Induced pluripotent stem cells literatures

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Abstract: All animal cells come from stem cells. Stem cell pluripotency means a stem cell having the potential to differentiate into any of the three germ layers: endoderm, mesoderm or ectoderm. Induced pluripotent stem (iPS) cells can be differentiated to any fetal or adult cell type. However, the pluripotent stem cells cannot develop into a fetal or adult organism alone because they are lack of the potential to contribute to extraembryonic tissue, such as the placenta. iPS cells are genetically reprogrammed adult cells that exhibit a pluripotent stem cell-like state similar to embryonic stem cells. While these artificially generated cells are not known to exist in the human body, they show qualities remarkably similar to those of embryonic stem cells (ESCs); thus, iPSCs are an invaluable resource for drug discovery, cell therapy, and basic research. Here are the xxx academic literature collections on iPS.
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1. Introduction

Induced pluripotent stem cells (iPSCs) are genetically reprogrammed adult cells that exhibit a pluripotent stem cell-like state similar to embryonic stem cells.[1] While these artificially generated cells are not known to exist in the human body, they show qualities remarkably similar to those of embryonic stem cells (ESCs); thus, iPSCs are an invaluable resource for drug discovery, cell therapy, and basic research. After an egg is fertilized by a sperm, a single cell comes out. This fertilized egg is totipotent which has the potential ability to create an entire organism. However, the totipotent cells change to pluripotent cells that lost the ability of totipotent – the pluripotent cannot differentiate to an entire body.

There are several key types of pluripotent stem cells: (1) Embryonic stem cells are isolated from the inner cell mass of the blastocyst. (2) Embryonic germ cells are taken from aborted foetuses and these pluripotent cells are derived from very early cells. (3) Embryonic carcinoma or cancer cells are isolated from a type of tumour that sometimes occurs in a foetus.

Yamanaka and colleagues first demonstrated that retrovirus-mediated delivery and expression of Oct4, Sox2, c-Myc and Klf4 is capable of inducing the pluripotent state in mouse fibroblasts, and they also reported the successful reprogramming of human somatic cells into induced pluripotent stem (iPS) cells using human versions of the same transcription factors delivered by retroviral vectors. The generation of patient-specific iPS cells circumvents an important roadblock to personalized regenerative medicine therapies by eliminating the potential for immune rejection of non-autologous transplanted cells. (Wu, Hamilton et al. 2009).

Mouse and human fibroblasts have been transformed into induced pluripotent stem (iPS) cells by retroviral transduction or plasmid transfection with four genes. Tumor formation has been found in offspring of mice generated from blastocysts made mosaic with iPS cells. The adenoviral vectors can reprogram human fibroblasts to pluripotent stem cells for use in individualized cell therapy without the risk for viral or oncogene incorporation (Zhou and Freed 2009).

Domesticated ungulate pluripotent embryonic stem (ES) cell lines would be useful for generating precise gene-modified animals. Many efforts have been made to establish domesticated ungulate pluripotent ES cells from early embryos without success. Wu, et al, reported that properties of porcine pluripotent stem cells that may facilitate the eventual establishment of porcine ES cells (Wu, Chen et al. 2009).

Pluripotent stem cells have the potential for treatment of many diseases. Pluripotent stem cells can evolve into specialized cells that ultimately can replace diseased cells and tissues. The positive uses of pluripotent stem cells are enormous but new research and ethical challenges must be taken into account before the public can reap the full benefits. For those who suffer from the many diseases that may be treated by pluripotent stem cells, additional knowledge and research will hopefully come sooner rather than later. The positive uses of pluripotent stem cells are enormous but new research and ethical challenges must be taken into account before the public can reap the full benefits.

Human induced pluripotent stem (iPS) cells hold great promise for cardiovascular research and therapeutic applications, but the ability of human iPS

cells to differentiate into functional cardiomyocytes has not yet been demonstrated (Zhang, Wilson et al. 2009). Reprogramming differentiated human cells to induced pluripotent stem (iPS) cells has applications in basic biology, drug development, and transplantation. Human iPS cell derivation previously required vectors that integrate into the genome, which can create mutations and limit the utility of the cells in both research and clinical applications (Yu, Hu et al. 2009). Human induced pluripotent stem (iPS) cells derived from somatic cells hold promise to develop novel patient-specific cell therapies and research models for inherited and acquired diseases (Ye, Zhan et al. 2009).

In 2006, Kazutoshi Takahashi and Shinya Yamanaka established murine ES-like cell lines from mouse embryonic fibroblasts (MEFs) and skin fibroblasts by expressing four transcription factor genes encoding **Oct4, Sox2, Klf4, and c-Myc** (Takahashi & Yamanaka 2006). They called these somatic cell-derived cell lines induced pluripotent stem (iPS) cells. These iPS cell lines perform similar properties as ES cells and express ES cell-specific genes. In the years after Takahashi and Yamanaka's initial success in reprogramming mouse cells, several groups used the same strategy to generate human iPS cells. The set of transcription factors to reprogram is same in both mouse and human somatic cells, which means that the transcription factor networks are conserved in these two species to control self-renewal and pluripotency.

iPS cell lines could be generated from different cell types, such as fibroblasts, progenitor cells, hepatocytes, B cells, neuronals kidneys, muscles, keratinocytes, and adrenal glands, etc. The efficiency of cell reprogramming varies among different cell types.

To get the patient-specific iPS cell lines is important in the clincal appliction. Reprogramming of fibroblasts from patients allows the establishment of disease-specific iPS cell lines. To study the disease mechanism, a key issue is whether the affected cell type derived from iPS cells can recapitulate the disease phenotype (Colman & Dreesen 2009).

It is important and useful in scientific research and clinical medicine to apply the fact that somatic cells can be reprogrammed into iPS cells. However, there are many problems in the technology and theory (Saha & Jaenisch 2009). One of the problems is the use of retroviral and lentiviral vectors to introduce the 4 transcription factor genes into somatic cells for cell reprogramming. These viral vectors preferentially integrate into active genes and therefore have the potential to activate flanking cellular genes. And, the 4 introduced transcription factors may have oncogenic potentials. Although the iPS cells may be suitable for the study of disease mechanisms or for drug screening and validation, they have potentail danger for cell replacement therapy.

Literautres

The following are about 200 recent reference papers on iSP.

Alfano, R., B. A. Youngblood, et al. "Human leukemia inhibitory factor produced by the ExpressTec method from rice (Oryza sativa L.) is active in human neural stem cells and mouse induced pluripotent stem cells." Bioengineered. 2014 May-Jun;5(3):180-5. doi: 10.4161/bioe.28996. Epub 2014 Apr 28.

Stem cell-based therapy has the potential to treat an array of human diseases. However, to study the therapeutic potential and safety of these cells, a scalable cell culture medium is needed that is free of human or bovine-derived serum proteins. Thus, cost-effective recombinant serum proteins and cytokines are needed to produce such mediums. One such cytokine, leukemia inhibitory factor (LIF), has been shown to be a critical paracrine factor that maintains stem cell pluripotency in murine embryonic stem cells and human naive stem cells while simultaneously inhibiting differentiation. Alfano et al in this project recently produced recombinant human LIF (rhLIF) in a rice-based protein expression system known as ExpressTec. They described expression of rice-derived rhLIF and demonstrated its biological equivalency to E. coli-derived rhLIF in traditional and embryonic mouse stem cell systems. Here they describe the expression yield of rice-derived rhLIF and the scale up production capacity. The authors provide further evidence of the efficacy of rice-derived rhLIF in additional stem cell systems including human neural stem cells and mouse induced pluripotent stem (iPS) cells. The expression level, biological activity, and potential for production at commercial scale of rice-derived rhLIF provides a proof-of-principal for ExpressTec-derived proteins to produce regulatory-friendly, high performance, and dependable stem cell media.

Ao, Y., J. D. Mich-Basso, et al. "High efficient differentiation of functional hepatocytes from porcine induced pluripotent stem cells." PLoS One. 2014 Jun 20;9(6):e100417. doi: 10.1371/journal.pone.0100417. eCollection 2014.

Hepatocyte transplantation is considered to be a promising therapy for patients with liver diseases. Induced pluripotent stem cells (iPSCs) provide an unlimited source for the generation of functional hepatocytes. In this study, Ao et al generated iPSCs from porcine ear fibroblasts (PEFs) by overexpressing Sox2, Klf4, Oct4, and c-Myc (SKOM), and developed a novel strategy for the efficient differentiation of hepatocyte-like cells from porcine iPSCs by following the processes of early liver development. The differentiated cells displayed the phenotypes of hepatocytes, exhibited classic hepatocyte-associated bio-functions, such as LDL uptake, glycogen storage and urea secretion, as well as possessed the metabolic activities of cytochrome P-450 (CYP) 3A and 2C. Furthermore, we compared the hepatocyte differentiation efficacy of our protocol with another published method, and the results demonstrated that our differentiation strategy could significantly improve the generation of morphological and functional hepatocyte-like cells from porcine iPSCs. In conclusion, this study establishes an efficient method for in vitro generation of functional hepatocytes from porcine iPSCs, which could represent a promising cell source for preclinical testing of cell-based therapeutics for liver failure and for pharmacological applications.

Ardeshirylajimi, A., M. Soleimani, et al. "A Comparative Study of Osteogenic Differentiation of Human Induced Pluripotent Stem cells and Adipose Tissue Derived Mesenchymal Stem Cells." Cell J. 2013 Sep 1;16(3). pii: CellJ.2014.16(3).5.

Human induced pluripotent stem cells (iPSCs) have been shown to have promising capacity for stem cells therapy and tissue engineering applications. Therefore, it is essential to compare the ability of these cells with the commonly used mesenchymal stem cells (MSC) for bone tissue engineering in vitro. In the present study, the biological behavior and osteogenic capacity of the iPSCs were compared with MSC isolated from human adipose tissue (AT-MSC) using 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, Alizarin red staining, alkaline phosphatase (ALP) activity measurements, calcium content assay and common osteogenic-related genes. Data were reported as the mean +/- SD. One-way analysis of variance (ANOVA) was used to compare the results. A p-value of less than 0.05 was considered statistically significant. Results: According to the results, there was significant difference between the rate of proliferation of two stem cells, as iPSCs showed increased proliferation compared to AT-MSCs. During osteogenic differentiation, ALP activity and mineralization were demonstrated to be significantly higher in iPSCs.

Although AT-MSC expressed higher levels of Runx2, iPSCs expressed higher amount of osteonection and osteocalcin during differentiation. Conclusion: Taking together, iPSCs showed a higher capacity for osteogenic differentiation and it hold promising potential for bone tissue engineering and cell therapy applications.

Badenes, S. M., T. G. Fernandes, et al. "Scalable Expansion of Human-Induced Pluripotent Stem Cells in Xeno-Free Microcarriers." Methods Mol Biol. 2014 Aug 10.

The expansion of human-induced pluripotent stem cells (hiPSCs) is commonly performed using feeder layers of mouse embryonic fibroblasts or in feeder-free conditions in two-dimensional culture platforms, which are associated with low production yields and lack of process control. Robust large-scale production of these cells under defined conditions has been one of the major challenges to fulfil the large cell number requirement for drug screening applications, toxicology assays, disease modeling and potential cellular therapies. Microcarrier-based systems, in particular, are a promising culture format since they provide a high surface-to-volume ratio and allow the scale-up of the process to stirred suspension bioreactors. In this context, this chapter describes a detailed methodology for the scalable expansion of hiPSCs in spinner flasks and using xeno-free microcarriers to allow further translation to Good Manufacturing Practice (GMP) conditions.

Bobo-Ruiz, J. "[Incidence of the characteristics of induced pluripotent stem cells in vitro in administrative regime of their lines]." Rev Derecho Genoma Hum. 2013 Jul-Dec;(39):61-82.

Recently achieved techniques in the field of stem cell research have permitted the development of human induced pluripotent stem cells (hiPSCs). The characteristics of these cells and their appearance after the passage of Order SCO/393/2006, of 8 February, on Functioning and Organization of a National Bank of Cell Lines and Act 14/2007, of 3 July, on Biomedical Research raise the questions of the legal regime of their deposition at the National Bank of Cell Lines and their control by the Commission of Guarantees for the Donation and Use of Human Cells and Tissues. Drawing on a wider legal frame, the author defends these requirements on the grounds that the legal classification comes from the origin and potentiality of the cells rather than from the techniques employed to obtain them.

Borestrom, C., S. Simonsson, et al. "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. 2014 Apr;3(4):433-47. doi: 10.5966/sctm.2013-0138. Epub 2014 Mar 6.

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.

Brandl, C., S. J. Zimmermann, et al. "In-Depth Characterisation of Retinal Pigment Epithelium (RPE) Cells Derived from Human Induced Pluripotent Stem Cells (hiPSC)." Neuromolecular Med. 2014 Sep;16(3):551-64. doi: 10.1007/s12017-014-8308-8. Epub 2014 May 7.

Induced pluripotent stem cell (iPSC)-derived retinal pigment epithelium (RPE) has widely been appreciated as a promising tool to model human ocular disease emanating from primary RPE pathology. Here, we describe the successful reprogramming of adult human dermal fibroblasts to iPSCs and their differentiation to pure expandable RPE cells with structural and functional features characteristic for native RPE. Fibroblast cultures were established from skin biopsy material and subsequently reprogrammed following polycistronic lentiviral transduction with OCT4, SOX2, KLF4 and L-Myc. Fibroblast-derived iPSCs showed typical morphology, chromosomal integrity and a distinctive stem cell marker profile. Subsequent differentiation resulted in expandable pigmented hexagonal RPE cells. The cells revealed stable RNA expression of mature RPE markers RPE65, RLBP and BEST1. Immunolabelling verified localisation of BEST1 at the basolateral plasma membrane, and scanning electron microscopy showed typical microvilli at the apical side of iPSC-derived RPE cells. Transepithelial resistance was maintained at high levels during cell culture indicating functional formation of tight junctions. Secretion capacity was demonstrated for VEGF-A. Feeding of porcine photoreceptor outer segments revealed the proper ability of these cells for phagocytosis. IPSC-derived RPE cells largely maintained these properties after cryopreservation.

Budniatzky, I. and L. Gepstein "Concise review: reprogramming strategies for cardiovascular regenerative medicine: from induced pluripotent stem cells to direct reprogramming." Stem Cells Transl Med. 2014 Apr;3(4):448-57. doi: 10.5966/sctm.2013-0163. Epub 2014 Mar 3.

Myocardial cell-replacement therapies are emerging as novel therapeutic paradigms for myocardial repair but are hampered by the lack of sources of autologous human cardiomyocytes. The recent advances in stem cell biology and in transcription factor-based reprogramming strategies may provide exciting solutions to this problem. In the current review, we describe the different reprogramming strategies that can give rise to cardiomyocytes for regenerative medicine purposes. Initially, we describe induced pluripotent stem cell technology, a method by which adult somatic cells can be reprogrammed to yield pluripotent stem cells that could later be coaxed ex vivo to differentiate into cardiomyocytes. The generated induced pluripotent stem cell-derived cardiomyocytes could then be used for myocardial cell transplantation and tissue engineering strategies. We also describe the more recent direct reprogramming approaches that aim to directly convert the phenotype of one mature cell type (fibroblast) to another (cardiomyocyte) without going through a pluripotent intermediate cell type. The advantages and shortcomings of each strategy for cardiac regeneration are discussed, along with the hurdles that need to be overcome on the road to clinical translation.

Cao, L., L. Tan, et al. "Induced Pluripotent Stem Cells for Disease Modeling and Drug Discovery in Neurodegenerative Diseases." Mol Neurobiol. 2014 Aug 23.

Although most neurodegenerative diseases have been closely related to aberrant accumulation of aggregation-prone proteins in neurons, understanding their pathogenesis remains incomplete, and there is no treatment to delay the onset or slow the progression of many neurodegenerative diseases. The availability of induced pluripotent stem cells (iPSCs) in recapitulating the phenotypes of several late-onset neurodegenerative diseases marks the new era in in vitro modeling. The iPSC collection represents a unique and well-characterized resource to elucidate disease mechanisms in these diseases and provides a novel human stem cell platform for screening new candidate therapeutics. Modeling human diseases using iPSCs has created novel opportunities for both mechanistic studies as well as for the discovery of new disease therapies. In this review, we introduce iPSC-based disease modeling in neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis. In addition, we discuss the implementation of iPSCs in drug discovery associated with some new techniques.

Casado, M. and I. de Lecuona "[Unresolved issues in the evaluation of research projects involving induced pluripotent stem cells (iPS)]." Rev Derecho Genoma Hum. 2013 Jul-Dec;(39):15-40.

This paper identifies problems and analyzes those conflicts posed by the evaluation of research projects involving the collection and use of human induced pluripotent stem cells (iPS) in Spain. Current legislation is causing problems of interpretation, circular and unnecessary referrals, legal uncertainty and undue delays. Actually, this situation may cause a lack of control and monitoring, and even some paralysis in regenerative medicine and cell therapy research, that is a priority nowadays. The analysis of the current legislation and its bioethical implications, led us to conclude that the review of iPS research projects cannot be assimilated to the evaluation of research projects that involve human embryonic stem cell (hESC). In this context, our proposal is based on the review by the Research Ethics Committees and the checkout by the Spanish Comission of Guarantees for Donation and Use of Human Cells and Tissues (CGDUCTH) of human iPS cells research projects. Moreover, this article claims for a more transparent research system, by effectively articulating the Registry on Research Projects.

Chang, C. W., Y. S. Lai, et al. "Broad T-cell receptor repertoire in T-lymphocytes derived from human induced pluripotent stem cells." PLoS One. 2014 May 14;9(5):e97335. doi: 10.1371/journal.pone.0097335. eCollection 2014.

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.

Chang, Y. C., W. C. Chang, et al. "The generation of induced pluripotent stem cells for macular degeneration as a drug screening platform: identification of curcumin as a protective agent for retinal pigment epithelial cells against oxidative stress." Front Aging Neurosci. 2014 Aug 1;6:191. doi: 10.3389/fnagi.2014.00191. eCollection 2014.

Age-related macular degeneration (AMD) is one retinal aging process that may lead to irreversible vision loss in the elderly. Its pathogenesis remains unclear, but oxidative stress inducing retinal pigment epithelial (RPE) cells damage is perhaps responsible for the aging sequence of retina and may play an important role in macular degeneration. In this study, we have reprogrammed T cells from patients with dry type AMD into induced pluripotent stem cells (iPSCs) via integration-free episomal vectors and differentiated them into RPE cells that were used as an expandable platform for investigating pathogenesis of the AMD and in-vitro drug screening. These patient-derived RPEs with the AMD-associated background (AMD-RPEs) exhibited reduced antioxidant ability, compared with normal RPE cells. Among several screened candidate drugs, curcumin caused most significant reduction of ROS in AMD-RPEs.

Chen, H. M., C. J. DeLong, et al. "Transcripts involved in calcium signaling and telencephalic neuronal fate are altered in induced pluripotent stem cells from bipolar disorder patients." Transl Psychiatry. 2014 Mar 25;4:e375. doi: 10.1038/tp.2014.12.

Bipolar disorder (BP) is a chronic psychiatric condition characterized by dynamic, pathological mood fluctuations from mania to depression. To date, a major challenge in studying human neuropsychiatric conditions such as BP has been limited access to viable central nervous system tissue to examine disease progression. Patient-derived induced pluripotent stem cells (iPSCs) now offer an opportunity to analyze the full compliment of neural tissues and the prospect of identifying novel disease mechanisms. We have examined changes in gene expression as iPSC derived from well-characterized patients differentiate into neurons; there was little difference in the transcriptome of iPSC, but BP neurons were significantly different than controls in their transcriptional profile. Expression of transcripts for membrane bound receptors and ion channels was significantly increased in BP-derived neurons compared with controls, and we found that lithium pretreatment of BP neurons significantly altered their calcium transient and wave amplitude. The expression of transcription factors involved in the specification of telencephalic neuronal identity was also altered. Control neurons expressed transcripts that confer dorsal telencephalic fate, whereas BP neurons expressed genes involved in the differentiation of ventral (medial ganglionic eminence) regions.

Chestkov, I. V., E. A. Vasilieva, et al. "Patient-Specific Induced Pluripotent Stem Cells for SOD1-Associated Amyotrophic Lateral Sclerosis Pathogenesis Studies." Acta Naturae. 2014 Jan;6(1):54-60.

The genetic reprogramming technology allows one to generate pluripotent stem cells for individual patients. These cells, called induced pluripotent stem cells (iPSCs), can be an unlimited source of specialized cell types for the body. Thus, autologous somatic cell replacement therapy becomes possible, as well as the generation of in vitro cell models for studying the mechanisms of disease pathogenesis and drug discovery. Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disorder that leads to a loss of upper and lower motor neurons. About 10% of cases are genetically inherited, and the most common familial form of ALS is associated with mutations in the SOD1 gene. We used the reprogramming technology to generate induced pluripotent stem cells with patients with familial ALS. Patient-specific iPS cells were obtained by both integration and transgene-free delivery methods of reprogramming transcription factors. These iPS cells have the properties of pluripotent cells and are capable of direct differentiation into motor neurons.

Choi, I. Y., H. Lim, et al. "Efficient generation human induced pluripotent stem cells from human somatic cells with Sendai-virus." J Vis Exp. 2014 Apr 23;(86). doi: 10.3791/51406.

A few years ago, the establishment of human induced pluripotent stem cells (iPSCs) ushered in a new era in biomedicine. Potential uses of human iPSCs include modeling pathogenesis of human genetic diseases, autologous cell therapy after gene correction, and personalized drug screening by providing a source of patient-specific and symptom relevant cells. However, there are several hurdles to overcome, such as eliminating the remaining reprogramming factor transgene expression after human iPSCs production. More importantly, residual transgene expression in undifferentiated human iPSCs could hamper proper differentiations and misguide the interpretation of disease-relevant in vitro phenotypes. With this reason, integration-free and/or transgene-free human iPSCs have been developed using several methods, such as adenovirus, the piggyBac system, minicircle vector, episomal vectors, direct protein delivery and synthesized mRNA. However, efficiency of reprogramming using integration-free methods is quite low in most cases. Here, we present a method to isolate human iPSCs by using Sendai-virus (RNA virus) based reprogramming system. This reprogramming method shows consistent results and high efficiency in cost-effective manner.

Connelly, J. P., E. M. Kwon, et al. "Targeted correction of RUNX1 mutation in FPD patient-specific induced pluripotent stem cells rescues megakaryopoietic defects." Blood. 2014 Aug 11. pii: blood-2014-01-550525.

