curr Stem Cell Res Ther **11**(2): 141-148.

Mesenchymal Stem/stromal cell (MSCs) transplantation procedures have been used since the 1960's to treat leukemia and other diseases, but due to the risks involved only patients with life threatening illnesses were typically subjected to the transplantation procedure until the last decade. Recent advancements in transplantation techniques have made it more feasible to use it for non-life-threatening diseases. However, the potential uses for stem cells are still limited by their rarity, and, in the case of allogeneic transplants, graft-vs.-host complications.

Yamazaki, K., et al. (2016). "Functional Comparison of Neuronal Cells Differentiated from Human Induced Pluripotent Stem Cell-Derived Neural Stem Cells under Different Oxygen and Medium Conditions." J Biomol Screen **21**(10): 1054-1064.

Because neurons are difficult to obtain from humans, generating functional neurons from human induced pluripotent stem cells (hiPSCs) is important for establishing physiological or disease-relevant screening systems for drug discovery. To examine the culture conditions leading to efficient differentiation of functional neural cells, we investigated the effects of oxygen stress (2% or 20% O2) and differentiation medium (DMEM/F12:Neurobasal-based [DN] or commercial [PhoenixSongs Biologicals; PS]) on the expression of genes related to neural differentiation, glutamate receptor function, and the formation of networks of neurons differentiated from hiPSCs (201B7) via long-term self-renewing neuroepithelial-like stem (lt-NES) cells. Expression of genes related to neural differentiation occurred more quickly in PS and/or 2% O2 than in DN and/or 20% O2, resulting in high responsiveness of neural cells to glutamate, N-methyl-d-aspartate (NMDA), alpha-amino-3-hydroxy-

5-methyl-4-isoxazolepropionate (AMPA), and (S)-3,5-dihydroxyphenylglycine (an agonist for mGluR1/5), as revealed by calcium imaging assays. NMDA receptors, AMPA receptors, mGluR1, and mGluR5 were functionally validated by using the specific antagonists MK-801, NBQX, JNJ16259685, and 2-methyl-6-(phenylethynyl)-pyridine, respectively. Multielectrode array analysis showed that spontaneous firing occurred earlier in cells cultured in 2% O2 than in 20% O2. Optimization of O2 tension and culture medium for neural differentiation of hiPSCs can efficiently generate physiologically relevant cells for screening systems.

Yamazoe, T., et al. (2015). "Potent tumor tropism of induced pluripotent stem cells and induced pluripotent stem cell-derived neural stem cells in the mouse intracerebral glioma model." Int J Oncol **46**(1): 147-152.

Although neural and mesenchymal stem cells have been well-known to have a strong glioma tropism, this activity in induced pluripotent stem cells (iPSCs) has not yet been fully studied. In the present study, we tested tumor tropic activity of mouse iPSCs and neural stem cells derived from the iPSC (iPS-NSCs) using in vitro Matrigel invasion chamber assay and in vivo mouse intracranial tumor model. Both iPSC and iPS-NSC had a similar potent in vitro tropism for glioma conditioned media. The migrated iPSCs to the gliomas kept expressing Nanog-GFP gene, suggesting no neuronal or glial differentiation. iPSCs or iPS-NSCs labeled with 5-bromo-2-deoxyuridine were intracranially implanted in the contralateral hemisphere to the GL261 glioma cell implantation in the allogeneic C57BL/6 mouse. Active migration of both stem cells was observed 7 days after implantation. Again, the iPSCs located in the tumor area expressed Nanog-GFP gene, suggesting that the migrated cells were still iPSCs. These findings demonstrated that both iPSCs and iPS-NSCs had potent glioma tropism and could be candidates as vehicles in stem cell-based glioma therapy.

Yan, B. and D. K. Singla (2013). "Transplanted induced pluripotent stem cells mitigate oxidative stress and improve cardiac function through the Akt cell survival pathway in diabetic cardiomyopathy." Mol Pharm **10**(9): 3425-3432.