Familial platelet disorder with predisposition to acute myeloid leukemia (FPD/AML) is an autosomal dominant disease of the hematopoietic system, which is caused by heterozygous mutations in RUNX1. FPD/AML patients have a bleeding disorder characterized by thrombocytopenia with reduced platelet numbers and functions, and a tendency to develop AML. Currently no suitable animal models exist for FPD/AML as Runx1+/- mice and zebrafish do not develop bleeding disorders or leukemia. Here we derived induced pluripotent stem cells (iPSCs) from two patients in a family with FPD/AML, and found that the FPD iPSCs display defects in megakaryocytic differentiation in vitro. We corrected the RUNX1 mutation in one FPD iPSC line through gene targeting, which led to normalization of megakaryopoiesis of the iPSCs in culture. Our results demonstrate successful in vitro modeling of FPD with patient-specific iPSCs and confirm that RUNX1 mutations are responsible for megakaryopoietic defects in FPD patients.

Csobonyeiova, M., S. Polak, et al. "Induced pluripotent stem cells and their implication for regenerative medicine." Cell Tissue Bank. 2014 Jul 19.

In 2006 Yamanaka's group showed that stem cells with properties similar to embryonic stem cells could be generated from mouse fibroblasts by introducing four genes. These cells were termed induced pluripotent stem cells (iPSCs). Because iPSCs avoid many of ethical concerns associated with the use of embryonic material, they have great potential in cell-based regenerative medicine. They are suitable also for other various purposes, including disease modelling, personalized cell therapy, drug or toxicity screening and basic research. Moreover, in the future, there might become possible to generate organs for human transplantation. Despite these progresses, several studies have raised the concern for genetic and epigenetic abnormalities of iPSCs that could contribute to immunogenicity of some cells differentiated from iPSCs. Recent methodological improvements are increasing the ease and efficacy of reprogramming, and reducing the genomic modification. However, to minimize or eliminate genetic alternations in the derived iPSC line creation, factor-free human iPSCs are necessary.

Dang, L. T., N. T. Feric, et al. "Inhibition of apoptosis in human induced pluripotent stem cells during expansion in a defined culture using angiopoietin-1 derived peptide QHREDGS." Biomaterials. 2014 Sep;35(27):7786-99. doi: 10.1016/j.biomaterials.2014.05.018. Epub 2014 Jun 13.

Adhesion molecule signaling is critical to human pluripotent stem cell (hPSC) survival, self-renewal, and differentiation. Thus, hPSCs are grown as clumps of cells on feeder cell layers or poorly defined extracellular matrices such as Matrigel. We sought to define a small molecule that would initiate adhesion-based signaling to serve as a basis for a defined substrate for hPSC culture. Soluble angiopoeitin-1 (Ang-1)-derived peptide QHREDGS added to defined serum-free media increased hPSC colony cell number and size during long- and short-term culture when grown on feeder cell layers or Matrigel, i.e. on standard substrates, without affecting hPSC morphology, growth rate or the ability to differentiate into multiple lineages both in vitro and in vivo. Importantly, QHREDGS treatment decreased hPSC apoptosis during routine passaging and single-cell dissociation.

de Almeida, P. E., E. H. Meyer, et al. "Transplanted terminally differentiated induced pluripotent stem cells are accepted by immune mechanisms similar to self-tolerance." Nat Commun. 2014 May 30;5:3903. doi: 10.1038/ncomms4903.

The exact nature of the immune response elicited by autologous-induced pluripotent stem cell (iPSC) progeny is still not well understood. Here we show in murine models that autologous iPSC-derived endothelial cells (iECs) elicit an immune response that resembles the one against a comparable somatic cell, the aortic endothelial cell (AEC). These cells exhibit long-term survival in vivo and prompt a tolerogenic immune response

characterized by elevated IL-10 expression. In contrast, undifferentiated iPSCs elicit a very different immune response with high lymphocytic infiltration and elevated IFN-gamma, granzyme-B and perforin intragraft. Furthermore, the clonal structure of infiltrating T cells from iEC grafts is statistically indistinguishable from that of AECs, but is different from that of undifferentiated iPSC grafts. Taken together, our results indicate that the differentiation of iPSCs results in a loss of immunogenicity and leads to the induction of tolerance, despite expected antigen expression differences between iPSC-derived versus original somatic cells.

De Bonis, M. L., S. Ortega, et al. "SIRT1 Is Necessary for Proficient Telomere Elongation and Genomic Stability of Induced Pluripotent Stem Cells." Stem Cell Reports. 2014 Apr 17;2(5):690-706. doi: 10.1016/j.stemcr.2014.03.002. eCollection 2014 May 6.

The NAD-dependent deacetylase SIRT1 is involved in chromatin silencing and genome stability. Elevated SIRT1 levels in embryonic stem cells also suggest a role for SIRT1 in pluripotency. Murine SIRT1 attenuates telomere attrition in vivo and is recruited at telomeres in induced pluripotent stem cells (iPSCs). Because telomere elongation is an iPSC hallmark, we set out to study the role of SIRT1 in pluripotency in the setting of murine embryonic fibroblasts reprogramming into iPSCs. We find that SIRT1 is required for efficient postreprogramming telomere elongation, and that this effect is mediated by a c-MYC-dependent regulation of the mTert gene. We further demonstrate that SIRT1-deficient iPSCs accumulate chromosomal aberrations and show a derepression of telomeric heterochromatin. Finally, SIRT1-deficient iPSCs form larger teratomas that are poorly differentiated, highlighting a role for SIRT1 in exit from pluripotency. In summary, this work demonstrates a role for SIRT1 in the maintenance of pluripotency and modulation of differentiation.

de Lazaro, I., C. Bussy, et al. "Generation of induced pluripotent stem cells from virus-free in vivo reprogramming of BALB/c mouse liver cells." Biomaterials. 2014 Sep;35(29):8312-20. doi: 10.1016/j.biomaterials.2014.05.086. Epub 2014 Jul 4.

The in vivo cell reprogramming of terminally differentiated somatic cells to a pluripotent state by the ectopic expression of defined transcription factors has been previously shown in the BALB/c mouse liver upon plasmid DNA injection with no teratoma formation in the host tissue. Here, we hypothesized that the reprogrammed cells could be extracted from the tissue and cultured in vitro. We called these cells in vivo induced pluripotent stem (i(2)PS) cells because they showed pluripotent characteristics equivalent to a standard mouse ES cell line (E14TG2A). The pluripotent character of i(2)PS cells was determined by a battery of morphological, molecular and functional assays, including their contribution to adult tissues of chimeric mice upon blastocyst injection. These observations further confirm that terminally differentiated somatic cells in wild type, adult animals can be reprogrammed in vivo using virus-free methodologies. The reprogrammed cells can generate in vitro stem cell colonies that exhibit pluripotency similar to ES cells with numerous implications for the application of in vivo reprogramming for tissue regenerative purposes.

Dogaki, Y., S. Y. Lee, et al. "Efficient derivation of osteoprogenitor cells from induced pluripotent stem cells for bone regeneration." Int Orthop. 2014 Sep;38(9):1779-85. doi: 10.1007/s00264-014-2440-9. Epub 2014 Jul 6.

PURPOSE: There has been great interest in the use of induced pluripotent stem cells (iPSCs) in bone regenerative strategies. To generate osteoprogenitor cells from iPSCs, the most widely used protocol relies on an intermediate using embryoid body (EB) formation. We hypothesized that an osteoprogenitor cell population could be efficiently generated from iPSCs by employing a "direct-plating method" without the EB formation step. METHODS: Murine iPSC colonies were dissociated with trypsin-EDTA, and obtained single cells were cultured on gelatin-coated plates in MSC medium and FGF-2. Adherent homogeneous fibroblast-like cells obtained by this direct-plating technique were termed as direct-plated cells (DPCs). Expression levels of Oct-3/4 mRNA were analysed by real-time PCR. DPCs were evaluated for cell-surface protein expression using flow cytometry. After osteogenic induction, osteogenic differentiation ability of DPCs was evaluated. RESULTS: The expression level of Oct-3/4 in DPCs was significantly down-regulated compared to that observed in iPSCs, suggesting that the cells lost pluripotency. Flow cytometry analysis revealed that DPCs exhibited cell-surface antigens similar to those of bone marrow stromal cells. Furthermore, the cells proved to have a high osteogenic differentiation capacity, which was confirmed by the significant increase in alkaline phosphatase activity, the expression levels of osteogenic genes, and calcium mineralization after 14-day osteogenic induction.

Du, D. and X. Lou "Generation of induced pluripotent stem cells from neonatal mouse cochlear cells." Differentiation. 2014 Mar-Apr;87(3-4):127-33. doi: 10.1016/j.diff.2014.02.004. Epub 2014 Feb 28.

The sensory epithelium (SE) within the mammalian cochleae has a limited capacity for regeneration, and the loss of mammalian cochlear hair cells always lead to permanent hearing loss. Previous reports show that early postnatal cochlea harbors stem/progenitor-like cells nominated otospheres which have a limited regenerative/repair capacity, while these cell populations are progressively lost during the postnatal development. Induced pluripotent stem cells (iPS cells) directly reprogrammed from non-embryonic cells have captured great attentions in the scientific community. In the present study, we determine whether Yamanakas factors can induce the reprogramming of cochlear cells into iPS cells. We introduce defined factors Oct3/4, Sox2 and Klf4 into otospheres derived from postnatal day-1 (P1) mouse SE, and analyze characteristics alterations in cochlear cells. After transduction, otospheres generated colonies exhibiting a normal karyotype and morphology similar to that of mouse embryonic stem cells (ESCs). Moreover, these cochlear iPS cells also express ESC-like markers. Importantly, the cochlear iPS cells show pluripotency in vitro and in vivo, as evidenced by differentiation into three germ layers by embryoid body formation, as well as high efficient formation of teratomas containing three germ layers in immunodeficient mice.

Duan, L., Z. Wang, et al. "Comparison of reprogramming genes in induced pluripotent stem cells and nuclear transfer cloned embryos." Stem Cell Rev. 2014 Aug;10(4):548-60. doi: 10.1007/s12015-014-9516-1.

The most effective reprogramming methods, somatic cell nuclear transfer (SCNT) and induced pluripotent stem cells (iPSCs), are widely used in biological research and regenerative medicine, yet the mechanism that reprograms somatic cells to totipotency remains unclear and thus reprogramming efficiency is still low. Microarray technology has been employed in analyzing the transcriptomes changes during iPS reprogramming. Unfortunately, it is difficult to obtain enough DNA from SCNT reconstructed embryos to take advantage of this technology. In this study, we aimed to identify critical genes from the transcriptional profile for iPS reprogramming and compared expression levels of these genes in SCNT reprogramming. By integrating gene expression information from microarray databases and published studies comparing somatic cells with either miPSCs or mouse embryonic stem cells (ESCs), we obtained two lists of co-upregulated genes. The gene ontology (GO) enriched analysis of these two lists demonstrated that the reprogramming process is associated with numerous biological processes. Specifically, we selected 32 genes related to heterochromatin, embryonic development, and cell cycle from our co-upregulated gene datasets and examined the gene expression level in iPSCs and SCNT embryos by qPCR.

Durruthy-Durruthy, J., S. F. Briggs, et al. "Rapid and efficient conversion of integration-free human induced pluripotent stem cells to GMP-grade culture conditions." PLoS One. 2014 Apr 9;9(4):e94231. doi: 10.1371/journal.pone.0094231. eCollection 2014.

Data suggest that clinical applications of human induced pluripotent stem cells (hiPSCs) will be realized. Nonetheless, clinical applications will require hiPSCs that are free of exogenous DNA and that can be manufactured through Good Manufacturing Practice (GMP). Optimally, derivation of hiPSCs should be rapid and efficient in order to minimize manipulations, reduce potential for accumulation of mutations and minimize financial costs. Previous studies reported the use of modified synthetic mRNAs to reprogram fibroblasts to a pluripotent state. Here, we provide an optimized, fully chemically defined and feeder-free protocol for the derivation of hiPSCs using synthetic mRNAs. The protocol results in derivation of fully reprogrammed hiPSC lines from adult dermal fibroblasts in less than two weeks. The hiPSC lines were successfully tested for their identity, purity, stability and safety at a GMP facility and cryopreserved. To our knowledge, as a proof of principle, these are the first integration-free iPSCs lines that were reproducibly generated through synthetic mRNA reprogramming that could be putatively used for clinical purposes.

Efthymiou, A. G., G. Chen, et al. "Self-renewal and cell lineage differentiation strategies in human embryonic stem cells and induced pluripotent stem cells." Expert Opin Biol Ther. 2014 Sep;14(9):1333-44. doi: 10.1517/14712598.2014.922533. Epub 2014 May 31.

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.

Egusa, H., H. Kayashima, et al. "Comparative analysis of mouse-induced pluripotent stem cells and mesenchymal stem cells during osteogenic differentiation in vitro." Stem Cells Dev. 2014 Sep 15;23(18):2156-69. doi: 10.1089/scd.2013.0344. Epub 2014 May 27.

Induced pluripotent stem cells (iPSCs) can differentiate into mineralizing cells and are, therefore, expected to be useful for bone regenerative medicine; however, the characteristics of iPSC-derived osteogenic cells remain unclear. Here, we provide a direct in vitro comparison of the osteogenic differentiation process in mesenchymal stem cells (MSCs) and iPSCs from adult C57BL/6J mice. After 30 days of culture in osteogenic medium, both MSCs and iPSCs produced robustly mineralized bone nodules that contained abundant calcium phosphate with hydroxyapatite crystal formation. Mineral deposition was significantly higher in iPSC cultures than in MSC cultures. Scanning electron microscopy revealed budding matrix vesicles in early osteogenic iPSCs; subsequently, the vesicles propagated to exhibit robust mineralization without rich fibrous structures. Early osteogenic MSCs showed deposition of many matrix vesicles in abundant collagen fibrils that became solid mineralized structures. Both cell types demonstrated increased expression of osteogenic marker genes, such as runx2, osterix, dlx5, bone sialoprotein (BSP), and osteocalcin, during osteogenesis; however, real-time reverse transcription-polymerase chain reaction array analysis revealed that osteogenesis-related genes encoding mineralization-associated molecules, bone morphogenetic proteins, and extracellular matrix collagens were differentially expressed between iPSCs and MSCs. These data suggest that iPSCs are capable of differentiation into mature osteoblasts whose associated hydroxyapatite has a crystal structure similar to that of MSC-associated hydroxyapatite; however, the transcriptional differences between iPSCs and MSCs could result in differences in the mineral and matrix environments of the bone nodules.

Fang, I. M., C. H. Yang, et al. "Induced Pluripotent Stem Cells Without c-Myc Ameliorate Retinal Oxidative Damage via Paracrine Effects and Reduced Oxidative Stress in Rats." J Ocul Pharmacol Ther. 2014 Aug 14.

Abstract Purpose: To investigate the efficacy and mechanisms of non-c-Myc induced pluripotent stem cell (iPSC) transplantation in a rat model of retinal oxidative damage. Methods: Paraquat was intravitreously injected into Sprague-Dawley rats. After non-c-Myc iPSC transplantation, retinal function was evaluated by electroretinograms (ERGs). The generation of reactive oxygen species (ROS) was determined by lucigenin- and luminol-enhanced chemiluminescence. The expression of brain-derived neurotrophic factor, ciliary neurotrophic factor, basic fibroblast growth factor (bFGF), stromal cell-derived factor (SDF)-1alpha, and CXCR4 was measured by immunohistochemistry and ELISA. An in vitro study using SH-SY5Y cells was performed to verify the protective effects of SDF-1alpha. Results: Transplantation of non-c-Myc iPSCs effectively promoted the recovery of the b-wave ratio in ERGs and significantly ameliorated retinal damage. Non-c-Myc iPSC transplantation decreased ROS production and increased the activities of superoxide dismutase and catalase, thereby reducing retinal oxidative damage and apoptotic cells. Moreover, non-c-Myc iPSC transplantation resulted in significant upregulation of SDF-1alpha, followed by bFGF, accompanied by a significant improvement in the ERG.

Faravelli, I., M. Bucchia, et al. "Motor neuron derivation from human embryonic and induced pluripotent stem cells: experimental approaches and clinical perspectives." Stem Cell Res Ther. 2014 Jul 14;5(4):87. doi: 10.1186/scrt476.

Motor neurons are cells located in specific areas of the central nervous system, such as brain cortex (upper motor neurons), brain stem, and spinal cord (lower motor neurons), which maintain control over voluntary actions. Motor neurons are affected primarily by a wide spectrum of neurological disorders, generally indicated as motor neuron diseases (MNDs): these disorders share symptoms related to muscular atrophy and paralysis leading to death. No effective treatments are currently available. Stem cell-derived motor neurons represent a promising research tool in disease modeling, drug screening, and development of therapeutic approaches for MNDs and spinal cord injuries. Directed differentiation of human pluripotent stem cells - human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) - toward specific lineages is the first crucial step in order to extensively employ these cells in early human development investigation and potential clinical applications. Induced pluripotent stem cells (iPSCs) can be generated from patients' own somatic cells (for example, fibroblasts) by reprogramming them with specific factors. They can be considered embryonic stem cell-like cells, which express stem cell markers and have the ability to give rise to all three germ layers, bypassing the ethical concerns. Thus, hiPSCs constitute an appealing alternative source of motor neurons. These motor neurons might be a great research tool, creating a model for investigating the cellular and molecular interactions underlying early human brain development and pathologies during neurodegeneration. Patient-specific iPSCs may also provide the premises for autologous cell replacement therapies without related risks of immune rejection.

Felgentreff, K., L. Du, et al. "Differential role of nonhomologous end joining factors in the generation, DNA damage response, and myeloid differentiation of human induced pluripotent stem cells." Proc Natl Acad Sci U S A. 2014 Jun 17;111(24):8889-94. doi: 10.1073/pnas.1323649111. Epub 2014 Jun 2.

Nonhomologous end-joining (NHEJ) is a key pathway for efficient repair of DNA double-strand breaks (DSBs) and V(D)J recombination. NHEJ defects in humans cause immunodeficiency and increased cellular sensitivity to ionizing irradiation (IR) and are variably associated with growth retardation, microcephaly, and neurodevelopmental delay. Repair of DNA DSBs is important for reprogramming of somatic cells into induced pluripotent stem cells (iPSCs). To compare the specific contribution of DNA ligase 4 (LIG4), Artemis, and DNA-protein kinase catalytic subunit (PKcs) in this process and to gain insights into phenotypic variability associated with these disorders, we reprogrammed patient-derived fibroblast cell lines with NHEJ defects. Deficiencies of LIG4 and of DNA-PK catalytic activity, but not Artemis deficiency, were associated with markedly reduced reprogramming efficiency, which could be partially rescued by genetic complementation. Moreover, we identified increased genomic instability in LIG4-deficient iPSCs. Cell cycle synchronization revealed a severe defect of DNA repair and a G0/G1 cell cycle arrest, particularly in LIG4- and DNA-PK catalytically deficient iPSCs. Impaired myeloid differentiation was observed in LIG4-, but not Artemis- or DNA-PK-mutated iPSCs. These results indicate a critical importance of the NHEJ pathway for somatic cell reprogramming, with a major role for LIG4 and DNA-PKcs and a minor, if any, for Artemis.

Fink, K. D., A. T. Crane, et al. "Intrastriatal transplantation of adenovirus-generated induced pluripotent stem cells for treating neuropathological and functional deficits in a rodent model of Huntington's disease." Stem Cells Transl Med. 2014 May;3(5):620-31. doi: 10.5966/sctm.2013-0151. Epub 2014 Mar 21.

Induced pluripotent stem cells (iPSCs) show considerable promise for cell replacement therapies for Huntington's disease (HD). Our laboratory has demonstrated that tail-tip fibroblasts, reprogrammed into iPSCs via two adenoviruses, can survive and differentiate into neuronal lineages following transplantation into healthy adult rats. However, the ability of these cells to survive, differentiate, and restore function in a damaged brain is unknown. To this end, adult rats received a regimen of 3-nitropropionic acid (3-NP) to induce behavioral and neuropathological deficits that resemble HD. At 7, 21, and 42 days after the initiation of 3-NP or vehicle, the rats received intrastriatal bilateral transplantation of iPSCs. All rats that received 3-NP and vehicle treatment displayed significant motor impairment, whereas those that received iPSC transplantation after 3-NP treatment had preserved motor function. Histological analysis of the brains of these rats revealed significant decreases in optical densitometric measures in the striatum, lateral ventricle enlargement, as well as an increase in striosome size in all rats receiving 3-NP when compared with sham rats. The 3-NP-treated rats given transplants of iPSCs in the 7- or 21-day groups did not exhibit these deficits. Transplantation of iPSCs at the late-stage (42-day) time point did not protect against the 3-NP-induced neuropathology, despite preserving motor function.

Focosi, D., G. Amabile, et al. "Induced pluripotent stem cells in hematology: current and future applications." Blood Cancer J. 2014 May 9;4:e211. doi: 10.1038/bcj.2014.30.

Reprogramming somatic cells into induced pluripotent stem (iPS) cells is nowadays approaching effectiveness and clinical grade. Potential uses of this technology include predictive toxicology, drug screening, pathogenetic studies and transplantation. Here, we review the basis of current iPS cell technology and potential applications in hematology, ranging from disease modeling of congenital and acquired hemopathies to hematopoietic stem and other blood cell transplantation.

Fujita, J. and K. Fukuda "Future prospects for regenerated heart using induced pluripotent stem cells." J Pharmacol Sci. 2014;125(1):1-5. Epub 2014 Apr 16.

Induced pluripotent stem cell (iPSC) generation is an epoch-making technology. The potential applications for iPSCs are wide-ranging from in vitro disease models to drug discovery. For regenerative medicine in particular, the technology provides great hope for patients with incurable diseases or potentially fatal disorders such as heart failure (HF). However, the true realization of that promise for HF remains uncertain and moving toward the clinical application of iPSCs needs to be stepwise and careful. The establishment of "safe" iPSCs must be a major premise, while genome integration-free and oncogene-free reprogramming is also necessary. Teratoma formation also remains a risk with undifferentiated iPSCs, but it must not happen in patients' bodies. Thus, regardless of the target organ, the differentiated cells from iPSCs must be purified to exclude any possibility of

tumorigenicity. The transplantation strategies used for iPSC-derived cells are very important for the recovery of lost cardiac function. Longer engraftment of transplanted iPSCs-derived cardiomyocytes is essential particularly because their survival could be hampered by ischemia, inflammation, apoptosis, immunological rejection, and other cardiac phenomena. Providing these multistep solutions will open the new frontier of regenerative therapies with iPSCs for patients with severe HF.

Gieseck, R. L., 3rd, J. Colquhoun, et al. Disease modeling using human induced pluripotent stem cells: Lessons from the liver. Biochim Biophys Acta. 2014 Jun 2. pii: S1388-1981(14)00101-2. doi: 10.1016/j.bbalip.2014.05.010.

Human pluripotent stem cells (hPSCs) have the capacity to differentiate into any of the hundreds of distinct cell types that comprise the human body. This unique characteristic has resulted in considerable interest in the field of regenerative medicine, given the potential for these cells to be used to protect, repair, or replace diseased, injured, and aged cells within the human body. In addition to their potential in therapeutics, hPSCs can be used to study the earliest stages of human development and to provide a platform for both drug screening and disease modeling using human cells. Recently, the description of human induced pluripotent stem cells (hIPSCs) has allowed the field of disease modeling to become far more accessible and physiologically relevant, as pluripotent cells can be generated from patients of any genetic background. Disease models derived from hIPSCs that manifest cellular disease phenotypes have been established to study several monogenic diseases; furthermore, hIPSCs can be used for phenotype-based drug screens to investigate complex diseases for which the underlying genetic mechanism is unknown. As a result, the use of stem cells as research tools has seen an unprecedented growth within the last decade as researchers look for in vitro disease models which closely mimic in vivo responses in humans.

Gilpin, S. E., X. Ren, et al. "Enhanced Lung Epithelial Specification of Human Induced Pluripotent Stem Cells on Decellularized Lung Matrix." Ann Thorac Surg. 2014 Aug 19. pii: S0003-4975(14)01280-6. doi: 10.1016/j.athoracsur.2014.05.080.

Whole-lung scaffolds can be created by perfusion decellularization of cadaveric donor lungs. The resulting matrices can then be recellularized to regenerate functional organs. This study evaluated the capacity of acellular lung scaffolds to support recellularization with lung progenitors derived from human induced pluripotent stem cells (iPSCs). Whole rat and human lungs were decellularized by constant-pressure perfusion with 0.1% sodium dodecyl sulfate solution. Resulting lung scaffolds were cryosectioned into slices or left intact. Human iPSCs were differentiated to definitive endoderm, anteriorized to a foregut fate, and then ventralized to a population expressing NK2 homeobox 1 (Nkx2.1). Cells were seeded onto slices and whole lungs, which were maintained under constant perfusion biomimetic culture. Lineage specification was assessed by quantitative polymerase chain reaction and immunofluorescent staining. Regenerated left lungs were transplanted in an orthotopic position. Activin-A treatment, followed by transforming growth factor-beta inhibition, induced differentiation of human iPSCs to anterior foregut endoderm as confirmed by forkhead box protein A2 (FOXA2), SRY (Sex Determining Region Y)-Box 17 (SOX17), and SOX2 expression. Cells cultured on decellularized lung slices demonstrated proliferation and lineage commitment after 5 days. Cells expressing Nkx2.1 were identified at 40% to 60% efficiency. Within whole-lung scaffolds and under perfusion culture, cells further upregulated Nkx2.1 expression. After orthotopic transplantation, grafts were perfused and ventilated by host vasculature and airways.

Goh, P. A. and P. J. Verma "Generation of induced pluripotent stem cells from mouse adipose tissue." Methods Mol Biol. 2014;1194:253-70. doi: 10.1007/978-1-4939-1215-5_14.

The discovery that embryonic stem (ES) cell-like cells can be generated by simply over-expressing four key genes in adult somatic cells has changed the face of regenerative medicine. These induced pluripotent stem (iPS) cells have a wide range of potential uses from drug testing and in vitro disease modeling to personalized cell therapies for patients. However, prior to the realization of their potential, many issues need to be considered. One of these is the low-efficiency formation of iPSC. It has been extensively demonstrated that the somatic cell type can greatly influence reprogramming outcomes. We have shown that adipose tissue-derived cells (ADCs) can be easily isolated from adult animals and can be reprogrammed to a pluripotent state with high efficiency. Here, we describe a protocol for the high-efficiency derivation of ADCs and their subsequent use to generate mouse iPSC using Oct4, Sox2, Klf4, and cMyc retroviral vectors.

Graham, B., J. Stevens, et al. "Enhancement of arsenic trioxide-mediated changes in human induced pluripotent stem cells (IPS)." Int J Environ Res Public Health. 2014 Jul 22;11(7):7524-36. doi: 10.3390/ijerph110707524.

Induced pluripotent stem cells (IPS) are an artificially derived type of pluripotent stem cell, showing many of the same characteristics as natural pluripotent stem cells. IPS are a hopeful therapeutic model; however there is a critical need to determine their response to environmental toxins. Effects of arsenic on cells have been studied extensively; however, its effect on IPS is yet to be elucidated. Arsenic trioxide (ATO) has been shown to inhibit cell proliferation, induce apoptosis and genotoxicity in many cells. Based on ATOs action in other cells, we hypothesize that it will induce alterations in morphology, inhibit cell viability and induce a genotoxic effect on IPS. Cells were treated for 24 hours with ATO (0-9 microg/mL). Cell morphology, viability and DNA damage were documented. Results indicated sufficient changes in morphology of cell colonies mainly in cell ability to maintain grouping and ability to remain adherent. Cell viability decreased in a dose dependent manner.

Guo, Z., C. A. Higgins, et al. "Building a microphysiological skin model from induced pluripotent stem cells." Stem Cell Res Ther. 2013;4 Suppl 1:S2. doi: 10.1186/scrt363. Epub 2013 Dec 20.

The discovery of induced pluripotent stem cells (iPSCs) in 2006 was a major breakthrough for regenerative medicine. The establishment of patient-specific iPSCs has created the opportunity to model diseases in culture systems, with the potential to rapidly advance the drug discovery field. Current methods of drug discovery are inefficient, with a high proportion of drug candidates failing during clinical trials due to low efficacy and/or high toxicity. Many drugs fail toxicity testing during clinical trials, since the cells on which they have been tested do not adequately model three-dimensional tissues or their interaction with other organs in the body. There is a need to develop microphysiological systems that reliably represent both an intact tissue and also the interaction of a particular tissue with other systems throughout the body. As the port of entry for many drugs is via topical delivery, the skin is the first line of exposure, and also one of the first organs to demonstrate a reaction after systemic drug delivery. In this review, we discuss our strategy to develop a microphysiological system using iPSCs that recapitulates human skin for analyzing the interactions of drugs with the skin.

Gupta, P., M. Z. Ismadi, et al. "Optimization of agitation speed in spinner flask for microcarrier structural integrity and expansion of induced pluripotent stem cells." Cytotechnology. 2014 Jul 26.

In recent times, the study and use of induced pluripotent stem cells (iPSC) have become important in order to avoid the ethical issues surrounding the use of embryonic stem cells. Therapeutic, industrial and research based use of iPSC requires large quantities of cells generated in vitro. Mammalian cells, including pluripotent stem cells, have been expanded using 3D culture, however current limitations have not been overcome to allow a uniform, optimized platform for dynamic culture of pluripotent stem cells to be achieved. In the current work, we have expanded mouse iPSC in a spinner flask using Cytodex 3 microcarriers. We have looked at the effect of agitation on the microcarrier survival and optimized an agitation speed that supports bead suspension and iPS cell expansion without any bead breakage. Under the optimized conditions, the mouse iPSC were able to maintain their growth, pluripotency and differentiation capability. We demonstrate that microcarrier survival and iPS cell expansion in a spinner flask are reliant on a very narrow range of spin rates, highlighting the need for precise control of such set ups and the need for improved design of more robust systems.

Hasegawa, Y., D. Tang, et al. "CCL2 enhances pluripotency of human induced pluripotent stem cells by activating hypoxia related genes." Sci Rep. 2014 Jun 24;4:5228. doi: 10.1038/srep05228.

Standard culture of human induced pluripotent stem cells (hiPSCs) requires basic Fibroblast Growth Factor (bFGF) to maintain the pluripotent state, whereas hiPSC more closely resemble epiblast stem cells than true naive state ES which requires LIF to maintain pluripotency. Here we show that chemokine (C-C motif) ligand 2 (CCL2) enhances the expression of pluripotent marker genes through the phosphorylation of the signal transducer and activator of transcription 3 (STAT3) protein. Moreover, comparison of transcriptomes between hiPSCs cultured with CCL2 versus with bFGF, we found that CCL2 activates hypoxia related genes, suggesting that CCL2 enhanced pluripotency by inducing a hypoxic-like response. Further, we show that hiPSCs cultured with CCL2 can differentiate at a higher efficiency than culturing with just bFGF and we show CCL2 can be used in feeder-free conditions in the absence of LIF. Taken together, our finding indicates the novel functions of CCL2 in enhancing its pluripotency in hiPSCs.

Heng, B. C. and M. Fussenegger "Integration-free reprogramming of human somatic cells to induced pluripotent stem cells (iPSCs) without viral vectors, recombinant DNA, and genetic modification." Methods Mol Biol. 2014;1151:75-94. doi: 10.1007/978-1-4939-0554-6_6.

Stem cells are envisaged to be integral components of multicellular systems engineered for therapeutic applications. The reprogramming of somatic cells to induced pluripotent stem cells (iPSCs) via

recombinant expression of a limited number of transcription factors, which was first achieved by Yamanaka and colleagues in 2007, heralded a major breakthrough in the stem cell field. Since then, there has been rapid progress in the field of iPSC generation, including the identification of various small molecules that can enhance reprogramming efficiency and reduce the number of different transcription factors required for reprogramming. Nevertheless, the major obstacles facing clinical applications of iPSCs are safety concerns associated with the use of viral vectors and recombinant DNA for expressing the appropriate transcription factors during reprogramming. In particular, permanent genetic modifications to newly reprogrammed iPSCs have to be avoided in order to meet stringent safety requirements for clinical therapy. These safety challenges can be overcome by new technology platforms that enable cellular reprogramming to iPSCs without the need to utilize either recombinant DNA or viral vectors.

Higuchi, T., S. Kawagoe, et al. "The generation of induced pluripotent stem cells (iPSCs) from patients with infantile and late-onset types of Pompe disease and the effects of treatment with acid-alpha-glucosidase in Pompe's iPSCs." Mol Genet Metab. 2014 May;112(1):44-8. doi: 10.1016/j.ymgme.2014.02.012. Epub 2014 Mar 4.

Pompe disease (PD), which is also called glycogen storage disease type II (GSDII), is one of the lysosomal storage diseases (LSDs) caused by a deficiency in acid-alpha-glucosidase (GAA) in the lysosome and is characterized by the accumulation of glycogen in various cells. PD has been treated by enzyme replacement therapy (ERT). We generated induced pluripotent stem cells (iPSCs) from the cells of patients with infantile-type and late-onset-type PD using a retrovirus vector to deliver transgenes encoding four reprogramming factors, namely, OCT4, SOX2, c-MYC, and KLF4. We confirmed that the two types of PD-iPSCs exhibited an undifferentiated state, alkaline phosphatase staining, and the presence of SSEA-4, TRA-1-60, and TRA-1-81. The PD-iPSCs exhibited strong positive staining with Periodic acid-Schiff (PAS). Moreover, ultrastructural features of these iPSCs exhibited massive glycogen granules in the cytoplasm, particularly in the infantile-type but to a lesser degree in the late-onset type. Glycogen granules of the infantile-type iPSCs treated with rhGAA were markedly decreased in a dose-dependent manner. Human induced pluripotent stem cell provides an opportunity to build up glycogen storage of Pompe disease in vitro. It represents a promising resource to study disease mechanisms, screen new drug compounds and develop new therapies for Pompe disease.

Hirschi, K. K., S. Li, et al. "Induced pluripotent stem cells for regenerative medicine." Annu Rev Biomed Eng. 2014 Jul 11;16:277-94. doi: 10.1146/annurev-bioeng-071813-105108. Epub 2014 May 29.

With the discovery of induced pluripotent stem (iPS) cells, it is now possible to convert differentiated somatic cells into multipotent stem cells that have the capacity to generate all cell types of adult tissues. Thus, there is a wide variety of applications for this technology, including regenerative medicine, in vitro disease modeling, and drug screening/discovery. Although biological and biochemical techniques have been well established for cell reprogramming, bioengineering technologies offer novel tools for the reprogramming, expansion, isolation, and differentiation of iPS cells. In this article, we review these bioengineering approaches for the derivation and manipulation of iPS cells and focus on their relevance to regenerative medicine.

Horiguchi, I., M. M. Chowdhury, et al. "Proliferation, morphology, and pluripotency of mouse induced pluripotent stem cells in three different types of alginate beads for mass production." Biotechnol Prog. 2014 Jul-Aug;30(4):896-904. doi: 10.1002/btpr.1891. Epub 2014 Mar 11.

Induced pluripotent stem cells (iPSCs) are expected to be an ideal cell source for biomedical applications, but such applications usually require a large number of cells. Suspension culture of iPSC aggregates can offer high cell yields but sometimes results in excess aggregation or cell death by shear stress. Hydrogel-based microencapsulation can solve such problems observed in Suspension culture, but there is no systematic evaluation of the possible capsule formulations. In addition, their biological effects on entrapped cells are still poorly studied so far. We, therefore, immobilized mouse iPSCs in three different types of calcium-alginate (Alg-Ca) hydrogel-based microcapsules; (i) Alg-Ca capsules without further treatment (Naked), (ii) Alg-Ca capsules with poly-l-lysine (PLL) coating (Coated), and (iii) Alg-PLL membrane capsules with liquid cores (Hollow). After 10 days of culture within the medium containing serum and leukemia inhibitory factor, we obtained good cellular expansions (10-13-fold) in Coated and Hollow capsules that were similar to Suspension culture. However, 32 +/- 9% of cellular leakage and lower cell yield (about threefold) were observed in Naked capsules. This was not observed in Coated and Hollow capsules. In addition, immunostaining and quantitative RT-PCR showed that the formation of primitive endodermal layers was suppressed in Coated capsules contrary to all other formulations. This agenesis of primitive endoderm layers in Coated capsules is likely to be the main cause of the significantly better pluripotency maintenance in hydrogel-based encapsulation culture. These results are helpful in further optimizing hydrogel-based iPSC culture, which can maintain better local cellular environments and be compatible with mass culture.

Hosoi, M., K. Kumano, et al. "Generation of induced pluripotent stem cells derived from primary and secondary myelofibrosis patient samples." Exp Hematol. 2014 May 20. pii: S0301-472X(14)00195-7. doi: 10.1016/j.exphem.2014.03.010.

Induced pluripotent stem cells (iPS) derived from disease cells are expected to provide a new experimental material, especially for diseases from which samples are difficult to obtain. In this study, we generated iPS from samples from patients with primary and secondary myelofibrosis. The primary myelofibrosis cells had chromosome 13q deletions, and the secondary myelofibrosis (SMF) cells had JAK2V617F mutations. The myelofibrosis patient cell-derived iPS (MF-iPS) were confirmed as possessing these parental disease-specific genomic markers. The capacity to form three germ layers was confirmed by teratoma assay. By co-culture with specific feeder cells and cytokines, MF-iPS can re-differentiate into blood progenitor cells and finally into megakaryocytes. We found that mRNA levels of interleukin-8, one of the candidate cytokines related to the pathogenesis of myelofibrosis, was elevated predominantly in megakaryocytes derived from MF-iPS. Because megakaryocytes from myelofibrosis clones are considered to produce critical mediators to proliferate fibroblasts in the bone marrow and iPS can provide differentiated cells abundantly, the disease-specific iPS we established should be a good research tool for this intractable disease.

Hoveizi, E., S. Khodadadi, et al. "Small Molecules Differentiate Definitive Endoderm from Human Induced Pluripotent Stem Cells on PCL Scaffold." Appl Biochem Biotechnol. 2014 Aug;173(7):1727-36. doi: 10.1007/s12010-014-0960-9. Epub 2014 May 27.

Human induced pluripotent stem cells (hiPSCs) are attractive sources of cells for disease modeling in vitro, and they may eventually provide access to cells/tissues for the treatment of many degenerative diseases. Stepwise differentiation from hiPSCs to definitive endoderm (DE) will identify a key step in hepatocytes and beta cell development and may prove useful for transplantation therapy for liver diseases and diabetes. Inducer of definitive endoderm 1 (IDE1) is known to play an important role in the regional specification of DE. Here, we have investigated the effect of stimulation with IDE1 on the development of hiPSCs into DE cells in three-dimensional (3D) cultures. The differentiation was determined by immunofluorescence staining with Sox17, FoxA2, and goosecoid (Gsc) and also by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) analysis. In this study, we showed that hiPSCs with 6-day IDE1 treatment (as chemical tool) on poly(epsilon-caprolactone) (PCL) nanofibrous scaffold were able to differentiate into DE cells.

Hu, B., Y. Guo, et al. "Repression of SIRT1 promotes the differentiation of mouse induced pluripotent stem cells into neural stem cells." Cell Mol Neurobiol. 2014 Aug;34(6):905-12. doi: 10.1007/s10571-014-0071-8. Epub 2014 May 15.

The use of transplanting functional neural stem cells (NSCs) derived from induced pluripotent stem cells (iPSCs) has increased for the treatment of brain diseases. As such, it is important to understand the molecular mechanisms that promote NSCs differentiation of iPSCs for future NSC-based therapies. Sirtuin 1 (SIRT1), a NAD(+)-dependent protein deacetylase, has attracted significant attention over the past decade due to its prominent role in processes including organ development, longevity, and cancer. However, it remains unclear whether SIRT1 plays a role in the differentiation of mouse iPSCs toward NSCs. In this study, we produced NSCs from mouse iPSCs using serum-free medium supplemented with retinoic acid. We then assessed changes in the expression of SIRT1 and microRNA-34a, which regulates SIRT1 expression. Moreover, we used a SIRT1 inhibitor to investigate the role of SIRT1 in NSCs differentiation of iPSCs.

Huang, C. J., P. N. Nguyen, et al. "Frequent co-expression of miRNA-5p and -3p species and cross-targeting in induced pluripotent stem cells." Int J Med Sci. 2014 Jun 5;11(8):824-33. doi: 10.7150/ijms.8358. eCollection 2014.

BACKGROUND: A miRNA precursor generally gives rise to one major miRNA species derived from the 5' arm, and are called miRNA-5p. However, more recent studies have shown co-expression of miRNA-5p and -3p, albeit in different concentrations, in cancer cells targeting different sets of transcripts. Co-expression and regulation of the -5p and -3p miRNA species in stem cells, particularly in the reprogramming process, have not been studied. METHODS: In this work, we investigated co-expression and regulation of miRNA-5p and -3p species in human induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs) and embryonic stem cells (ESC) using a nanoliter-scale real-time PCR microarray platform that

included 1,036 miRNAs. RESULTS: In comparing iPSC and ESC, only 32 miRNAs were found to be differentially expressed, in agreement of the ESC-like nature of iPSC. In the analysis of reprogramming process in iPSCs, 261 miRNAs were found to be differentially expressed compared with the parental MSC and pre-adipose tissue, indicating significant miRNA alternations in the reprogramming process. In iPSC reprogrammed from MSC, there were 88 miRNAs (33.7%), or 44 co-expressed 5p/3p pairs, clearly indicating frequent co-expression of both miRNA species on reprogramming. Of these, 40 pairs were either co-up- or co-downregulated indicating concerted 5p/3p regulation. The 5p/3p species of only 4 pairs were regulated in reverse directions. Furthermore, some 5p/3p species of the same miRNAs were found to target the same transcript and the same miRNA may cross-target different transcripts of proteins of the G1/S transition of the cell cycle; 5p/3p co-targeting was confirmed in stem-loop RT-PCR. CONCLUSION: The observed cross- and co-regulation by paired miRNA species suggests a fail-proof scheme of miRNA regulation in iPSC, which may be important to iPSC pluripotency.

Huang, K., Z. Wu, et al. "Selective demethylation and altered gene expression are associated with ICF syndrome in human-induced pluripotent stem cells and mesenchymal stem cells." Hum Mol Genet. 2014 Jul 15. pii: ddu365.

Immunodeficiency, centromeric instability and facial anomalies type I (ICF1) syndrome is a rare genetic disease caused by mutations in DNA methyltransferase (DNMT) 3B, a de novo DNA methyltransferase. However, the molecular basis of how DNMT3B deficiency leads to ICF1 pathogenesis is unclear. Induced pluripotent stem cell (iPSC) technology facilitates the study of early human developmental diseases via facile in vitro paradigms. Here, we generate iPSCs from ICF Type 1 syndrome patient fibroblasts followed by directed differentiation of ICF1-iPSCs to mesenchymal stem cells (MSCs). By performing genome-scale bisulfite sequencing, we find that DNMT3B-deficient iPSCs exhibit global loss of non-CG methylation and select CG hypomethylation at gene promoters and enhancers. Further unbiased scanning of ICF1-iPSC methylomes also identifies large megabase regions of CG hypomethylation typically localized in centromeric and subtelomeric regions.

Imaizumi, M., Y. Sato, et al. "In vitro epithelial differentiation of human induced pluripotent stem cells for vocal fold tissue engineering." Ann Otol Rhinol Laryngol. 2013 Dec;122(12):737-47.

OBJECTIVES: We determined the feasibility and optimization of differentiating human induced pluripotent stem cells (hiPS) into nonkeratinized stratified squamous epithelial cells for vocal fold engineering. METHODS: hiPS were cultured and assessed for differentiation in 3 conditions: a 3-dimensional (3D) hyaluronic acid (HA) hydrogel scaffold, a 3D HA hydrogel scaffold with epidermal growth factor (EGF), and a 3D HA hydrogel scaffold cocultured with human vocal fold fibroblasts (hVFF). After 1, 2, and 4 weeks of cultivation, hiPS were selected for histology, immunohistochemistry, and/or transcript expression analysis. RESULTS: At 4 weeks, hiPS cultivated with hVFF or with EGF had significantly decreased levels of Oct 3/4, indicating loss of pluripotency. Immunofluorescence revealed the presence of pancytokeratin and of cytokeratin (CK) 13 and 14 epithelial-associated proteins at 4 weeks after cultivation in hiPS EGF and hiPS hVFF cultures. The transcript expression level of CK14 was significantly increased for hiPS hVFF cultures only and was measured concomitantly with cell morphology that was clearly cohesive and displayed a degree of nuclear polarity suggestive of epithelial differentiation. CONCLUSIONS: We found that hiPS cultivated in 3D HA hydrogel with hVFF demonstrated the most robust conversion evidence to date of epithelial differentiation. Further work is necessary to focus on amplification of these progenitors for application in vocal fold regenerative biology.

Ishizuka, T., H. Goshima, et al. "Involvement of beta-adrenoceptors in the differentiation of human induced pluripotent stem cells into mesodermal progenitor cells." Eur J Pharmacol. 2014 Oct 5;740:28-34. doi: 10.1016/j.ejphar.2014.06.056. Epub 2014 Jul 9.