Recent evidence suggests transplanted stem cells improve left ventricular function in diabetic induced cardiomyopathy (DICM). However, little is known about the mechanisms by which induced pluripotent stem (iPS) cells or factors released from these cells inhibit adverse cardiac remodeling in DICM. The present study was designed to determine molecular mediators and pathways regulated by transplanted iPS cells and their conditioned media (CM) in DICM. Animals were divided into four experimental groups such as control, streptozotocin (STZ), STZ+iPS-CM, and STZ+iPS cells. Experimental diabetes was induced in C57BL/6 mice by intraperitoneal STZ injections (100 mg/kg body weight for 2 consecutive days). Following STZ injections, iPS cells or CM was given intravenously for 3 consecutive days. Animals were humanely killed, and hearts were harvested at D14. Animals transplanted with iPS cells or CM demonstrated a significant reduction in apoptosis, mediated by Akt upregulation and ERK1/2 downregulation, and inhibition of interstitial fibrosis via MMP-9 suppression compared with the STZ group. Oxidative stress was significantly hindered in iPS cell and CM groups as evidenced by diminished pro-oxidant expression and enhanced antioxidant (catalase and MnSOD) concentration.

Yang, J., et al. (2012). "Tumor tropism of intravenously injected human-induced pluripotent stem cell-derived neural stem cells and their gene therapy application in a metastatic breast cancer model." Stem Cells **30**(5): 1021-1029.

Human pluripotent stem cells can serve as an accessible and reliable source for the generation of functional human cells for medical therapies. In this study, we used a conventional lentiviral transduction method to derive human-induced pluripotent stem (iPS) cells from primary human fibroblasts and then generated neural stem cells (NSCs) from the iPS cells. Using a dual-color whole-body imaging technology, we demonstrated that after tail vein injection, these human NSCs displayed a robust migratory capacity outside the central nervous system in both immunodeficient and immunocompetent mice and homed in on established orthotopic 4T1 mouse mammary tumors. To investigate whether the iPS cell-derived NSCs can be used as a cellular delivery vehicle for cancer gene therapy, the cells were transduced with a baculoviral vector containing the herpes simplex virus thymidine kinase suicide gene and injected through tail vein into 4T1 tumor-bearing mice. The transduced NSCs were effective in inhibiting the growth of the orthotopic 4T1 breast tumor and the metastatic spread of the cancer cells in the presence of ganciclovir, leading to prolonged survival of the tumor-bearing mice. The use of iPS cell-derived NSCs for cancer gene therapy bypasses the sensitive ethical issue surrounding the use of cells derived from human fetal tissues or human embryonic stem cells. This approach may also help to overcome problems associated with allogeneic transplantation of other types of human NSCs.

Yang, J., et al. (2016). "Induced pluripotent stem cells in Alzheimer's disease: applications for disease modeling and cell-replacement therapy." Mol Neurodegener **11**(1): 39.

Alzheimer's disease (AD) is the most common cause of dementia in those over the age of 65. While a numerous of disease-causing genes and risk factors have been identified, the exact etiological mechanisms of AD are not yet completely understood, due to the inability to test theoretical hypotheses on non-postmortem and patient-specific research systems. The use of recently developed and optimized induced pluripotent stem cells (iPSCs) technology may provide a promising platform to create reliable models, not only for better understanding the etiopathological process of AD, but also for efficient anti-AD drugs screening. More importantly, human-sourced iPSCs may also provide a beneficial tool for cell-replacement therapy against AD. Although considerable progress has been achieved, a number of key challenges still require to be addressed in iPSCs research, including the identification of robust disease phenotypes in AD modeling and the clinical availabilities of iPSCs-based cell-replacement therapy in human. In this review, we highlight recent progresses of iPSCs research and discuss the translational challenges of AD patients-derived iPSCs in disease modeling and cell-replacement therapy.

Yokobayashi, S., et al. (2017). "Clonal variation of human induced pluripotent stem cells for induction into the germ cell fate." Biol Reprod **96**(6): 1154-1166.