Previous studies suggest that beta-adrenoceptor stimulation may enhance the cardiac differentiation of mouse embryonic stem (ES) cells. It remains unclear whether the differentiations of ES cells and induced pluripotent stem (iPS) cells rely on similar molecular mechanisms. In addition, no previous studies have shown that human iPS cells express beta-adrenoceptors. Therefore, in the present study, we determined the involvement of beta-adrenoceptors in the differentiation of human iPS cells into mesodermal progenitor cells. The induction of differentiation of human iPS cells into kinase insert domain receptor (KDR)-positive mesodermal progenitor cells was performed on feeder cells in a differentiation medium with basic fibroblast growth factor (bFGF), bone morphogenetic protein-4 (BMP-4), and activin A. When the iPS cells that were exposed to bFGF, BMP-4, and activin A were treated with L-isoproterenol (a beta-adrenoceptor agonist) for 4 days, the expression of KDR was significantly increased compared to that in the cells that were not treated with L-isoproterenol.

Ishizuka, T., H. Goshima, et al. "Stimulation of 5-HT4 receptor enhances differentiation of mouse induced pluripotent stem cells into neural progenitor cells." Clin Exp Pharmacol Physiol. 2014 May;41(5):345-50. doi: 10.1111/1440-1681.12224.

Activation of serotonin (5-hydroxytryptamine; 5-HT) receptors plays a role in adult neurogenesis and differentiation of neural progenitor cells (NPC). Herein, we examined the involvement of 5-HT receptors in the differentiation of mouse induced pluripotent stem (iPS) cells into NPC. To induce embryoid body (EB) formation, mouse iPS cells were cultured on ultralow-attachment dishes. All-trans retinoic acid (ATRA; 1 mumol/L) and/or 5-HT (0.03 or 0.1 mumol/L) was added to the EB cultures for 4 days and then EB plated on gelatin-coated plates were cultured for 7 or 14 days. Immunofluorescence staining revealed that mouse iPS cells expressed both 5-HT2A and 5-HT4 receptors and, to a lesser extent, 5-HT1A receptors. Treatment with 5-HT significantly enhanced the ATRA-induced expression of nestin, a specific marker for NPC, and phosphorylation of cAMP response element-binding protein (CREB). Pretreatment of EB cultures with either 1 mumol/L GR113808 (a selective 5-HT4 receptor antagonist) or 1 mumol/L H89 (a protein kinase (PKA) inhibitor) significantly inhibited these effects of 5-HT. These findings suggest that stimulation of 5-HT4 receptors may enhance ATRA-induced neural differentiation of mouse iPS cells through activation of PKA and CREB.

Itaba, N., P. M. Wairagu, et al. "Nuclear receptor gene alteration in human induced pluripotent stem cells with hepatic differentiation propensity." Hepatol Res. 2014 Mar 18. doi: 10.1111/hepr.12329.

Human induced pluripotent stem (hiPS) cells are an alternative cell source of regenerative medicine for liver disease. Because variations in hepatic differentiation efficacy among hiPS cells exist, it is important to select a hiPS cell line with hepatic differentiation propensity. In addition, nuclear receptors (NR) regulate essential biological processes including differentiation and development. In this study, we identified the hiPS cell line with hepatic differentiation propensity and examined expression levels of 48 NR during this process. We screened 28 hiPS cell lines, which are established from various tissues of healthy persons with various reprogramming methods, using a three-step differentiation method, and examined expression levels of 48 NR by quantitative real-time polymerase chain reaction during the differentiation process in the selected cells. RESULTS: hiPS-RIKEN-2B and hiPS-RIKEN-2F cells have hepatic differentiation propensity. Differentiation propensity towards endoderm was affected by donor origin but not by reprogramming methods or cell type of origins. Expression levels of NR were closely associated with those of hepatic differentiation markers. Furthermore, expression patterns of NR were categorized as five patterns. In particular, seven NR such as chicken ovalbumin upstream promoter transcription factor 1, retinoic acid receptor alpha, peroxisome proliferator-activated receptor-gamma, progesterone receptor, photoreceptor cell-specific nuclear receptor, tailless homolog orphan receptor and glucocorticoid receptor were identified as the genes of which expression gradually goes up with differentiation.

Jang, J., Z. Quan, et al. "Induced pluripotent stem cells for modeling of pediatric neurological disorders." Biotechnol J. 2014 Jul;9(7):871-81. doi: 10.1002/biot.201400010. Epub 2014 Jun 25.

The pathophysiological mechanisms underlying childhood neurological disorders have remained obscure due to a lack of suitable disease models reflecting human pathogenesis. Using induced pluripotent stem cell (iPSC) technology, various neurological disorders can now be extensively modeled. Specifically, iPSC technology has aided the study and treatment of early-onset pediatric neurodegenerative diseases such as Rett syndrome, Down syndrome, Angelman syndrome. Prader-Willi syndrome, Friedreich's ataxia, spinal muscular atrophy (SMA), fragile X syndrome, X-linked adrenoleukodystrophy (ALD), and SCN1A gene-related epilepsies. In this paper, we provide an overview of various gene delivery systems for generating iPSCs, the current state of modeling early-onset neurological disorders and the ultimate application of these in vitro models in cell therapy through the correction of disease-specific mutations.

Jia, B., S. Chen, et al. "Modeling of hemophilia A using patient-specific induced pluripotent stem cells derived from urine cells." Life Sci. 2014 Jul 11;108(1):22-9. doi: 10.1016/j.lfs.2014.05.004. Epub 2014 May 13.

Hemophilia A (HA) is a severe, congenital bleeding disorder caused by the deficiency of clotting factor VIII (FVIII). For years, traditional laboratory animals have been used to study HA and its therapies, although animal models may not entirely mirror the human pathophysiology. Human induced pluripotent stem cells (iPSCs) can undergo unlimited self-renewal and differentiate into all cell types. This study aims to generate hemophilia A (HA) patient-specific iPSCs that differentiate into disease-affected hepatocyte cells. These hepatocytes are potentially useful for in vitro disease modeling and provide an applicable cell source for autologous cell therapy after genetic correction. In this study, we mainly generated iPSCs from urine collected

from HA patients with integration-free episomal vectors PEP4-EO2S-ET2K containing human genes OCT4, SOX2, SV40LT and KLF4, and differentiated these iPSCs into hepatocyte-like cells. We further identified the genetic phenotype of the FVIII genes and the FVIII activity in the patient-specific iPSC derived hepatic cells.

Jiang, G., J. Di Bernardo, et al. "Human Transgene-Free Amniotic-Fluid-Derived Induced Pluripotent Stem Cells for Autologous Cell Therapy." Stem Cells Dev. 2014 Aug 18.

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.

Kadari, A., M. Lu, et al. "Excision of viral reprogramming cassettes by Cre protein transduction enables rapid, robust and efficient derivation of transgene-free human induced pluripotent stem cells." Stem Cell Res Ther. 2014 Apr 8;5(2):47. doi: 10.1186/scrt435.

Integrating viruses represent robust tools for cellular reprogramming; however, the presence of viral transgenes in induced pluripotent stem cells (iPSCs) is deleterious because it holds the risk of insertional mutagenesis leading to malignant transformation. Here, we combine the robustness of lentiviral reprogramming with the efficacy of Cre recombinase protein transduction to derive iPSCs devoid of transgenes. By genome-wide analysis and targeted differentiation towards the cardiomyocyte lineage, we show that transgene-free iPSCs are superior to iPSCs before Cre transduction. Our study provides a simple, rapid and robust protocol for the generation of clinical-grade iPSCs suitable for disease modeling, tissue engineering and cell replacement therapies.

Kang, H., Y. R. Shih, et al. "Mineralized gelatin methacrylate-based matrices induce osteogenic differentiation of human induced pluripotent stem cells." Acta Biomater. 2014 Aug 18. pii: S1742-7061(14)00346-8. doi: 10.1016/j.actbio.2014.08.010.

Human induced pluripotent stem cells (hiPSC) are a promising cell source with pluripotency and self-renewal properties. Design of simple and robust biomaterials with an innate ability to induce lineage-specificity of hiPSC is desirable to realize their application in regenerative medicine. In this study, the potential of biomaterials containing calcium phosphate minerals to induce osteogenic differentiation of hiPSC was investigated. hiPSC cultured using mineralized gelatin methacrylate-based matrices underwent osteogenic differentiation ex vivo, in both two-dimensional and three-dimensional cultures, in growth medium devoid of any osteogenic-inducing chemical components or growth factors. The findings that osteogenic differentiation of hiPSC can be achieved through biomaterial-based cues alone present new avenues for personalized regenerative medicine. Such biomaterials that could not only act as structural scaffolds, but could also provide tissue-specific functions such as directing stem cell differentiation commitment, have great potential in bone tissue engineering.

Kang, S. J., Y. I. Park, et al. "Sodium Butyrate Efficiently Converts Fully Reprogrammed Induced Pluripotent Stem Cells from Mouse Partially Reprogrammed Cells." Cell Reprogram. 2014 Aug 5.

Abstract Partially reprogrammed cells [preinduced pluripotent stem cells (pre-iPSCs)] commonly stall at epigenetic barriers, and this is one of the major failures in the reprogramming process. These cells can be converted to the fully reprogrammed state by reducing epigenetic blocks. In this study, we established three iPSC lines and two pre-iPSC lines induced by the doxycycline (dox)-inducible lentiviral system. In the pre-iPSC lines maintained under dox treatment (dox+), a small portion of embryonic stem cell (ESC)-like colonies spontaneously emerged after dox withdrawal (dox-), and major differentiation into fibroblast-like cells occurred. The spontaneous conversions based on the number of stage-specific embryonic antigen-1-positive (SSEA-1+) colonies were 0.006+/-0.004% [mean+/-standard deviation (SD)] for the #89-7D line and 0.016+/-0.004% for the #102-2D line. The SSEA-1+ colonies did not express the Nanog protein. However, the colonies showed characteristics typical of fully reprogrammed iPSCs after further expansion. To determine whether spontaneous conversion could be improved by epigenetic modification, we applied four small molecules-valproic acid (VPA), sodium butyrate (SB), trichostatin (TSA), and 5-aza-2'-deoxycytidine (5-Aza)-in both pre-iPSC lines. SB was the most effective molecule in enhancing the number of SSEA-1+ colonies (32- to 39-fold) at day 5 of dox- treatment. In addition, the expression of pluripotent genes (sox2 and nanog) was increased by SB.

Kidder, B. L. "Generation of induced pluripotent stem cells using chemical inhibition and three transcription factors." Methods Mol Biol. 2014;1150:227-36. doi: 10.1007/978-1-4939-0512-6_15.

Generation of induced pluripotent stem (iPS) cells from differentiated cells has traditionally been performed by overexpressing four transcription factors: Oct4, Sox2, Klf4, and c-Myc. However, inclusion of c-Myc in the reprogramming cocktail can lead to expansion of transformed cells that are not fully reprogrammed, and studies have demonstrated that c-Myc reactivation increases tumorigenicity in chimeras and progeny mice. Moreover, chemical inhibition of Wnt signaling has been shown to enhance reprogramming efficiency. Here, we describe a modified protocol for generating iPS cells from murine fibroblasts using chemical inhibition and overexpression of three transcription factors. Using this protocol, we observed robust conversion to iPS cells while maintaining minimal contamination of partially reprogrammed transformed colonies.

Kim, D. S., P. J. Ross, et al. "Optimizing neuronal differentiation from induced pluripotent stem cells to model ASD." Front Cell Neurosci. 2014 Apr 11;8:109. doi: 10.3389/fncel.2014.00109. eCollection 2014.

Autism spectrum disorder (ASD) is an early-onset neurodevelopmental disorder characterized by deficits in social communication, and restricted and repetitive patterns of behavior. Despite its high prevalence, discovery of pathophysiological mechanisms underlying ASD has lagged due to a lack of appropriate model systems. Recent advances in induced pluripotent stem cell (iPSC) technology and neural differentiation techniques allow for detailed functional analyses of neurons generated from living individuals with ASD. Refinement of cortical neuron differentiation methods from iPSCs will enable mechanistic studies of specific neuronal subpopulations that may be preferentially impaired in ASD. In this review, we summarize recent accomplishments in differentiation of cortical neurons from human pluripotent stems cells and efforts to establish in vitro model systems to study ASD using personalized neurons.

Kim, M. H. and M. Kino-Oka "Maintenance of undifferentiated state of human induced pluripotent stem cells through cytoskeleton-driven force acting to secreted fibronectin on a dendrimer-immobilized surface." J Biosci Bioeng. 2014 Jun 16. pii: S1389-1723(14)00181-9. doi: 10.1016/j.jbiosc.2014.05.011.

Understanding of the fundamental mechanisms that govern adhesive properties of human induced pluripotent stem cells (hiPSCs) to culture environments provides surface design strategies for maintaining their undifferentiated state during cell expansion. Polyamidoamine dendrimer surface with first-generation (G1) with dendron structure was used for co-cultures of hiPSCs and SNL feeder cells that formed tightly packed compact hiPSC colonies, similar to those on a conventional gelatin-coated surface. hiPSCs passaged up to 10 times on the G1 surface maintained their undifferentiated state. Immunostaining and reverse transcriptase PCR analysis of fibronectin showed that the secreted fibronectin matrix from feeder cells on the G1 surface contributed to hiPSC attachment. Compared with cells on the gelatin-coated surface, F-actin and paxillin immunostaining revealed a well-organized network of actin stress fibers and focal adhesion formation at cell-substrate sites in hiPSC colonies on the G1 surface. E-cadherin expression levels on these surfaces were almost same, but paxillin and Rac1 expression levels on the G1 surface were significantly higher than those on the gelatin-coated surface. Zyxin showed prominent expression on the G1 surface at sites of focal adhesion and cell-cell contact in colonies, whereas zyxin expression on the gelatin-coated surface was not observed in regions of cell-cell contact. These findings indicate that transduction of mechanical stimuli through actin polymerization at sites of focal adhesion and cell-cell contact results in maintenance of undifferentiated hiPSC colonies on G1 surface. The G1 surface enables a substrate design based on the mechanical cues in the microenvironment from feeder cells to expand undifferentiated hiPSCs in long-term culture.

Kim, M. H. and M. Kino-oka "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. 2014 Jul;35(22):5670-8. doi: 10.1016/j.biomaterials.2014.03.085. Epub 2014 Apr 18.

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.

Kim, T., M. Bershteyn, et al. Chromosome therapy: Correction of large chromosomal aberrations by inducing ring chromosomes induced pluripotent stem cells (iPSCs). Nucleus. 2014 Sep 3;5(5).

The fusion of the short (p) and long (q) arms of a chromosome is referred to as a "ring chromosome." Ring chromosome disorders occur in approximately 1 in 50 000-100 000 patients. Ring chromosomes can result in birth defects, mental disabilities, and growth retardation if additional genes are deleted during the formation of the ring. Due to the severity of these large-scale aberrations affecting multiple contiguous genes, no possible therapeutic strategies for ring chromosome disorders have so far been proposed. Our recent study (Bershteyn et al.) using patient-derived fibroblast lines containing ring chromosomes, found that cellular reprogramming of these fibroblasts into induced pluripotent stem cells (iPSCs) resulted in the cell-autonomous correction of the ring chromosomal aberration via compensatory uniparental disomy (UPD).

Kishino, Y., T. Seki, et al. "Derivation of transgene-free human induced pluripotent stem cells from human peripheral T cells in defined culture conditions." PLoS One. 2014 May 13;9(5):e97397. doi: 10.1371/journal.pone.0097397. eCollection 2014.

Recently, induced pluripotent stem cells (iPSCs) were established as promising cell sources for revolutionary regenerative therapies. The initial culture system used for iPSC generation needed fetal calf serum in the culture medium and mouse embryonic fibroblast as a feeder layer, both of which could possibly transfer unknown exogenous antigens and pathogens into the iPSC population. Therefore, the development of culture systems designed to minimize such potential risks has become increasingly vital for future applications of iPSCs for clinical use. On another front, although donor cell types for generating iPSCs are wide-ranging, T cells have attracted attention as unique cell sources for iPSCs generation because T cell-derived iPSCs (TiPSCs) have a unique monoclonal T cell receptor genomic rearrangement that enables their differentiation into antigen-specific T cells, which can be applied to novel immunotherapies. In the present study, we generated transgene-free human TiPSCs using a combination of activated human T cells and Sendai virus under defined culture conditions. These TiPSCs expressed pluripotent markers by quantitative PCR and immunostaining, had a normal karyotype, and were capable of differentiating into cells from all three germ layers. This method of TiPSCs generation is more suitable for the therapeutic application of iPSC technology because it lowers the risks associated with the presence of undefined, animal-derived feeder cells and serum. Therefore this work will lead to establishment of safer iPSCs and extended clinical application.

Ko, J. Y., S. Park, et al. "Osteogenesis from human induced pluripotent stem cells: an in vitro and in vivo comparison with mesenchymal stem cells." Stem Cells Dev. 2014 Aug 1;23(15):1788-97. doi: 10.1089/scd.2014.0043. Epub 2014 Apr 28.

The purpose of this study was to examine the in vitro and in vivo osteogenic potential of human induced pluripotent stem cells (hiPSCs) against that of human bone marrow mesenchymal stem cells (hBMMSCs). Embryoid bodies (EBs), which were formed from undifferentiated hiPSCs, were dissociated into single cells and underwent osteogenic differentiation using the same medium as hBMMSCs for 14 days. Osteoinduced hiPSCs were implanted on the critical-size calvarial defects and long bone segmental defects in rats. The healing of defects was evaluated after 8 weeks and 12 weeks of implantation, respectively. Osteoinduced hiPSCs showed relatively lower and delayed in vitro expressions of the osteogenic marker COL1A1 and bone sialoprotein, as well as a weaker osteogenic differentiation through alkaline phosphatase staining and mineralization through Alizarin red staining compared with hBMMSCs.

Kobayashi, J., M. Yoshida, et al. "Directed differentiation of patient-specific induced pluripotent stem cells identifies the transcriptional repression and epigenetic modification of NKX2-5, HAND1, and NOTCH1 in hypoplastic left heart syndrome." PLoS One. 2014 Jul 22;9(7):e102796. doi: 10.1371/journal.pone.0102796. eCollection 2014.

The genetic basis of hypoplastic left heart syndrome (HLHS) remains unknown, and the lack of animal models to reconstitute the cardiac maldevelopment has hampered the study of this disease. This study investigated the altered control of transcriptional and epigenetic programs that may affect the development of HLHS by using disease-specific induced pluripotent stem (iPS) cells. Cardiac progenitor cells (CPCs) were isolated from patients with congenital heart diseases to generate patient-specific iPS cells. Comparative gene expression analysis of HLHS- and biventricle (BV) heart-derived iPS cells was performed to dissect the complex genetic circuits that may promote the disease phenotype. Both HLHS- and BV heart-derived CPCs were reprogrammed to generate disease-specific iPS cells, which showed characteristic human embryonic stem cell signatures, expressed pluripotency markers, and could give rise to cardiomyocytes. However, HLHS-iPS cells exhibited lower cardiomyogenic differentiation potential than BV-iPS cells.

Koido, S., M. Ito, et al. "Vaccination with vascular progenitor cells derived from induced pluripotent stem cells elicits antitumor immunity targeting vascular and tumor cells." Cancer Immunol Immunother. 2014 May;63(5):459-68. doi: 10.1007/s00262-014-1531-1. Epub 2014 Mar 14.

Vaccination of BALB/c mice with dendritic cells (DCs) loaded with the lysate of induced vascular progenitor (iVP) cells derived from murine-induced pluripotent stem (iPS) cells significantly suppressed the tumor of CMS-4 fibrosarcomas and prolonged the survival of CMS-4-inoculated mice. This prophylactic antitumor activity was more potent than that of immunization with DCs loaded with iPS cells or CMS-4 tumor cells. Tumors developed slowly in mice vaccinated with DCs loaded with iVP cells (DC/iVP) and exhibited a limited vascular bed. Immunohistochemistry and a tomato-lectin perfusion study demonstrated that the tumors that developed in the iVP-immunized mice showed a marked decrease in tumor vasculature. Immunization with DC/iVP induced a potent suppressive effect on vascular-rich CMS-4 tumors, a weaker effect on BNL tumors with moderate vasculature, and nearly no effect on C26 tumors with poor vasculature. Treatment of DC/iVP-immunized mice with a monoclonal antibody against CD4 or CD8, but not anti-asialo GM1, inhibited the antitumor activity. CD8(+) T cells from DC/iVP-vaccinated mice showed significant cytotoxic activity against murine endothelial cells and CMS-4 cells, whereas CD8(+) T cells from DC/iPS-vaccinated mice did not.

Kondo, Y., T. Iwao, et al. "Histone deacetylase inhibitor valproic Acid promotes the differentiation of human induced pluripotent stem cells into hepatocyte-like cells." PLoS One. 2014 Aug 1;9(8):e104010. doi: 10.1371/journal.pone.0104010. eCollection 2014.

In this study, we aimed to elucidate the effects and mechanism of action of valproic acid on hepatic differentiation from human induced pluripotent stem cell-derived hepatic progenitor cells. Human induced pluripotent stem cells were differentiated into endodermal cells in the presence of activin A and then into hepatic progenitor cells using dimethyl sulfoxide. Hepatic progenitor cells were matured in the presence of hepatocyte growth factor, oncostatin M, and dexamethasone with valproic acid that was added during the maturation process. After 25 days of differentiation, cells expressed hepatic marker genes and drug-metabolizing enzymes and exhibited drug-metabolizing enzyme activities. These expression levels and activities were increased by treatment with valproic acid, the timing and duration of which were important parameters to promote differentiation from human induced pluripotent stem cell-derived hepatic progenitor cells into hepatocytes. Valproic acid inhibited histone deacetylase activity during differentiation of human induced pluripotent stem cells, and other histone deacetylase inhibitors also enhanced differentiation into hepatocytes. In conclusion, histone deacetylase inhibitors such as valproic acid can be used to promote hepatic differentiation from human induced pluripotent stem cell-derived hepatic progenitor cells.

Kondo, Y., S. Yoshihashi, et al. "Selective Culture Method for Hepatocyte-like Cells Differentiated from Human Induced Pluripotent Stem Cells." Drug Metab Pharmacokinet. 2014 Apr 29.

This study aimed to establish culture conditions which are able to give the differentiation of induced pluripotent (iPS) cells to the hepatocytes. To this end, we examined the usefulness of culture medium containing the components involved in the intermediary metabolism in the liver. More specifically, we examined the effect of the 'modified L-15 medium' containing galactose, phenylalanine and ornitine, but deprived of glucose, tyrosine, arginine and pyruvic acid. The medium was altered according to changes in the expression of enzymes that participate in liver-specific pathways. After 25 days of differentiation, the differentiated cells expressed

hepatocyte markers and drug-metabolizing enzymes. These expression levels were increased using modified L-15 medium. The survival of human fetal liver cells and the death of human fibroblasts were observed during culture in modified L-15 medium. Most of the cells that differentiated from human iPS cells using modified L-15 medium were stained by anti-human albumin antibody. These results suggest that iPS cells can be converted to high purity-differentiated hepatocytes by cultivating them in modified L-15 medium.

Kruta, M., M. Seneklova, et al. "Mutation Frequency Dynamics in HPRT Locus in Culture-Adapted Human Embryonic Stem Cells and Induced Pluripotent Stem Cells Correspond to Their Differentiated Counterparts." Stem Cells Dev. 2014 Jul 25.

The genomic destabilization associated with the adaptation of human embryonic stem cells (hESCs) to culture conditions or the reprogramming of induced pluripotent stem cells (iPSCs) increases the risk of tumorigenesis upon the clinical use of these cells and decreases their value as a model for cell biology studies. Base excision repair (BER), a major genomic integrity maintenance mechanism, has been shown to fail during hESC adaptation. Here, we show that the increase in the mutation frequency (MF) caused by the inhibition of BER was similar to that caused by the hESC adaptation process. The increase in MF reflected the failure of DNA maintenance mechanisms and the subsequent increase in MF rather than being due solely to the accumulation of mutants over a prolonged period, as was previously suggested. The increase in the ionizing-radiation-induced MF in adapted hESCs exceeded the induced MF in nonadapted hESCs and differentiated cells.

Kuise, T., H. Noguchi, et al. "Establishment of a pancreatic stem cell line from fibroblast-derived induced pluripotent stem cells." Biomed Eng Online. 2014 May 27;13:64. doi: 10.1186/1475-925X-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.

Kuroda, T., S. Yasuda, et al. "In vitro detection of residual undifferentiated cells in retinal pigment epithelial cells derived from human induced pluripotent stem cells." Methods Mol Biol. 2014;1210:183-92. doi: 10.1007/978-1-4939-1435-7_14.

Human pluripotent stem cells (hPSCs) such as human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) are a leading candidate for regenerative medicine/cell therapies because of their capacity for pluripotency and unlimited self-renewal. However, there are significant obstacles preventing the clinical use of hPSCs. A significant safety issues is the presence of residual undifferentiated cells that have the potential to form tumors in vivo. Here, we describe the highly sensitive qRT-PCR methods for detection of residual undifferentiated cells in retinal pigment epithelial (RPE) cells derived from hiPSCs. qRT-PCR using probes and primers targeting LIN28A (LIN28) transcripts can detect residual undifferentiated cell levels as low as 0.002 % in hiPSC-derived RPE cells. We expect this method to contribute to process validation and quality control of hiPSC-derived cell therapy product.

Lalit, P. A., D. J. Hei, et al. "Induced pluripotent stem cells for post-myocardial infarction repair: remarkable opportunities and challenges." Circ Res. 2014 Apr 11;114(8):1328-45. doi: 10.1161/CIRCRESAHA.114.300556.

Coronary artery disease with associated myocardial infarction continues to be a major cause of death and morbidity around the world, despite significant advances in therapy. Patients who have large myocardial infarctions are at highest risk for progressive heart failure and death, and cell-based therapies offer new hope for these patients. A recently discovered cell source for cardiac repair has emerged as a result of a breakthrough reprogramming somatic cells to induced pluripotent stem cells (iPSCs). The iPSCs can proliferate indefinitely in culture and can differentiate into cardiac lineages, including cardiomyocytes, smooth muscle cells, endothelial cells, and cardiac progenitors. Thus, large quantities of desired cell products can be generated without being limited by cellular senescence. The iPSCs can be obtained from patients to allow autologous therapy or, alternatively, banks of human leukocyte antigen diverse iPSCs are possible for allogeneic therapy. Preclinical animal studies using a variety of cell preparations generated from iPSCs have shown evidence of cardiac repair. Methodology for the production of clinical grade products from human iPSCs is in place.

Leung, K. S., V. W. Cheng, et al. "The involvement of DNA methylation and histone modification on the epigenetic regulation of embryonic stem cells and induced pluripotent stem cells." Curr Stem Cell Res Ther. 2014;9(5):388-95.

Stem cell research has been developing rapidly in diverse areas such as the fields of genetics and molecular biology over the past decades. Genomic studies on both embryonic stem cells (ESCs) and terminally-differentiated cells illustrated that factors apart from their hereditary information disparity are associated with gene expression patterns of ESCs. Therefore, current research is trying to explore the effects of epigenetic processes in stem cell physiology and phenotypic changes. In-depth analyses of the molecular mechanisms underpinning such epigenetic-mediated functions have also been conducted. These findings suggest the importance of understanding the epigenetic influences in stem cell activities. Accordingly this review will describe the regulatory machineries of stem cells development targeting the two epigenetic processes: (1) DNA methylation and (2) histones modification. In addition, up-to-date findings concerning the functional roles of these processes in stem cells homeostasis will be covered.

Li, J., W. Song, et al. "Advances in understanding the cell types and approaches used for generating induced pluripotent stem cells." J Hematol Oncol. 2014 Jul 19;7(1):50.

Successfully reprogramming somatic cells to a pluripotent state generates induced pluripotent stem (iPS) cells (or iPSCs), which have extensive self-renewal capacity like embryonic stem cells (ESCs). iPSCs can also generate daughter cells that can further undergo differentiation into various lineages or terminally differentiate to reach their final functional state. The discovery of how to produce iPSCs opened a new field of stem cell research with both intellectual and therapeutic benefits. The huge potential implications of disease-specific or patient-specific iPSCs have impelled scientists to solve problems hindering their applications in clinical medicine, especially the issues of convenience and safety. To determine the range of tissue types amenable to reprogramming as well as their particular characteristics, cells from three embryonic germ layers have been assessed, and the advantages that some tissue origins have over fibroblast origins concerning efficiency and accessibility have been elucidated. To provide safe iPSCs in an efficient and convenient way, the delivery systems and combinations of inducing factors as well as the chemicals used to generate iPSCs have also been significantly improved in addition to the efforts on finding better donor cells. Currently, iPSCs can be generated without c-Myc and Klf4 oncogenes, and non-viral delivery integration-free chemically mediated reprogramming methods have been successfully employed with relatively satisfactory efficiency. This paper will review recent advances in iPS technology by highlighting tissue origin and generation of iPSCs. The obstacles that need to be overcome for clinical applications of iPSCs are also discussed.

Li, J. Q., M. Cheng, et al. [Retraction] Generation of induced pluripotent stem cells using skin fibroblasts from patients with myocardial infarction under feeder-free conditions, Mol Med Rep. 2014 Aug;10(2):1170. doi: 10.3892/mmr.2014.2272. Epub 2014 May 27.

After the publication of the article, the authors decided they wished to retract their manuscript for the following reasons. We wish to retract our research article entitled 'Generation of induced pluripotent stem cells using skin fibroblasts from patients with myocardial infarction under feeder-free conditions' published on the Molecular Medicine Reports 9: 837-842, 2014. In this article, we generated human iPSCs from skin fibroblasts from myocardial infarction patients in feeder-independent conditions. However, in subsequent researches, all of the cells generated and believed to be iPSCs showed negative expression of the pluripotent markers, Nanog and Rex1, and the cell surface marker, SSEA-1 and SSEA-4. Therefore we think the established iPS cells might not be real pluripotent stem cells. Based on the above mentioned, we ascertained that there must have some serious disadvantages in our design of experiment fundamentally. As a result, all

authors involved unanimously agreed to retract this article and redesign our experiment. We deeply apologize to the readers for any inconvenience caused by this retraction. [the original article was published in the Molecular Medicine Reports 9: 837-842, 2014 DOI: 10.3892/mmr.2014.1885].

Lin, Y. C., K. K. Kuo, et al. "Bovine induced pluripotent stem cells are more resistant to apoptosis than testicular cells in response to mono-(2-ethylhexyl) phthalate." Int J Mol Sci. 2014 Mar 20;15(3):5011-31. doi: 10.3390/ijms15035011.

Although the androgen receptor (AR) has been implicated in the promotion of apoptosis in testicular cells (TSCs), the molecular pathway underlying AR-mediated apoptosis and its sensitivity to environmental hormones in TSCs and induced pluripotent stem cells (iPSCs) remain unclear. We generated the iPSCs from bovine TSCs via the electroporation of OCT4. The established iPSCs were supplemented with leukemia inhibitory factor and bone morphogenetic protein 4 to maintain and stabilize the expression of stemness genes and their pluripotency. Apoptosis signaling was assessed after exposure to mono-(2-ethylhexyl) phthalate (MEHP), the active metabolite of di-(2-ethylhexyl) phthalate. Here, we report that iPSCs were more resistant to MEHP-induced apoptosis than were original TSCs. MEHP also repressed the expression of AR and inactivated WNT signaling, and then led to the commitment of cells to apoptosis via the cyclin dependent kinase inhibitor p21CIP1. The loss of the frizzed receptor 7 and the gain of p21CIP were responsible for the stimulatory effect of MEHP on AR-mediated apoptosis. Our results suggest that testicular iPSCs can be used to study the signaling pathways involved in the response to environmental disruptors, and to assess the toxicity of environmental endocrine disruptors in terms of the maintenance of stemness and pluripotency.

Lin, Y. C., Y. Murayama, et al. "Role of tumor suppressor genes in the cancer-associated reprogramming of human induced pluripotent stem cells." Stem Cell Res Ther. 2014;5(2):58.

Because of their pluripotent characteristics, human induced pluripotent stem cells (iPSCs) possess great potential for therapeutic application and for the study of degenerative disorders. These cells are generated from normal somatic cells, multipotent stem cells, or cancer cells. They express embryonic stem cell markers, such as OCT4, SOX2, NANOG, SSEA-3, SSEA-4, and REX1, and can differentiate into all adult tissue types, both in vitro and in vivo. However, some of the pluripotency-promoting factors have been implicated in tumorigenesis. Here, we describe the merits of tumor suppresser genes as reprogramming factors for the generation of iPSCs without tumorigenic activity. The initial step of reprogramming is induction of the exogenous pluripotent factors to generate the oxidative stress that leads to senescence by DNA damage and metabolic stresses, thus inducing the expression of tumor suppressor genes such as p21CIP1 and p16INK4a through the activation of p53 to be the pre-induced pluripotent stem cells (pre-iPSCs). The later stage includes overcoming the barrier of reprogramming-induced senescence or cell-cycle arrest by shutting off the function of these tumor suppressor genes, followed by the induction of endogenous stemness genes for the full commitment of iPSCs (full-iPSCs). Thus, the reactive oxygen species (ROS) produced by oxidative stress might be critical for the induction of endogenous reprogramming-factor genes via epigenetic changes or antioxidant reactions. We also discuss the critical role of tumor suppressor genes in the evaluation of the tumorigenicity of human cancer cell-derived pluripotent stem cells, and describe how to overcome their tumorigenic properties for application in stem cell therapy in the field of regenerative medicine.

Liu, J., B. Shi, et al. "Applications of Induced Pluripotent Stem Cells in the Modeling of Human Inflammatory Bowel Diseases." Curr Stem Cell Res Ther. 2014 Aug 20.

Inflammatory bowel diseases (IBDs) are chronic and involve the gastrointestinal tract; the two primary IBDs are ulcerative colitis and Crohn's disease. Existing treatments for IBD include control of active inflammation and regulation of immune disorders, and commonly used drugs include salicylates, corticosteroids, and immunosuppressants. At the same time, an in-depth study of IBD pathogenesis promoted the acceptance of bioimmunotherapy by increasing numbers of people. However, long-term use of these drugs can cause adverse reactions that are difficult for patients to overcome, with limited efficacy for critically ill patients. Recent studies have found that stem cell transplantation is a new and effective therapy and IBD treatment, particularly for refractory cases. Stem cells, especially induced pluripotent stem cells (iPSCs), can differentiate into functional intestinal epithelia and their use avoids ethical issues arising from embryonic stem cells, providing a new kind of seed cell for alternative treatments for IBD. This paper reviews iPSCs as a potential new treatment for IBDs in order to provide an experimental and clinical reference.

Liu, S. P., Y. X. Li, et al. "[An improved method for generating integration-free human induced pluripotent stem cells]." Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2014 Jun;22(3):580-7. doi: 10.7534/j.issn.1009-2137.2014.03.002.

The genome instability and tumorigenicity of induced pluripotent stem cells (iPSC) hinder their great potentials for clinical application. Using episomal vectors to generate iPSC is the best way to solve safety issues at present. This method is simple and the exogenous gene was not integrated into the host genome. However, the reprogramming efficiency for this method is very low and thus limits its usage. This study was purposed to improve episomal method for generating induced pluripotent stem cells from cord blood mononuclear cells (CB MNC), to establish integration-free iPSC technology system, and to lay the foundation for individualized iPSC for future clinical uses. To improve the reprogramming efficiency for iPSC, episomal method was used at various combinations of episomal vectors, pre-stimulating culture mediums and oxygen condition were tested to optimize the method. The results showed that using erythroid culture medium for culturing 8 days, transfecting with episomal vectors with SFFV (spleen focus forming virus) promoter under the hypoxic condition (3%), CB MNC could be mostly efficiently reprogrammed with the efficiency 0.12%. Furthermore, the results showed that erythroblasts (CD36(+)CD71(+)CD235a(low)) were the cells that are reprogrammed with high efficiency after culture for 8 days. It is concluded that a highly efficient and safe method for generation of integration-free iPSC is successfully established, which is useable in clinical study.

Liu, Y., S. Fu, et al. "Native nucleus pulposus tissue matrix promotes notochordal differentiation of human induced pluripotent stem cells with potential for treating intervertebral disc degeneration." J Biomed Mater Res A. 2014 Jun 2. doi: 10.1002/jbm.a.35243.

Native porcine nucleus pulposus (NP) tissue harbors a number of notochordal cells (NCs). Whether the native NP matrix supports the homeostasis of notochordal cells is poorly understood. We hypothesized the NP matrix alone may contain sufficient regulatory factors and can serve as stimuli to generate notochordal cells (NCs) from human pluripotent stem cells. NCs are a promising cell sources for cell-based therapy to treat some types of intervertebral disc (IVD) degeneration. One major limitation of this emerging technique is the lack of available NCs as a potential therapeutic cell source. Human pluripotent stem cells derived from reprogramming or somatic cell nuclear transfer technique may yield stable and unlimited source for therapeutic use. We devised a new method to use porcine NP matrix to direct notochordal differentiation of human induced pluripotent stem cells (hiPSCs). The results showed that hiPSCs successfully differentiated into NC-like cells under the influence of devitalized porcine NP matrix. The NC-like cells expressed typical notochordal marker genes including brachyury (T), cytokeratin-8 (CK-8) and cytokeratin-18 (CK-18), and they displayed the ability to generate NP-like tissue in vitro, which was rich in aggrecan and collagen type II. These findings demonstrated the proof of concept for using native NP matrix to direct notochordal differentiation of hiPSCs. It provides a foundation for further understanding the biology of NCs, and eventually towards regenerative therapies for disc degeneration. (c) 2014 Wiley Periodicals, Inc. J Biomed Mater Res Part A, 2014.

Liu, Y., M. N. Rahaman, et al. "Modulating notochordal differentiation of human induced pluripotent stem cells using natural nucleus pulposus tissue matrix." PLoS One. 2014 Jul 23;9(7):e100885. doi: 10.1371/journal.pone.0100885. eCollection 2014.

Human induced pluripotent stem cells (hiPSCs) can differentiate into notochordal cell (NC)-like cells when cultured in the presence of natural porcine nucleus pulposus (NP) tissue matrix. The method promises massive production of high-quality, functional cells to treat degenerative intervertebral discs (IVDs). Based on our previous work, we further examined the effect of cell-NP matrix contact and culture medium on the differentiation, and further assessed the functional differentiation ability of the generated NC-like. The study showed that direct contact between hiPSCs and NP matrix can promote the differentiation yield, whilst both the contact and non-contact cultures can generate functional NC-like cells. The generated NC-like cells are highly homogenous regarding the expression of notochordal marker genes. A culture medium containing a cocktail of growth factors (FGF, EGF, VEGF and IGF-1) also supported the notochordal differentiation in the presence of NP matrix. The NC-like cells showed excellent functional differentiation ability to generate NP-like tissue which was rich in aggrecan and collagen type II; and particularly, the proteoglycan to collagen content ratio was as high as 12.5-17.5 which represents a phenotype close to NP rather than hyaline cartilage. Collectively, the present study confirmed the effectiveness and flexibility of using natural NP tissue matrix to direct notochordal differentiation of hiPSCs, and the potential of using the generated NC-like cells for treating IVD degeneration.

Lu, P., G. Woodruff, et al. "Long-distance axonal growth from human induced pluripotent stem cells after spinal cord injury." Neuron. 2014 Aug 20;83(4):789-96. doi: 10.1016/j.neuron.2014.07.014. Epub 2014 Aug 7.

Human induced pluripotent stem cells (iPSCs) from a healthy 86-year-old male were differentiated into neural stem cells and grafted into adult immunodeficient rats after spinal cord injury. Three months after C5 lateral hemisections, iPSCs survived and differentiated into neurons and glia and extended tens of thousands of axons from the lesion site over virtually the entire length of the rat CNS. These iPSC-derived axons extended through adult white matter of the injured spinal cord, frequently penetrating gray matter and forming synapses with rat neurons. In turn, host supraspinal motor axons penetrated human iPSC grafts and formed synapses. These findings indicate that intrinsic neuronal mechanisms readily overcome the inhibitory milieu of the adult injured spinal cord to extend many axons over very long distances; these capabilities persist even in neurons reprogrammed from very aged human cells. VIDEO ABSTRACT:

Luo, Y., C. Liu, et al. "Stable Enhanced Green Fluorescent Protein Expression After Differentiation and Transplantation of Reporter Human Induced Pluripotent Stem Cells Generated by AAVS1 Transcription Activator-Like Effector Nucleases." Stem Cells Transl Med. 2014 Jul;3(7):821-35. doi: 10.5966/sctm.2013-0212. Epub 2014 May 15.

Human induced pluripotent stem (hiPS) cell lines with tissue-specific or ubiquitous reporter genes are extremely useful for optimizing in vitro differentiation conditions as well as for monitoring transplanted cells in vivo. The adeno-associated virus integration site 1 (AAVS1) locus has been used as a "safe harbor" locus for inserting transgenes because of its open chromatin structure, which permits transgene expression without insertional mutagenesis. However, it is not clear whether targeted transgene expression at the AAVS1 locus is always protected from silencing when driven by various promoters, especially after differentiation and transplantation from hiPS cells. In this paper, we describe a pair of transcription activator-like effector nucleases (TALENs) that enable more efficient genome editing than the commercially available zinc finger nuclease at the AAVS1 site. Using these TALENs for targeted gene addition, we find that the cytomegalovirus-immediate early enhancer/chicken beta-actin/rabbit beta-globin (CAG) promoter is better than cytomegalovirus 7 and elongation factor 1alpha short promoters in driving strong expression of the transgene. The two independent AAVS1, CAG, and enhanced green fluorescent protein (EGFP) hiPS cell reporter lines that we have developed do not show silencing of EGFP either in undifferentiated hiPS cells or in randomly and lineage-specifically differentiated cells or in teratomas. Transplanting cardiomyocytes from an engineered AAVS1-CAG-EGFP hiPS cell line in a myocardial infarcted mouse model showed persistent expression of the transgene for at least 7 weeks in vivo. Our results show that high-efficiency targeting can be obtained with open-source TALENs and that careful optimization of the reporter and transgene constructs results in stable and persistent expression in vitro and in vivo.

Luo, Y., M. Rao, et al. "Generation of GFP Reporter Human Induced Pluripotent Stem Cells Using AAVS1 Safe Harbor Transcription Activator-Like Effector Nuclease." Curr Protoc Stem Cell Biol. 2014 May 16;29:5A.7.1-5A.7.18. doi: 10.1002/9780470151808.sc05a07s29.

Generation of a fluorescent GFP reporter line in human induced pluripotent stem cells (hiPSCs) provides enormous potentials in both basic stem cell research and regenerative medicine. A protocol for efficiently generating such an engineered reporter line by gene targeting is highly desired. Transcription activator-like effector nucleases (TALENs) are a new class of artificial restriction enzymes that have been shown to significantly promote homologous recombination by >1000-fold. The AAVS1 (adeno-associated virus integration site 1) locus is a "safe harbor" and has an open chromatin structure that allows insertion and stable expression of transgene. Here, we describe a step-by-step protocol from determination of TALENs activity, hiPSC culture, and delivery of a donor into AAVS1 targeting site, to validation of targeted integration by PCR and Southern blot analysis using hiPSC line, and a pair of open-source AAVS1 TALENs. Curr. Protoc. Stem Cell Biol. 29:5A.7.1-5A.7.18. (c) 2014 by John Wiley & Sons, Inc.

Ma, M. S., M. Czepiel, et al. "Generation of Induced Pluripotent Stem Cells from Hair Follicle Bulge Neural Crest Stem Cells." Cell Reprogram. 2014 Aug 1.

Abstract Induced pluripotent stem cells (iPSCs) are promising candidates for the study of disease models as well as for tissue engineering purposes. Part of a strategy to develop safe reprogramming technique is reducing the number of exogenous reprogramming factors. Some cells types are more prone to reprogramming than others. iPSC induction with less reprogramming factors has been described in cells with endogenous expression levels of pluripotency genes, such as neural stem cells. Because multipotent neural crest stem cells (NCSCs) from mammalian hair follicle bulges also express pluripotency genes, we argued that this property would facilitate reprogramming of hair follicle bulge NCSCs and could substitute for the use of exogenous reprogramming factors. Although we confirmed the expression of pluripotency genes in hair follicle bulge cells, our results show that these cells do require a full set of reprogramming factors for iPSC induction. Hair follicle bulge-derived iPSCs were created with efficiencies similar to fibroblasts. We conclude that high endogenous levels of pluripotency factors are no guarantee for facilitated induction of pluripotency.

Madonna, R., Y. J. Geng, et al. "High glucose-induced hyperosmolarity impacts proliferation, cytoskeleton remodeling and migration of human induced pluripotent stem cells via aquaporin-1." Biochim Biophys Acta. 2014 Aug 7. pii: S0925-4439(14)00251-8. doi: 10.1016/j.bbadis.2014.07.030.