The mechanisms for human germ cell development have remained largely unknown, due to the difficulty in obtaining suitable experimental materials. The establishment of an in vitro system to reconstitute human germ cell development will thus provide a critical opportunity to understand its mechanisms at a molecular level. It has previously been shown that human induced pluripotent stem cells (hiPSCs) are first induced into incipient mesoderm-like cells (iMeLCs), which are in turn induced into primordial germ-cell like cells (PGCLCs) with gene expression properties similar to early migratory PGCs. Here, we report that the efficiency of PGCLC induction varies among hiPSC clones, and, interestingly, the clonal variations in PGCLC induction efficiency are reflected in the gene expression states of the iMeLCs. Remarkably, the expression levels of EOMES, MIXL1, or T in the iMeLCs are positively correlated with the efficiency of subsequent PGCLC generation, while the expressions of CDH1, SOX3, or FGF2 are negatively correlated. These results indicate that the expression changes of these genes occurring during iMeLC induction are key markers indicative of successful induction of PGCLCs, and furthermore, that hiPSC clones have different properties that influence their responsivity to the iMeLC induction. Our study thus provides important insights into the mechanism of hPGC specification as well as the development of a better strategy for inducing human germ cell fate from PSCs in vitro.

Zanella, F. and F. Sheikh (2016). "Patient-Specific Induced Pluripotent Stem Cell Models: Generation and Characterization of Cardiac Cells." Methods Mol Biol **1353**: 147-162.

The generation of human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes has been of utmost interest for the study of cardiac development, cardiac disease modeling, and evaluation of cardiotoxic effects of novel candidate drugs. Several protocols have been developed to guide human stem cells toward the cardiogenic path. Pioneering work used serum to promote cardiogenesis; however, low cardiogenic throughputs, lack of chemical definition, and batch-to-batch variability of serum lots constituted a considerable impediment to the implementation of those protocols to large-scale cell biology. Further work focused on the manipulation of pathways that mouse genetics indicated to be fundamental in cardiac development to promote cardiac differentiation in stem cells. Although extremely elegant, those serum-free protocols involved the use of human recombinant cytokines that tend to be quite costly and which can also be variable between lots. The latest generation of cardiogenic protocols aimed for a more cost-effective and reproducible definition of the conditions driving cardiac differentiation, using small molecules to manipulate cardiogenic pathways overriding the need for cytokines. This chapter details methods based on currently available cardiac differentiation protocols for the generation and characterization of robust numbers of hiPSC-derived cardiomyocytes under chemically defined conditions.

Zanotelli, M. R., et al. (2016). "Stable engineered vascular networks from human induced pluripotent stem cell-derived endothelial cells cultured in synthetic hydrogels." Acta Biomater **35**: 32-41.

UNLABELLED: Here, we describe an in vitro strategy to model vascular morphogenesis where human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) are encapsulated in peptide-functionalized poly (ethylene glycol) (PEG) hydrogels, either on standard well plates or within a passive pumping polydimethylsiloxane (PDMS) tri-channel microfluidic device. PEG hydrogels permissive towards cellular remodeling were fabricated using thiol-ene photopolymerization to incorporate matrix metalloproteinase (MMP)-degradable crosslinks and CRGDS cell adhesion

peptide. Time lapse microscopy, immunofluorescence imaging, and RNA sequencing (RNA-Seq) demonstrated that iPSC-ECs formed vascular networks through mechanisms that were consistent with in vivo vasculogenesis and angiogenesis when cultured in PEG hydrogels. Migrating iPSC-ECs condensed into clusters, elongated into tubules, and formed polygonal networks through sprouting. Genes upregulated for iPSC-ECs cultured in PEG hydrogels relative to control cells on tissue culture polystyrene (TCP) surfaces included adhesion, matrix remodeling, and Notch signaling pathway genes relevant to in vivo vascular development. Vascular networks with lumens were stable for at least 14days when iPSC-ECs were encapsulated in PEG hydrogels that were polymerized within the central channel of the microfluidic device. Therefore, iPSC-ECs cultured in peptide-functionalized PEG hydrogels offer a defined platform for investigating vascular morphogenesis in vitro using both standard and microfluidic formats. STATEMENT OF SIGNIFICANCE: Human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) cultured in synthetic hydrogels self-assemble into capillary networks through mechanisms consistent with in vivo vascular morphogenesis.

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