Background and objective: Hyperglycemia leads to adaptive cell responses in part due to hyperosmolarity. In endothelial and epithelial cells, hyperosmolarity induces aquaporin-1 (AQP1) which plays a role in cytoskeletal remodeling, cell proliferation and migration. Whether such impairments also occur in human induced pluripotent stem cells (iPS) is not known. We therefore investigated whether high glucose-induced hyperosmolarity impacts proliferation, migration, expression of pluripotency markers and actin skeleton remodeling in iPS cells in an AQP1-dependent manner. Methods and results: Human iPS cells were generated from skin fibroblasts by lentiviral transduction of four reprogramming factors (Oct4, Sox2, Klf4, c-Myc). After reprogramming, iPS cells were characterized by their adaptive responses to high glucose-induced hyperosmolarity by incubation with 5.5mmol/L glucose, high glucose (HG) at 30.5mM, or with the hyperosmolar control mannitol (HM). Exposure to either HG or HM increased the expression of AQP1. AQP1 co-immunoprecipitated with beta-catenin. HG and HM induced the expression of beta-catenin. Under these conditions, iPS cells showed increased ratios of F-actin to G-actin and formed increased tubing networks. Inhibition of AQP1 with small interfering RNA (siRNA) reverted the inducing effects of HG and HM. Conclusions: High glucose enhances human iPS cell proliferation and cytoskeletal remodeling due to hyperosmolarity-induced upregulation of AQP1.

Mahairaki, V., J. Ryu, et al. "Induced Pluripotent Stem Cells from Familial Alzheimer's Disease Patients Differentiate into Mature Neurons with Amyloidogenic Properties." Stem Cells Dev. 2014 Aug 21.

Although the majority of Alzheimer's disease (AD) cases are sporadic, about 5% of cases are inherited in an autosomal dominant pattern as familial AD (FAD) and manifest at an early age. Mutations in the presenilin 1 (PSEN1) gene account for the majority of early-onset FAD. Here, we describe the generation of virus-free human induced pluripotent stem cells (hiPSCs) derived from fibroblasts of patients harboring the FAD PSEN1 mutation A246E and fibroblasts from healthy age-matched controls using nonintegrating episomal vectors. We have differentiated these hiPSC lines to the neuronal lineage and demonstrated that hiPSC-derived neurons have mature phenotypic and physiological properties. Neurons from mutant hiPSC lines express PSEN1-A246E mutations themselves and show AD-like biochemical features, that is, amyloidogenic processing of amyloid precursor protein (APP) indicated by an increase in beta-amyloid (Abeta)42/Abeta40 ratio. FAD hiPSCs harboring disease properties can be used as humanized models to test novel diagnostic methods and therapies and explore novel hypotheses for AD pathogenesis.

Malecki, M., E. Putzer, et al. "Directed cardiomyogenesis of autologous human induced pluripotent stem cells recruited to infarcted myocardium with bioengineered antibodies." Mol Cell Ther. 2014 May 1;2. pii: 13.

OBJECTIVE: Myocardial infarctions constitute a major factor contributing to non-natural mortality world-wide. Clinical trials of myocardial regenerative therapy, currently pursued by cardiac surgeons, involve administration of stem cells into the hearts of patients suffering from myocardial infarctions. Unfortunately, surgical acquisition of these cells from bone marrow or heart is traumatic, retention of these cells to sites of therapeutic interventions is low, and directed differentiation of these cells in situ into cardiomyocytes is difficult. The specific aims of this work were: (1) to generate autologous, human, pluripotent, induced stem cells (ahiPSCs) from the peripheral blood of the patients suffering myocardial infarctions; (2) to bioengineer heterospecific antibodies (htAbs) and use them for recruitment of the ahiPSCs to infarcted myocardium; (3) to initiate in situ directed cardiomyogenesis of the ahiPSCs retained to infarcted myocardium. METHODS: Peripheral blood was drawn from six patients scheduled for heart transplants. Mononuclear cells were isolated and reprogrammed, with plasmids carrying six genes (NANOG, POU5F1, SOX2, KLF4, LIN28A, MYC), to yield the ahiPSCs. Cardiac tissues were excised from the injured hearts of the patients, who received transplants during orthotopic surgery. These tissues were used to prepare in vitro models of stem cell therapy of infarcted myocardium. The htAbs were bioengineered, which simultaneously targeted receptors displayed on pluripotent stem cells (SSEA-4, SSEA-3, TRA-1-60, TRA-1-81) and proteins of myocardial sarcomeres (myosin, alpha-actinin, actin, titin). They were used to bridge the ahiPSCs to the

infarcted myocardium. The retained ahiPSCs were directed with bone morphogenetic proteins and nicotinamides to differentiate towards myocardial lineage. RESULTS: The patients' mononuclear cells were efficiently reprogrammed into the ahiPSCs. These ahiPSCs were administered to infarcted myocardium in in vitro models. They were recruited to and retained at the treated myocardium with higher efficacy and specificity, if were preceded with the htAbs, than with isotype antibodies or plain buffers. The retained cells differentiated into cardiomyocytes. CONCLUSIONS: The proof of concept has been attained, for reprogramming the patients' blood mononuclear cells (PBMCs) into the ahiPSCs, recruiting these cells to infarcted myocardium, and initiating their cardiomyogenesis. This novel strategy is ready to support the ongoing clinical trials aimed at regeneration of infarcted myocardium.

Martens, A., S. V. Rojas, et al. "Substantial Early Loss of Induced Pluripotent Stem Cells Following Transplantation in Myocardial Infarction." Artif Organs. 2014 Feb 21. doi: 10.1111/aor.12268.

The limited success of cardiac stem cell therapy has lately generated discussion regarding its effectiveness. We hypothesized that immediate cell loss after intramyocardial injection significantly obscures the regenerative potential of stem cell therapy. Therefore, our aim was to assess the distribution and quantity of induced pluripotent stem cells after intramyocardial delivery using in vivo bioluminescence analysis. In this context, we wanted to investigate if the injection of different cell concentrations would exert influence on cardiac cell retention. Murine-induced pluripotent stem cells were transfected for luciferase reporter gene expression and transplanted into infarcted myocardium in mice after left anterior descending coronary artery ligation. Cells were delivered constantly in aqueous media (15 muL) in different cell concentrations (group A, n = 10, 5.0 x 105 cells; group B, n = 10, 1.0 x 106 cells). Grafts were detected using bioluminescence imaging. Organ explants were imaged 10 min after injection to quantify early cardiac retention and cell biodistribution. Bioluminescence imaging showed a massive early displacement from the injection site to the pulmonary circulation, leading to lung accumulation. Mean cell counts of explanted organs in group A were 7.51 x 104 +/- 4.09 x 103 (heart), 6.44 x 104 +/- 2.48 x 103 (left lung), and 8.06 x 105 +/- 3.61 x 103 (right lung). Respective cell counts in group B explants were 1.69 x 105 +/- 7.69 x 104 (heart), 2.11 x 105 +/- 4.58 x 103 (left lung), and 3.25 x 105 +/- 9.35 x 103 (right lung). Applying bioluminescence imaging, we could unveil and quantify massive early cardiac stem cell loss and pulmonary cell accumulation following intramyocardial injection. Increased injection concentrations led to much higher intracardiac cell counts; however, pulmonary biodistribution of transplanted cells still persisted. Therefore, we recommend applying tissue engineering techniques for cardiac stem cell transplantations in order to improve cardiac retention and limit biodistribution.

Mastromonaco, G. F., L. A. Gonzalez-Grajales, et al. "Somatic cells, stem cells, and induced pluripotent stem cells: how do they now contribute to conservation?" Adv Exp Med Biol. 2014;753:385-427. doi: 10.1007/978-1-4939-0820-2_16.

More than a decade has now passed since the birth of the first endangered species produced from an adult somatic cell reprogrammed by somatic cell nuclear transfer. At that time, advances made in domestic and laboratory animal species provided the necessary foundation for attempting cutting-edge technologies on threatened and endangered species. In addition to nuclear transfer, spermatogonial stem cell transplantation and induction of pluripotent stem cells have also been explored. Although many basic scientific questions have been answered and more than 30 wild species have been investigated, very few successes have been reported. The majority of studies document numerous obstacles that still need to be overcome to produce viable gametes or embryos for healthy offspring production. This chapter provides an overview of somatic cell and stem cell technologies in different taxa (mammals, fishes, birds, reptiles and amphibians) and evaluates the potential and impact of these approaches for animal species conservation.

Maynard, K. M., U. Arvindam, et al. "Potentially immunogenic proteins expressed similarly in human embryonic stem cells and induced pluripotent stem cells." Exp Biol Med (Maywood). 2014 Apr;239(4):484-8. doi: 10.1177/1535370214522936. Epub 2014 Mar 4.

A major limitation of the use of cellular therapies is the loss of donor-derived cells because of immune incompatibility. While induced pluripotent stem (iPS) cells offer the potential for autologous transplant therapies, questions have been raised using a mouse model that specific antigens mediate the rejection of grafts after syngeneic transplants with iPS, but not embryonic stem (ES) cells. In this study, we examined whether the human homologs of these markers, HORMAD1, ZG16, and Cyp3A, are differentially expressed in human iPS versus ES cells, as well as undifferentiated and in vitro-differentiated cells. Both qRT-PCR and flow cytometric analyses demonstrated similar gene and protein expression profiles for iPS and ES cells regardless of differentiation state. Our data are consistent with a recent study in mice that showed no evidence of rejection of differentiated syngeneic iPS cells. Furthermore, our results suggest that expression of these gene products cannot predict differences in clinical outcomes between human iPS and ES-derived cells.

Medrano, J. V., C. Simon, et al. "Human germ cell differentiation from pluripotent embryonic stem cells and induced pluripotent stem cells." Methods Mol Biol. 2014;1154:563-78. doi: 10.1007/978-1-4939-0659-8_27.

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.

Mehta, A., V. Verma, et al. "A systemic evaluation of cardiac differentiation from mRNA reprogrammed human induced pluripotent stem cells." PLoS One. 2014 Jul 28;9(7):e103485. doi: 10.1371/journal.pone.0103485. eCollection 2014.

Genetically unmodified cardiomyocytes mandated for cardiac regenerative therapy is conceivable by "foot-print free" reprogramming of somatic cells to induced pluripotent stem cells (iPSC). In this study, we report generation of foot-print free hiPSC through messenger RNA (mRNA) based reprograming. Subsequently, we characterize cardiomyocytes derived from these hiPSC using molecular and electrophysiological methods to characterize their applicability for regenerative medicine. Our results demonstrate that mRNA-iPSCs differentiate ontogenetically into cardiomyocytes with increased expression of early commitment markers of mesoderm, cardiac mesoderm, followed by cardiac specific transcriptional and sarcomeric structural and ion channel genes. Furthermore, these cardiomyocytes stained positively for sarcomeric and ion channel proteins. Based on multi-electrode array (MEA) recordings, these mRNA-hiPSC derived cardiomyocytes responded predictably to various pharmacologically active drugs that target adrenergic, sodium, calcium and potassium channels. The cardiomyocytes responded chronotropically to isoproterenol in a dose dependent manner, inotropic activity of nifidipine decreased spontaneous contractions. Moreover, Sotalol and E-4031 prolonged QT intervals, while TTX reduced sodium influx. Our results for the first time show a systemic evaluation based on molecular, structural and functional properties of cardiomyocytes differentiated from mRNA-iPSC. These results, coupled with feasibility of generating patient-specific iPSCs hold great promise for the development of large-scale generation of clinical grade cardiomyocytes for cardiac regenerative medicine.

Mitani, K. "Gene targeting in human-induced pluripotent stem cells with adenoviral vectors." Methods Mol Biol. 2014;1114:163-7. doi: 10.1007/978-1-62703-761-7_10.

Helper-dependent adenoviral vector (HDAdV), which is also called gutless AdV, has been used to deliver donor DNA for gene targeting in human pluripotent stem cells. Surprisingly, the targeting efficacies, both per chromosomal integration (drug-resistant colony) and per treated cells, are much higher than those by standard electroporation and equivalent to those by utilizing artificial nucleases, such as TAL effector nucleases (Aizawa et al., Mol Ther 20:424-431, 2012; Suzuki, Proc Natl Acad Sci U S A 105:13781-13786, 2008). Importantly, gene targeting with HDAdVs was equally efficient in transcriptionally inactive loci in human ES/iPS cells. Therefore, multiple gene-targeted clones can be obtained from human embryonic stem cells (hESCs) and human-induced pluripotent stem cells (hiPSCs) cultured in one 100-mm dish. For virus-mediated gene targeting, it is not required to introduce artificial double-strand breaks. By using electroporation for gene targeting, target cells should be expanded to 10(6)-10(7) cells. In contrast, as an advantage of virus-mediated method, DNA delivery efficiency is high even in a smaller number of cells, resulting in minimizing the number of passages/cell divisions before performing gene targeting. The characteristics suggest that HDAdV-mediated gene targeting has potential advantages for manipulation of chromosomes of pluripotent stem cells for therapeutic applications.

Mizukami, Y., T. Abe, et al. "MHC-matched induced pluripotent stem cells can attenuate cellular and humoral immune responses but are still susceptible to innate immunity in pigs." PLoS One. 2014 Jun 13;9(6):e98319. doi: 10.1371/journal.pone.0098319. eCollection 2014.

Recent studies have revealed negligible immunogenicity of induced pluripotent stem (iPS) cells in syngeneic mice and in autologous monkeys. Therefore, human iPS cells would not elicit immune responses in the autologous setting. However, given that human leukocyte antigen (HLA)-

matched allogeneic iPS cells would likely be used for medical applications, a more faithful model system is needed to reflect HLA-matched allogeneic settings. Here we examined whether iPS cells induce immune responses in the swine leukocyte antigen (SLA)-matched setting. iPS cells were generated from the SLA-defined C1 strain of Clawn miniature swine, which were confirmed to develop teratomas in mice, and transplanted into the testes (n = 4) and ovary (n = 1) of C1 pigs. No teratomas were found in pigs on 47 to 125 days after transplantation. A Mixed lymphocyte reaction revealed that T-cell responses to the transplanted MHC-matched (C1) iPS cells were significantly lower compared to allogeneic cells. The humoral immune responses were also attenuated in the C1-to-C1 setting. More importantly, even MHC-matched iPS cells were susceptible to innate immunity, NK cells and serum complement. iPS cells lacked the expression of SLA class I and sialic acids. The in vitro cytotoxic assay showed that C1 iPS cells were targeted by NK cells and serum complement of C1. In vivo, the C1 iPS cells developed larger teratomas in NK-deficient NOG (T-B-NK-) mice (n = 10) than in NK-competent NOD/SCID (T-B-NK+) mice (n = 8) ($p<0.01$). In addition, C1 iPS cell failed to form teratomas after incubation with the porcine complement-active serum. Taken together, MHC-matched iPS cells can attenuate cellular and humoral immune responses, but still susceptible to innate immunity in pigs.

Mo, X., N. Li, et al. "Generation and characterization of bat-induced pluripotent stem cells." Theriogenology. 2014 Jul 15;82(2):283-93. doi: 10.1016/j.theriogenology.2014.04.001. Epub 2014 Apr 19.

Induced pluripotent stem cells (iPSCs) were first generated from mouse embryonic fibroblasts in the year 2006. These cells resemble the typical morphology of embryonic stem cells, express pluripotency markers, and are able to transmit through germlines. To date, iPSCs of many species have been generated, whereas generation of bat iPSCs (biPSCs) has not been reported. To facilitate in-depth study of bats at the molecular and cellular levels, we describe the successful derivation of biPSCs with a piggyBac (PB) vector that contains eight reprogramming factors Oct4, Sox2, Klf4, Nanog, cMyc, Lin28, Nr5a2, and miR302/367. These biPSCs were cultured in media containing leukemia inhibitory factor and three small molecule inhibitors (CHIR99021, PD0325901, and A8301). They retained normal karyotype, displayed alkaline phosphatase activity, and expressed pluripotency markers Oct4, Sox2, Nanog, TBX3, and TRA-1-60. They could differentiate in vitro to form embryoid bodies and in vivo to form teratomas that contained tissue cells of all three germ layers. Generation of biPSCs will facilitate future studies on the mechanisms of antiviral immunity and longevity of bats at the cellular level.

Mu, S., J. Wang, et al. "Transplantation of induced pluripotent stem cells improves functional recovery in Huntington's disease rat model." PLoS One. 2014 Jul 23;9(7):e101185. doi: 10.1371/journal.pone.0101185. eCollection 2014.

The purpose of this study was to determine the functional recovery of the transplanted induced pluripotent stem cells in a rat model of Huntington's disease with use of 18F-FDG microPET/CT imaging. METHODS: In a quinolinic acid-induced rat model of striatal degeneration, induced pluripotent stem cells were transplanted into the ipsilateral lateral ventricle ten days after the quinolinic acid injection. The response to the treatment was evaluated by serial 18F-FDG PET/CT scans and Morris water maze test. Histological analyses and Western blotting were performed six weeks after stem cell transplantation. RESULTS: After induced pluripotent stem cells transplantation, higher 18F-FDG accumulation in the injured striatum was observed during the 4 to 6-weeks period compared with the quinolinic acid-injected group, suggesting the metabolic recovery of injured striatum. The induced pluripotent stem cells transplantation improved learning and memory function (and striatal atrophy) of the rat in six week in the comparison with the quinolinic acid-treated controls. In addition, immunohistochemical analysis demonstrated that transplanted stem cells survived and migrated into the lesioned area in striatum, and most of the stem cells expressed protein markers of neurons and glial cells. CONCLUSION: Our findings show that induced pluripotent stem cells can survive, differentiate to functional neurons and improve partial striatal function and metabolism after implantation in a rat Huntington's disease model.

Mukherjee, S., C. Pipino, et al. "Emerging neuronal precursors from amniotic fluid-derived down syndrome induced pluripotent stem cells." Hum Gene Ther. 2014 Aug;25(8):682-3. doi: 10.1089/hum.2014.074.

Mukherjee, S. and A. J. Thrasher "Gene correction of induced pluripotent stem cells derived from a murine model of X-linked chronic granulomatous disorder." Methods Mol Biol. 2014;1114:427-40. doi: 10.1007/978-1-62703-761-7_28.

Gene therapy presents an attractive alternative to allogeneic haematopoietic stem cell transplantation (HSCT) for treating patients suffering from primary immunodeficiency disorder (PID). The conceptual advantage of gene correcting a patient's autologous HSCs lies in minimizing or completely avoiding immunological complications arising from allogeneic transplantation while conferring the same benefits of immune reconstitution upon long-term engraftment. Clinical trials targeting X-linked chronic granulomatous disorder (X-CGD) have shown promising results in this context. However, long-term clinical benefits in these patients have been limited by issues of poor engraftment of gene-transduced cells coupled with transgene silencing and vector induced clonal proliferation. Novel vectors incorporating safety features such as self-inactivating (SIN) mutations in the long terminal repeats (LTRs) along with synthetic promoters driving lineage-restricted sustainable expression of the gp91phox transgene are expected to resolve the current pitfalls and require rigorous preclinical testing. In this chapter, we have outlined a protocol in which X-CGD mouse model derived induced pluripotent stem cells (iPSCs) have been utilized to develop a platform for investigating the efficacy and safety profiles of novel vectors prior to clinical evaluation.

Naujock, M., N. Stanslowsky, et al. "Molecular and functional analyses of motor neurons generated from human cord blood derived induced pluripotent stem cells." Stem Cells Dev. 2014 Jul 9.

Induced pluripotent stem cells (iPSC) have become the most promising candidates for in-vitro modeling of motor neuron diseases (MND) such as Amyotrophic Lateral Sclerosis (ALS) and possibly for future therapeutic implementation in regenerative medicine. We here present for the first time the differentiation of human cord blood derived iPSC (hCBiPSC) into motor neurons (MN), the cell type primarily affected in ALS. In contrast to iPSC generated from adult tissue the hCBiPSC used in this study hold the promise of lower genetic mutations burden or epigenetic alterations which makes them ideal candidates for transplantation studies. After 18 days of in-vitro differentiation the MNs stained positive for neuronal and for MN specific markers accompanied by respective gene expression patterns. To demonstrate that hCBiPSC can be differentiated into functional MNs, the cells were characterized by calcium-imaging and patch-clamp analysis. Calcium-imaging detected the expression of functional voltage-dependent calcium and ligand-gated channels of several important neurotransmitters. Using whole-cell patch-clamp recordings, we observed functional neuronal properties like sodium-inward currents and action potentials. Some cells showed spontaneous action potentials and synaptic activity that are signs of essential functional maturation. Having established a rapid and efficient method to generate functional MNs from hCBiPSC, we demonstrate the differentiation potential of genetically unbiased hCBiPSC as promising source for transplantation studies and also create a framework for future in-vitro modelling.

Ni, A., M. J. Wu, et al. "Sphere Formation Permits Oct4 Reprogramming of Ciliary Body Epithelial Cells into Induced Pluripotent Stem Cells." Stem Cells Dev. 2014 Aug 18.

Somatic cells can be reprogrammed to induced pluripotent stem (iPS) cells by defined sets of transcription factors. We previously described reprogramming of monolayer-cultured adult mouse ciliary body epithelial (CE) cells by Oct4 and Klf4, but not with Oct4 alone. In this study, we report that Oct4 alone is sufficient to reprogram CE cells to iPS cells through sphere formation. Furthermore, we demonstrate that sphere formation induces a partial reprogramming state characterized by expression of retinal progenitor markers, upregulation of reprogramming transcription factors, such as Sall4 and Nanog, demethylation in the promoter regions of pluripotency associated genes, and mesenchymal to epithelial transition. The Oct4-iPS cells maintained normal karyotypes, expressed markers for pluripotent stem cells, and were capable of differentiating into derivatives of all three embryonic germ layers in vivo and in vitro. These findings suggest that sphere formation may render somatic cells more susceptible to reprogramming.

Ninomiya, H., K. Mizuno, et al. "Improved efficiency of definitive endoderm induction from human induced pluripotent stem cells in feeder and serum-free culture system." In Vitro Cell Dev Biol Anim. 2014 Aug 15.

Improvement of methods to produce endoderm-derived cells from pluripotent stem cells is important to realize high-efficient induction of endodermal tissues such as pancreas and hepatocyte. Difficulties hampering such efforts include the low efficiency of definitive endoderm cell induction and establishing appropriate defined culture conditions to ensure a safe cell source for human transplantation. Based on previous studies, we revised the experimental condition of definitive endoderm induction in feeder- and serum-free culture. Our results suggested that CHIR99021 is more effective than Wnt3A ligand in feeder- and serum-free conditions. In addition, keeping cell density low during endoderm induction is important for the efficiency. On the other hand, we showed that overtreatment with CHIR99021 converted the cells into BRACHYURY-expressing posterior mesoderm cells rather than endoderm, indicating strict CHIR99021 treatment requirements for endoderm differentiation. Nevertheless, these results should enable better control in the production of definitive endoderm-derived cells.

Nishimura, A. L., C. Shum, et al. "Allele-specific knockdown of ALS-associated mutant TDP-43 in neural stem cells derived from induced pluripotent stem cells." PLoS One. 2014 Mar 20;9(3):e91269. doi: 10.1371/journal.pone.0091269. eCollection 2014.

TDP-43 is found in cytoplasmic inclusions in 95% of amyotrophic lateral sclerosis (ALS) and 60% of frontotemporal lobar degeneration (FTLD). Approximately 4% of familial ALS is caused by mutations in TDP-43. The majority of these mutations are found in the glycine-rich domain, including the variant M337V, which is one of the most common mutations in TDP-43. In order to investigate the use of allele-specific RNA interference (RNAi) as a potential therapeutic tool, we designed and screened a set of siRNAs that specifically target TDP-43(M337V) mutation. Two siRNA specifically silenced the M337V mutation in HEK293T cells transfected with GFP-TDP-43(wt) or GFP-TDP-43(M337V) or TDP-43 C-terminal fragments counterparts. C-terminal TDP-43 transfected cells show an increase of cytosolic inclusions, which are decreased after allele-specific siRNA in M337V cells. We then investigated the effects of one of these allele-specific siRNAs in induced pluripotent stem cells (iPSCs) derived from an ALS patient carrying the M337V mutation. These lines showed a two-fold increase in cytosolic TDP-43 compared to the control. Following transfection with the allele-specific siRNA, cytosolic TDP-43 was reduced by 30% compared to cells transfected with a scrambled siRNA. We conclude that RNA interference can be used to selectively target the TDP-43(M337V) allele in mammalian and patient cells, thus demonstrating the potential for using RNA interference as a therapeutic tool for ALS.

Noto, F. K., M. R. Determan, et al. "Aneuploidy is permissive for hepatocyte-like cell differentiation from human induced pluripotent stem cells." BMC Res Notes. 2014 Jul 8;7:437. doi: 10.1186/1756-0500-7-437.

BACKGROUND: The characterization of induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) routinely includes analyses of chromosomal integrity. The belief is that pluripotent stem cells best suited to the generation of differentiated derivatives should display a euploid karyotype; although, this does not appear to have been formally tested. While aneuploidy is commonly associated with cell transformation, several types of somatic cells, including hepatocytes, are frequently aneuploid and variation in chromosomal content does not contribute to a transformed phenotype. This insight has led to the proposal that dynamic changes in the chromosomal environment may be important to establish genetic diversity within the hepatocyte population and such diversity may facilitate an adaptive response by the liver to various insults. Such a positive contribution of aneuploidy to liver function raises the possibility that, in contrast to existing dogma, aneuploid iPSCs may be capable of generating hepatocyte-like cells that display hepatic activities. RESULTS: We examined whether a human iPSC line that had multiple chromosomal aberrations was competent to differentiate into hepatocytes and found that loss of normal chromosomal content had little impact on the production of hepatocyte-like cells from iPSCs. CONCLUSIONS: iPSCs that harbor an abnormal chromosomal content retain the capacity to generate hepatocyte-like cells with high efficiency.

Ochiai-Shino, H., H. Kato, et al. "A novel strategy for enrichment and isolation of osteoprogenitor cells from induced pluripotent stem cells based on surface marker combination." PLoS One. 2014 Jun 9;9(6):e99534. doi: 10.1371/journal.pone.0099534. eCollection 2014.

In this study, we developed a new method to stimulate osteogenic differentiation in tissue-nonspecific alkaline phosphatase (TNAP)-positive cells liberated from human induced pluripotent stem cells (hiPSCs)-derived embryoid bodies (EBs) with 14 days long TGF-beta/IGF-1/FGF-2 treatment. TNAP is a marker protein of osteolineage cells. We analyzed and isolated TNAP-positive and E-cadherin-negative nonepithelial cells by fluorescence-activated cell sorting. Treating the cells with a combination of transforming growth factor (TGF)-beta, insulin-like growth factor (IGF)-1, and fibroblast growth factor (FGF)-2 for 14 days greatly enhanced TNAP expression and maximized expression frequency up to 77.3%. The isolated cells expressed high levels of osterix, which is an exclusive osteogenic marker. Culturing these TNAP-positive cells in osteoblast differentiation medium (OBM) led to the expression of runt-related transcription factor 2, type I collagen, bone sialoprotein, and osteocalcin (OCN). These cells responded to treatment with activated vitamin D3 by upregulating OCN. Furthermore, in OBM they were capable of generating many mineralized nodules with strong expression of receptor activator of NF-kappaB ligand and sclerostin (SOST). Real-time RT-PCR showed a significant increase in the expression of osteocyte marker genes, including SOST, neuropeptide Y, and reelin. Scanning electron microscopy showed dendritic morphology. Examination of semi-thin toluidine blue-stained sections showed many interconnected dendrites. Thus, TNAP-positive cells cultured in OBM may eventually become terminally differentiated osteocyte-like cells. In conclusion, treating hiPSCs-derived cells with a combination of TGF-beta, IGF-1, and FGF-2 generated TNAP-positive cells at high frequency. These TNAP-positive cells had a high osteogenic potential and could terminally differentiate into osteocyte-like cells. The method described here may reveal new pathways of osteogenesis and provide a novel tool for regenerative medicine and drug development.

Okada, M., S. Ikegawa, et al. "Modeling type II collagenopathy skeletal dysplasia by directed conversion and induced pluripotent stem cells." Hum Mol Genet. 2014 Sep 3. pii: ddu444.

Type II collagen is a major component of cartilage. Heterozygous mutations in the type II collagen gene (COL2A1) result in a group of skeletal dysplasias known as Type II collagenopathy (COL2pathy). The understanding of COL2pathy is limited by difficulties in obtaining live chondrocytes. In the present study, we converted COL2pathy patients' fibroblasts directly into induced chondrogenic (iChon) cells. The COL2pathy-iChon cells showed suppressed expression of COL2A1 and significant apoptosis. A distended endoplasmic reticulum (ER) was detected, thus suggesting the adaptation of gene expression and cell death caused by excess ER stress. Chondrogenic supplementation adversely affected the chondrogenesis due to forced elevation of COL2A1 expression, suggesting that the application of chondrogenic drugs would worsen the disease condition. The application of a chemical chaperone increased the secretion of type II collagen, and partially rescued COL2pathy-iChon cells from apoptosis, suggesting that molecular chaperons serve as therapeutic drug candidates. We next generated induced pluripotent stem cells from COL2pathy fibroblasts. Chondrogenically-differentiated COL2pathy-iPS cells showed apoptosis and increased expression of ER stress-markers. Finally, we generated teratomas by transplanting COL2pathy iPS cells into immunodeficient mice. The cartilage in the teratomas showed accumulation of type II collagen within cells, a distended ER, and sparse matrix, recapitulating the patient's cartilage. These COL2pathy models will be useful for pathophysiological studies and drug screening.

Osborn, S. L., R. Thangappan, et al. "Induction of human embryonic and induced pluripotent stem cells into urothelium." Stem Cells Transl Med. 2014 May;3(5):610-9. doi: 10.5966/sctm.2013-0131. Epub 2014 Mar 21.

In vitro generation of human urothelium from stem cells would be a major advancement in the regenerative medicine field, providing alternate nonurologic and/or nonautologous tissue sources for bladder grafts. Such a model would also help decipher the mechanisms of urothelial differentiation and would facilitate investigation of deviated differentiation of normal progenitors into urothelial cancer stem cells, perhaps elucidating areas of intervention for improved treatments. Thus far, in vitro derivation of urothelium from human embryonic stem cells (hESCs) or human induced pluripotent stem (hiPS) cells has not been reported. The goal of this work was to develop an efficient in vitro protocol for the induction of hESCs into urothelium through an intermediary definitive endoderm step and free of matrices and cell contact. During directed differentiation in a urothelial-specific medium ("Uromedium"), hESCs produced up to 60% urothelium, as determined by uroplakin expression; subsequent propagation selected for 90% urothelium. Alteration of the epithelial and mesenchymal cell signaling contribution through noncell contact coculture or conditioned media did not enhance the production of urothelium. Temporospatial evaluation of transcription factors known to be involved in urothelial specification showed association of IRF1, GET1, and GATA4 with uroplakin expression. Additional hESC and hiPS cell lines could also be induced into urothelium using this in vitro system. These results demonstrate that derivation and propagation of urothelium from hESCs and hiPS cells can be efficiently accomplished in vitro in the absence of matrices, cell contact, or adult cell signaling and that the induction process appears to mimic normal differentiation.

Payne, N. L., A. Sylvain, et al. "Application of human induced pluripotent stem cells for modeling and treating neurodegenerative diseases." N Biotechnol. 2014 May 9. pii: S1871-6784(14)00051-X. doi: 10.1016/j.nbt.2014.05.001.

The advent of human induced pluripotent stem cells (hiPSCs), reprogrammed in vitro from both healthy and disease-state human somatic cells, has triggered an enormous global research effort to realize personalized regenerative medicine for numerous degenerative conditions. hiPSCs have been generated from cells of many tissue types and can be differentiated in vitro to most somatic lineages, not only for the establishment of disease models that can be utilized as novel drug screening platforms and to study the molecular and cellular processes leading to degeneration, but also for the in vivo cell-based repair or modulation of a patient's disease profile. hiPSCs derived from patients with the neurodegenerative diseases amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease and multiple sclerosis have been successfully differentiated in vitro into disease-relevant cell types, including motor neurons, dopaminergic neurons and oligodendrocytes. However, the generation of functional iPSC-derived neural cells that are

capable of engraftment in humans and the identification of robust disease phenotypes for modeling neurodegeneration still require several key challenges to be addressed. Here, we discuss these challenges and summarize recent progress toward the application of iPSC technology for these four common neurodegenerative diseases.

Petkov, S., P. Hyttel, et al. "The Small Molecule Inhibitors PD0325091 and CHIR99021 Reduce Expression of Pluripotency-Related Genes in Putative Porcine Induced Pluripotent Stem Cells." Cell Reprogram. 2014 Aug;16(4):235-40. doi: 10.1089/cell.2014.0010. Epub 2014 Jun 24.

Abstract Small molecule inhibitors of the mitogen-activated protein kinase kinase (MEK) and glycogen synthesis kinase 3 (Gsk3) have been essential in the establishment and maintenance of embryonic stem cells (ESCs) from rats and from nonpermissive mouse strains. However, conflicting results have been reported regarding their efficacy in the establishment and maintenance of pluripotent stem cells from other species. Here, we investigated the effects of PD0325091 (PD; a MEK inhibitor) and CHIR99021 (CH; a Gsk3beta inhibitor) on the reprogramming of porcine fetal fibroblasts to induced pluripotent stem cells (piPSCs). Primary cultures treated with the two inhibitors (2i) showed a reduced number of alkaline phosphatase-positive colonies and a lower percentage of OCT4-expressing cells compared with the cultures grown with basic medium, which was supplemented with murine leukemia inhibitory factor (LIF). Moreover, the piPS-like cell lines established under 2i conditions expressed significantly lower levels of pluripotency markers, including OCT4, SOX2, REX1, UTF1, STELLA, TDH, and CHD1, compared with the controls. To test the short-term effects of the small molecule inhibitors, piPS-like cells that had been established in basic culture medium were cultured for five passages in medium supplemented with 2i or PD or CH individually. In accordance with the first experiment, expression levels of most pluripotency genes declined in cultures treated with inhibitors, although the response to each inhibitory molecule varied for the different genes. Results of this study concur with previous reports and cast doubts on the effectiveness of CH and PD in the reprogramming of porcine somatic cells to pluripotency.

Petrova, A., A. Celli, et al. "3D In Vitro Model of a Functional Epidermal Permeability Barrier from Human Embryonic Stem Cells and Induced Pluripotent Stem Cells." Stem Cell Reports. 2014 Apr 24;2(5):675-89. doi: 10.1016/j.stemcr.2014.03.009. eCollection 2014 May 6.

Cornification and epidermal barrier defects are associated with a number of clinically diverse skin disorders. However, a suitable in vitro model for studying normal barrier function and barrier defects is still lacking. Here, we demonstrate the generation of human epidermal equivalents (HEEs) from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). HEEs are structurally similar to native epidermis, with a functional permeability barrier. We exposed a pure population of hESC/iPSC-derived keratinocytes, whose transcriptome corresponds to the gene signature of normal primary human keratinocytes (NHKs), to a sequential high-to-low humidity environment in an air/liquid interface culture. The resulting HEEs had all of the cellular strata of the human epidermis, with skin barrier properties similar to those of normal skin. Such HEEs generated from disease-specific iPSCs will be an invaluable tool not only for dissecting molecular mechanisms that lead to epidermal barrier defects but also for drug development and screening.

Phillips, M. D., S. A. Kuznetsov, et al. "Directed differentiation of human induced pluripotent stem cells toward bone and cartilage: in vitro versus in vivo assays." Stem Cells Transl Med. 2014 Jul;3(7):867-78. doi: 10.5966/sctm.2013-0154. Epub 2014 May 22.

The ability to differentiate induced pluripotent stem cells (iPSCs) into committed skeletal progenitors could allow for an unlimited autologous supply of such cells for therapeutic uses; therefore, we attempted to create novel bone-forming cells from human iPSCs using lines from two distinct tissue sources and methods of differentiation that we previously devised for osteogenic differentiation of human embryonic stem cells, and as suggested by other publications. The resulting cells were assayed using in vitro methods, and the results were compared with those obtained from in vivo transplantation assays. Our results show that true bone was formed in vivo by derivatives of several iPSC lines, but that the successful cell lines and differentiation methodologies were not predicted by the results of the in vitro assays. In addition, bone was formed equally well from iPSCs originating from skin or bone marrow stromal cells (also known as bone marrow-derived mesenchymal stem cells), suggesting that the iPSCs did not retain a "memory" of their previous life. Furthermore, one of the iPSC-derived cell lines formed verifiable cartilage in vivo, which likewise was not predicted by in vitro assays.

Piao, Y., S. S. Hung, et al. "Efficient generation of integration-free human induced pluripotent stem cells from keratinocytes by simple transfection of episomal vectors." Stem Cells Transl Med. 2014 Jul;3(7):787-91. doi: 10.5966/sctm.2013-0036. Epub 2014 Jun 5.

Keratinocytes represent an easily accessible cell source for derivation of human induced pluripotent stem (hiPS) cells, reportedly achieving higher reprogramming efficiency than fibroblasts. However, most studies utilized a retroviral or lentiviral method for reprogramming of keratinocytes, which introduces undesirable transgene integrations into the host genome. Moreover, current protocols of generating integration-free hiPS cells from keratinocytes are mostly inefficient. In this paper, we describe a more efficient, simple-to-use, and cost-effective method for generating integration-free hiPS cells from keratinocytes. Our improved method using lipid-mediated transfection achieved a reprogramming efficiency of approximately 0.14% on average. Keratinocyte-derived hiPS cells showed no integration of episomal vectors, expressed stem cell-specific markers and possessed potentials to differentiate into all three germ layers by in vitro embryoid body formation as well as in vivo teratoma formation. To our knowledge, this represents the most efficient method to generate integration-free hiPS cells from keratinocytes.

Pipino, C., S. Mukherjee, et al. "Trisomy 21 Mid-Trimester Amniotic Fluid Induced Pluripotent Stem Cells Maintain Genetic Signatures During Reprogramming: Implications for Disease Modeling and Cryobanking." Cell Reprogram. 2014 Aug 27.

Abstract Trisomy 21 is the most common chromosomal abnormality and is associated primarily with cardiovascular, hematological, and neurological complications. A robust patient-derived cellular model is necessary to investigate the pathophysiology of the syndrome because current animal models are limited and access to tissues from affected individuals is ethically challenging. We aimed to derive induced pluripotent stem cells (iPSCs) from trisomy 21 human mid-trimester amniotic fluid stem cells (AFSCs) and describe their hematopoietic and neurological characteristics. Human AFSCs collected from women undergoing prenatal diagnosis were selected for c-KIT+ and transduced with a Cre-lox-inducible polycistronic lentiviral vector encoding SOX2, OCT4, KLF-4, and c-MYC (50,000 cells at a multiplicity of infection (MOI) 1-5 for 72 h). The embryonic stem cell (ESC)-like properties of the AFSC-derived iPSCs were established in vitro by embryoid body formation and in vivo by teratoma formation in RAG2-/-, gamma-chain-/-, C2-/- immunodeficient mice. Reprogrammed cells retained their cytogenetic signatures and differentiated into specialized hematopoietic and neural precursors detected by morphological assessment, immunostaining, and RT-PCR. Additionally, the iPSCs expressed all pluripotency markers upon multiple rounds of freeze-thawing. These findings are important in establishing a patient-specific cellular platform of trisomy 21 to study the pathophysiology of the aneuploidy and for future drug discovery.

Polinati, P., T. Ilmarinen, et al. "Patient specific induced pluripotent stem cells derived RPE cells: understanding the pathogenesis of retinopathy in LCHAD deficiency." Invest Ophthalmol Vis Sci. 2014 Jun 3. pii: IOVS-14-14007. doi: 10.1167/iovs.14-14007.

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 aims 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 characterised 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. Conclusion: We demonstrate that 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.

Pre, D., M. W. Nestor, et al. "A Time Course Analysis of the Electrophysiological Properties of Neurons Differentiated from Human Induced Pluripotent Stem Cells (iPSCs)." PLoS One. 2014 Jul 29;9(7):e103418. doi: 10.1371/journal.pone.0103418. eCollection 2014.

Many protocols have been designed to differentiate human embryonic stem cells (ESCs) and human induced pluripotent stem cells (iPSCs) into neurons. Despite the relevance of electrophysiological properties for proper neuronal function, little is known about the evolution over time of important neuronal electrophysiological parameters in iPSC-derived neurons. Yet, understanding the development of basic electrophysiological characteristics of iPSC-derived neurons is critical for evaluating their usefulness in basic and translational research. Therefore, we analyzed the basic electrophysiological parameters of forebrain neurons differentiated from human iPSCs, from day 31 to day 55 after the initiation of neuronal differentiation. We assayed the developmental progression of various properties, including resting membrane potential, action potential, sodium and potassium channel currents, somatic calcium transients and synaptic activity. During the maturation of iPSC-derived neurons, the resting membrane potential became more negative, the expression of voltage-gated sodium channels increased, the membrane became capable of generating action potentials following adequate depolarization and, at day 48-55, 50% of the cells were capable of firing action potentials in response to a prolonged depolarizing current step, of which 30% produced multiple action potentials. The percentage of cells exhibiting miniature excitatory post-synaptic currents increased over time with a significant increase in their frequency and amplitude. These changes were associated with an increase of Ca2+ transient frequency. Co-culturing iPSC-derived neurons with mouse glial cells enhanced the development of electrophysiological parameters as compared to pure iPSC-derived neuronal cultures. This study demonstrates the importance of properly evaluating the electrophysiological status of the newly generated neurons when using stem cell technology, as electrophysiological properties of iPSC-derived neurons mature over time.

Purwanti, Y. I., C. Chen, et al. "Antitumor effects of CD40 ligand-expressing endothelial progenitor cells derived from human induced pluripotent stem cells in a metastatic breast cancer model." Stem Cells Transl Med. 2014 Aug;3(8):923-35. doi: 10.5966/sctm.2013-0140. Epub 2014 Jun 27.

Given their intrinsic ability to home to tumor sites, endothelial progenitor cells (EPCs) are attractive as cellular vehicles for targeted cancer gene therapy. However, collecting sufficient EPCs is one of the challenging issues critical for effective clinical translation of this new approach. In this study, we sought to explore whether human induced pluripotent stem (iPS) cells could be used as a reliable and accessible cell source to generate human EPCs suitable for cancer treatment. We used an embryoid body formation method to derive CD133(+)CD34(+) EPCs from human iPS cells. The generated EPCs expressed endothelial markers such as CD31, Flk1, and vascular endothelial-cadherin without expression of the CD45 hematopoietic marker. After intravenous injection, the iPS cell-derived EPCs migrated toward orthotopic and lung metastatic tumors in the mouse 4T1 breast cancer model but did not promote tumor growth and metastasis. To investigate their therapeutic potential, the EPCs were transduced with baculovirus encoding the potent T cell costimulatory molecule CD40 ligand. The systemic injection of the CD40 ligand-expressing EPCs stimulated the secretion of both tumor necrosis factor-alpha and interferon-gamma and increased the caspase 3/7 activity in the lungs with metastatic tumors, leading to prolonged survival of the tumor bearing mice. Therefore, our findings suggest that human iPS cell-derived EPCs have the potential to serve as tumor-targeted cellular vehicles for anticancer gene therapy.

Qi, S. D., P. D. Smith, et al. "Nuclear reprogramming and induced pluripotent stem cells: a review for surgeons." ANZ J Surg. 2014 Jun;84(6):417-23. doi: 10.1111/ans.12419.

Induced pluripotent stem cells (iPSCs) are generated from somatic cells by the exogenous expression of defined transcription factors. iPSCs share the defining features of embryonic stem cells (ESCs) in that they are able to self renew indefinitely and maintain the potential to develop into all cell types of the body. These cells have key advantages over ESCs in that they are autologous to the donor cells and can be generated from individuals at any age. iPSCs also circumvent ethical and political issues surrounding the destruction of embryos that is necessary in the isolation of ESCs. This review briefly describes the advent of iPSC technology and the concepts of nuclear reprogramming, and discusses the potential application of this powerful biological tool in both surgical research and regenerative medicine.

Qin, J., S. Sontag, et al. "Cell Fusion Enhances Mesendodermal Differentiation of Human Induced Pluripotent Stem Cells." Stem Cells Dev. 2014 Aug 11.

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.

Ross, C. A. and S. S. Akimov Human-induced pluripotent stem cells: potential for neurodegenerative diseases, Hum Mol Genet. 2014 Sep 15;23(R1):R17-R26. Epub 2014 May 13.

The cell biology of human neurodegenerative diseases has been difficult to study till recently. The development of human induced pluripotent stem cell (iPSC) models has greatly enhanced our ability to model disease in human cells. Methods have recently been improved, including increasing reprogramming efficiency, introducing non-viral and non-integrating methods of cell reprogramming, and using novel gene editing techniques for generating genetically corrected lines from patient-derived iPSCs, or for generating mutations in control cell lines. In this review, we highlight accomplishments made using iPSC models to study neurodegenerative disorders such as Huntington's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, Fronto-Temporal Dementia, Alzheimer's disease, Spinomuscular Atrophy and other polyglutamine diseases. We review disease-related phenotypes shown in patient-derived iPSCs differentiated to relevant neural subtypes, often with stressors or cell "aging", to enhance disease-specific phenotypes. We also discuss prospects for the future of using of iPSC models of neurodegenerative disorders, including screening and testing of therapeutic compounds, and possibly of cell transplantation in regenerative medicine. The new iPSC models have the potential to greatly enhance our understanding of pathogenesis and to facilitate the development of novel therapeutics.

Rouhani, F., N. Kumasaka, et al. "Genetic background drives transcriptional variation in human induced pluripotent stem cells." PLoS Genet. 2014 Jun 5;10(6):e1004432. doi: 10.1371/journal.pgen.1004432. eCollection 2014 Jun.

Human iPS cells have been generated using a diverse range of tissues from a variety of donors using different reprogramming vectors. However, these cell lines are heterogeneous, which presents a limitation for their use in disease modeling and personalized medicine. To explore the basis of this heterogeneity we generated 25 iPS cell lines under normalised conditions from the same set of somatic tissues across a number of donors. RNA-seq data sets from each cell line were compared to identify the majority contributors to transcriptional heterogeneity. We found that genetic differences between individual donors were the major cause of transcriptional variation between lines. In contrast, residual signatures from the somatic cell of origin, so called epigenetic memory, contributed relatively little to transcriptional variation. Thus, underlying genetic background variation is responsible for most heterogeneity between human iPS cell lines. We conclude that epigenetic effects in hIPSCs are minimal, and that hIPSCs are a stable, robust and powerful platform for large-scale studies of the function of genetic differences between individuals. Our data also suggest that future studies using hIPSCs as a model system should focus most effort on collection of large numbers of donors, rather than generating large numbers of lines from the same donor.

Sachamitr, P., S. Hackett, et al. "Induced pluripotent stem cells: challenges and opportunities for cancer immunotherapy." Front Immunol. 2014 Apr 17;5:176. doi: 10.3389/fimmu.2014.00176. eCollection 2014.

Despite recent advances in cancer treatment over the past 30 years, therapeutic options remain limited and do not always offer a cure for malignancy. Given that tumor-associated antigens (TAA) are, by definition, self-proteins, the need to productively engage autoreactive T cells remains at the heart of strategies for cancer immunotherapy. These have traditionally focused on the administration of autologous monocyte-derived dendritic cells (moDC) pulsed with TAA, or the ex vivo expansion and adoptive transfer of tumor-infiltrating lymphocytes (TIL) as a source of TAA-specific cytotoxic T cells (CTL). Although such approaches have shown some efficacy, success has been limited by the poor capacity of moDC to cross present exogenous TAA to the CD8(+) T-cell repertoire and the potential for exhaustion of CTL expanded ex vivo. Recent advances in induced pluripotency offer opportunities to generate patient-specific stem cell lines with the potential to differentiate in vitro into cell types whose properties may help address these issues. Here, we review recent success in the differentiation of NK cells from human induced pluripotent stem (iPS) cells as well as minor subsets of dendritic cells (DCs) with therapeutic potential, including CD141(+)XCR1(+) DC, capable of cross presenting TAA to naive CD8(+) T cells. Furthermore, we review recent progress in the use of TIL as the starting material for the derivation of iPSC lines, thereby capturing their antigen specificity in a self-

renewing stem cell line, from which potentially unlimited numbers of naive TAA-specific T cells may be differentiated, free of the risks of exhaustion.

Saitta, B., J. Passarini, et al. "Patient-derived skeletal dysplasia induced pluripotent stem cells display abnormal chondrogenic marker expression and regulation by BMP2 and TGFbeta1." Stem Cells Dev. 2014 Jul 1;23(13):1464-78. doi: 10.1089/scd.2014.0014. Epub 2014 Apr 1.

Skeletal dysplasias (SDs) are caused by abnormal chondrogenesis during cartilage growth plate differentiation. To study early stages of aberrant cartilage formation in vitro, we generated the first induced pluripotent stem cells (iPSCs) from fibroblasts of an SD patient with a lethal form of metatropic dysplasia, caused by a dominant mutation (I604M) in the calcium channel gene TRPV4. When micromasses were grown in chondrogenic differentiation conditions and compared with control iPSCs, mutant TRPV4-iPSCs showed significantly ($P<0.05$) decreased expression by quantitative real-time polymerase chain reaction of COL2A1 (IIA and IIB forms), SOX9, Aggrecan, COL10A1, and RUNX2, all of which are cartilage growth plate markers. We found that stimulation with BMP2, but not TGFbeta1, up-regulated COL2A1 (IIA and IIB) and SOX9 gene expression, only in control iPSCs. COL2A1 (Collagen II) expression data were confirmed at the protein level by western blot and immunofluorescence microscopy. TRPV4-iPSCs showed only focal areas of Alcian blue stain for proteoglycans, while in control iPSCs the stain was seen throughout the micromass sample. Similar staining patterns were found in neonatal cartilage from control and patient samples. We also found that COL1A1 (Collagen I), a marker of osteogenic differentiation, was significantly ($P<0.05$) up-regulated at the mRNA level in TRPV4-iPSCs when compared with the control, and confirmed at the protein level. Collagen I expression in the TRPV4 model also may correlate with abnormal staining patterns seen in patient tissues. This study demonstrates that an iPSC model can recapitulate normal chondrogenesis and that mutant TRPV4-iPSCs reflect molecular evidence of aberrant chondrogenic developmental processes, which could be used to design therapeutic approaches for disorders of cartilage.

Sakurai, M., H. Kunimoto, et al. "Impaired hematopoietic differentiation of RUNX1-mutated induced pluripotent stem cells derived from FPD/AML patients." Leukemia. 2014 Apr 15. doi: 10.1038/leu.2014.136.

Somatic mutation of RUNX1 is implicated in various hematological malignancies, including myelodysplastic syndrome and acute myeloid leukemia (AML), and previous studies using mouse models disclosed its critical roles in hematopoiesis. However, the role of RUNX1 in human hematopoiesis has never been tested in experimental settings. Familial platelet disorder (FPD)/AML is an autosomal dominant disorder caused by germline mutation of RUNX1, marked by thrombocytopenia and propensity to acute leukemia. To investigate the physiological function of RUNX1 in human hematopoiesis and pathophysiology of FPD/AML, we derived induced pluripotent stem cells (iPSCs) from three distinct FPD/AML pedigrees (FPD-iPSCs) and examined their defects in hematopoietic differentiation. By in vitro differentiation assays, FPD-iPSCs were clearly defective in the emergence of hematopoietic progenitors and differentiation of megakaryocytes, and overexpression of wild-type (WT)-RUNX1 reversed most of these phenotypes. We further demonstrated that overexpression of mutant-RUNX1 in WT-iPSCs did not recapitulate the phenotype of FPD-iPSCs, showing that the mutations were of loss-of-function type. Taken together, this study demonstrated that haploinsufficient RUNX1 allele imposed cell-intrinsic defects on hematopoietic differentiation in human experimental settings and revealed differential impacts of RUNX1 dosage on human and murine megakaryopoiesis. FPD-iPSCs will be a useful tool to investigate mutant RUNX1-mediated molecular processes in hematopoiesis and leukemogenesis.Leukemia advance online publication, 13 May 2014; doi:10.1038/leu.2014.136.

Sanchez-Freire, V., A. S. Lee, et al. "Effect of human donor cell source on differentiation and function of cardiac induced pluripotent stem cells." J Am Coll Cardiol. 2014 Aug 5;64(5):436-48. doi: 10.1016/j.jacc.2014.04.056.

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.

Sareen, D., G. Gowing, et al. "Human induced pluripotent stem cells are a novel source of neural progenitor cells (iNPCs) that migrate and integrate in the rodent spinal cord." J Comp Neurol. 2014 Aug 15;522(12):2707-28. doi: 10.1002/cne.23578. Epub 2014 Apr 12.

Transplantation of human neural progenitor cells (NPCs) into the brain or spinal cord to replace lost cells, modulate the injury environment, or create a permissive milieu to protect and regenerate host neurons is a promising therapeutic strategy for neurological diseases. Deriving NPCs from human fetal tissue is feasible, although problematic issues include limited sources and ethical concerns. Here we describe a new and abundant source of NPCs derived from human induced pluripotent stem cells (iPSCs). A novel chopping technique was used to transform adherent iPSCs into free-floating spheres that were easy to maintain and were expandable (EZ spheres) (Ebert et al. [2013] Stem Cell Res 10:417-427). These EZ spheres could be differentiated towards NPC spheres with a spinal cord phenotype using a combination of all-trans retinoic acid (RA) and epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF-2) mitogens. Suspension cultures of NPCs derived from human iPSCs or fetal tissue have similar characteristics, although they were not similar when grown as adherent cells. In addition, iPSC-derived NPCs (iNPCs) survived grafting into the spinal cord of athymic nude rats with no signs of overgrowth and with a very similar profile to human fetal-derived NPCs (fNPCs). These results suggest that human iNPCs behave like fNPCs and could thus be a valuable alternative for cellular regenerative therapies of neurological diseases.

Sareen, D., M. Saghizadeh, et al. "Differentiation of human limbal-derived induced pluripotent stem cells into limbal-like epithelium." Stem Cells Transl Med. 2014 Sep;3(9):1002-12. doi: 10.5966/sctm.2014-0076. Epub 2014 Jul 28.

Limbal epithelial stem cell (LESC) deficiency (LSCD) leads to corneal abnormalities resulting in compromised vision and blindness. LSCD can be potentially treated by transplantation of appropriate cells, which should be easily expandable and bankable. Induced pluripotent stem cells (iPSCs) are a promising source of transplantable LESCs. The purpose of this study was to generate human iPSCs and direct them to limbal differentiation by maintaining them on natural substrata mimicking the native LESC niche, including feederless denuded human amniotic membrane (HAM) and de-epithelialized corneas. These iPSCs were generated with nonintegrating vectors from human primary limbal epithelial cells. This choice of parent cells was supposed to enhance limbal cell differentiation from iPSCs by partial retention of parental epigenetic signatures in iPSCs. When the gene methylation patterns were compared in iPSCs to parental LESCs using Illumina global methylation arrays, limbal-derived iPSCs had fewer unique methylation changes than fibroblast-derived iPSCs, suggesting retention of epigenetic memory during reprogramming. Limbal iPSCs cultured for 2 weeks on HAM developed markedly higher expression of putative LESC markers ABCG2, DeltaNp63alpha, keratins 14, 15, and 17, N-cadherin, and TrkA than did fibroblast iPSCs. On HAM culture, the methylation profiles of select limbal iPSC genes (including NTRK1, coding for TrkA protein) became closer to the parental cells, but fibroblast iPSCs remained closer to parental fibroblasts. On denuded air-lifted corneas, limbal iPSCs even upregulated differentiated corneal keratins 3 and 12. These data emphasize the importance of the natural niche and limbal tissue of origin in generating iPSCs as a LESC source with translational potential for LSCD treatment.

Sargent, R. G., S. Suzuki, et al. "Nuclease-mediated double-strand break (DSB) enhancement of small fragment homologous recombination (SFHR) gene modification in human-induced pluripotent stem cells (hiPSCs)." Methods Mol Biol. 2014;1114:279-90. doi: 10.1007/978-1-62703-761-7_18.

Recent developments in methods to specifically modify genomic DNA using sequence-specific endonucleases and donor DNA have opened the door to a new therapeutic paradigm for cell and gene therapy of inherited diseases. Sequence-specific endonucleases, in particular transcription activator-like (TAL) effector nucleases (TALENs), have been coupled with polynucleotide small/short DNA fragments (SDFs) to correct the most common mutation in the cystic fibrosis (CF) transmembrane conductance

regulator (CFTR) gene, a 3-base-pair deletion at codon 508 (delF508), in induced pluripotent stem (iPS) cells. The studies presented here describe the generation of candidate TALENs and their co-transfection with wild-type (wt) CFTR-SDFs into CF-iPS cells homozygous for the delF508 mutation. Using an allele-specific PCR (AS-PCR)-based cyclic enrichment protocol, clonal populations of corrected CF-iPS cells were isolated and expanded.

Satoh, D., T. Maeda, et al. "Establishment and directed differentiation of induced pluripotent stem cells from glycogen storage disease type Ib patient." Genes Cells. 2013 Dec;18(12):1053-69. doi: 10.1111/gtc.12101. Epub 2013 Oct 28.

Glycogen storage disease type Ib (GSDIb) is caused by a deficiency in the glucose-6-phosphate transporter (G6PT), which leads to neutrophil dysfunction. However, the underlying causes of these dysfunctions and their relationship with glucose homeostasis are unclear. Induced pluripotent stem cells (iPSCs) hold a great promise for advances in developmental biology, cell-based therapy and modeling of human disease. Here, we examined the use of iPSCs as a model for GSDIb. In this study, one 2-year-old patient was genetically screened and diagnosed with GSDIb. We established iPSCs and differentiated these cells into hepatocytes and neutrophils, which comprise the main pathological components of GSDIb. Cells that differentiated into hepatocytes exhibited characteristic albumin secretion and indocyanine green uptake. Moreover, iPSC-derived cells generated from patients with GSDIb metabolic abnormalities recapitulated key pathological features of the diseases affecting the patients from whom they were derived, such as glycogen, lactate, pyruvate and lipid accumulation. Cells that were differentiated into neutrophils also showed the GSDIb pathology. In addition to the expression of neutrophil markers, we showed increased superoxide anion production, increased annexin V binding and activation of caspase-3 and caspase-9, consistent with the GSDIb patient's neutrophils. These results indicate valuable tools for the analysis of this pathology and the development of future treatments.

Satoh, T., S. Sugiura, et al. "Inverting microwell array chip for the cultivation of human induced pluripotent stem cells with controlled aggregate size and geometrical arrangement." Biomicrofluidics. 2014 Apr 16;8(2):024112. doi: 10.1063/1.4871036. eCollection 2014 Mar.

We present a novel cell culture chip, namely, "inverting microwell array chip," for cultivation of human induced pluripotent stem cells. The chip comprises a lower hydrogel microwell array and an upper polystyrene culture surface. We demonstrate the formation of uniform cellular aggregates in the microwell array, and after inversion, a culture with controlled aggregate size and geometrical arrangement on the polystyrene surface. Here, we report effects of cell concentrations on a cultivation sequence in the chip.

Savla, J. J., B. C. Nelson, et al. Induced Pluripotent Stem Cells for the Study of Cardiovascular Disease, J Am Coll Cardiol. 2014 Aug 5;64(5):512-519. doi: 10.1016/j.jacc.2014.05.038.

Groundbreaking advances in stem cell research have led to techniques for the creation of human cardiomyocytes from cells procured from a variety of sources, including a simple skin biopsy. Since the advent of this technology, most research has focused on utilizing these cells for therapeutic purposes. However, recent studies have demonstrated that stem cell-derived cardiomyocytes generated from patients with inherited cardiovascular disorders recapitulate key phenotypic features of disease in vitro. Furthermore, these cells can be maintained in culture for prolonged periods of time and used for extensive biochemical and physiological analysis. By serving as models of inherited cardiac disorders, these systems have the potential to fundamentally change the manner in which cardiovascular disease is studied and new therapies are developed.

Sawai, T. "The moral value of induced pluripotent stem cells: a Japanese bioethics perspective on human embryo research." J Med Ethics. 2014 Jun 20. pii: medethics-2013-101838. doi: 10.1136/medethics-2013-101838.

In contemporary Japan, at least in the field of regenerative medicine, human induced pluripotent stem cells (hiPSCs) are given no moral status and are treated in a purely instrumental way. However, some authors have mentioned the potentiality of hiPSCs in that 'tetraploid complementation' would make it possible to create humans directly from human embryonic stem cells (hESCs) and hiPSCs. A blastocyst consists of inner cell mass (ICM) cells and a trophoblast. The tetraploid complementation technique demonstrates that hESCs and hiPSCs both have the same capacity as ICM cells. If ICM cells, hESCs and hiPSCs were all provided with a trophoblast or a substitute with the same function, which would work as a placenta, they would have the same potential to develop into embryos, fetuses and adult human beings. Thus hiPSCs could be regarded as potential humans. However, no authority or guideline in Japan has specifically considered the status and use of hiPSCs. In this paper, I will address the extent to which the existing recommendations apply to hiPSCs and develop a novel Japanese bioethical perspective on the status of hiPSCs and its implications for hiPSC research, based on the reasoning in the report, 'The fundamental way of thinking in treating the human embryo' presented by the Bioethics Committee of the Council for Science and Technology Policy in 2004, and broader consideration of Japanese culture.

Schnabel, L. V., C. M. Abratte, et al. "Induced pluripotent stem cells have similar immunogenic and more potent immunomodulatory properties compared with bone marrow-derived stromal cells in vitro." Regen Med. 2014 Apr 28.

Aim: To evaluate the in vitro immunogenic and immunomodulatory properties of induced pluripotent stem cells (iPSCs) compared with bone marrow-derived mesenchymal stromal cells (MSCs). Materials & Methods: Mouse embryonic fibroblasts (MEFs) were isolated from C3HeB/FeJ and C57BL/6J mice, and reprogrammed to generate iPSCs. Mixed leukocyte reactions were performed using MHC-matched and -mismatched responder leukocytes and stimulator leukocytes, iPSCs or MSCs. To assess immunogenic potential, iPSCs and MSCs were used as stimulator cells for responder leukocytes. To assess immunomodulatory properties, IPSCs and MSCs were cultured in the presence of stimulator and responder leukocytes. MEFs were used as a control. Results: iPSCs had similar immunogenic properties but more potent immunomodulatory effects than MSCs. Co-culture of MHC-mismatched leukocytes with MHC-matched iPSCs resulted in significantly less responder T-cell proliferation than observed for MHC-mismatched leukocytes alone and at more responder leukocyte concentrations than with MSCs. In addition, MHC-mismatched iPSCs significantly reduced responder T-cell proliferation when co-cultured with MHC-mismatched leukocytes, while MHC-mismatched MSCs did not. Conclusion: These results provide important information when considering the use of iPSCs in place of MSCs in both regenerative and transplantation medicine.

Sekine, K., T. Takebe, et al. "Fluorescent labeling and visualization of human induced pluripotent stem cells with the use of transcription activator-like effector nucleases." Transplant Proc. 2014 May;46(4):1205-7. doi: 10.1016/j.transproceed.2014.02.003.

BACKGROUND: The visualization of induced pluripotent stem (iPS) cells with the use of fluorescent techniques is useful for the in vivo evaluation of iPS-derived functional cells following differentiation and distribution of the transplanted cells. The data obtained from the fluorescently labeled iPS cells would lead to amelioration and validation of protocols directing the differentiation of iPS cells into functional cells. In this study, we established enhanced green fluorescent protein (EGFP)-labeled iPS cells to enable their easy visualization. METHODS: Human iPS cells were transfected with (a) 2 transcription activator-like effector nuclease (TALEN) vectors targeted to the adeno-associated virus integration site 1 (AAVS1) locus and (b) the targeting vector carrying the homology arms, EGFP gene, and a drug-selection marker. RESULTS: Several puromycin-resistant clones were obtained after transfection of the targeting vector and corresponding TALEN-expressing vectors. EGFP expression in these clones was observed with the use of a fluorescent microscope. Clones were examined for specific recombination, which revealed precise targeting at the AAVS1 locus. Furthermore, EGFP protein expression was sustained after directed differentiation into a hepatic lineage. CONCLUSIONS: We were successful in evaluating the behavior of iPS-derived hepatic cells. The data suggest that genomic knock-in at the AAVS1 locus is suitable for long-term observation of iPS-derived cells. Manipulation of the iPS genome can also be applied for the purification of hepatic cells during iPS cell differentiation by introducing the fluorescent protein under the regulation of a hepatic cell-specific promoter. Another application involves gene correction of iPS cells from patients with hepatic disease for regenerative medicine.

Shaer, A., N. Azarpira, et al. "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. 2014 Sep;174(1):242-58. doi: 10.1007/s12010-014-1045-5. Epub 2014 Jul 25.

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.

Shimamoto, R., N. Amano, et al. "Generation and characterization of induced pluripotent stem cells from Aid-deficient mice." PLoS One. 2014 Apr 9;9(4):e94735. doi: 10.1371/journal.pone.0094735. eCollection 2014.

It has been shown that DNA demethylation plays a pivotal role in the generation of induced pluripotent stem (iPS) cells. However, the underlying mechanism of this action is still unclear. Previous reports indicated that activation-induced cytidine deaminase (Aid, also known as Aicda) is involved in DNA demethylation in several developmental processes, as well as cell fusion-mediated reprogramming. Based on these reports, we hypothesized that Aid may be involved in the DNA demethylation that occurs during the generation of iPS cells. In this study, we examined the function of Aid in iPS cell generation using Aid knockout (Aid(-)/(-)) mice expressing a GFP reporter under the control of a pluripotent stem cell marker, Nanog. By introducing Oct3/4, Sox2, Klf4 and c-Myc, Nanog-GFP-positive iPS cells could be generated from the fibroblasts and primary B cells of Aid(-)/(-) mice. Their induction efficiency was similar to that of wild-type (Aid(+)/(+)) iPS cells. The Aid(-)/(-) iPS cells showed normal proliferation and gave rise to chimeras, indicating their capacity for self-renewal and pluripotency. A comprehensive DNA methylation analysis showed only a few differences between Aid(+)/(+) and Aid(-)/(-) iPS cells. These data suggest that Aid does not have crucial functions in DNA demethylation during iPS cell generation.

Shu, T., M. Pang, et al. "Effects of Salvia miltiorrhiza on neural differentiation of induced pluripotent stem cells." J Ethnopharmacol. 2014 Apr 11;153(1):233-41. doi: 10.1016/j.jep.2014.02.028. Epub 2014 Feb 22.

ETHNOPHARMACOLOGICAL RELEVANCE: Salvia miltiorrhiza, a well-known traditional Chinese medicine, is commonly used to treat some neurological diseases because of its anti-oxidative, anti-inflammatory and anti-apoptotic properties. We investigate whether Salvia miltiorrhiza can improve the differentiation of induced pluripotent stem cells (iPSCs) into neurons in vitro, and promote iPSCs-derived neural stem cells survival, integrate, and differentiation after their transplantation to the ischemic brain tissues. MATERIALS AND METHODS: Induced pluripotent stem cells were used to differentiate into neural stem cells, and further into neurons in induction medium with various concentrations of Salvia miltiorrhiza. The effects were assessed by immunofluorescence staining, quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and Western blotting. iPSC-derived neural stem cells were transplanted into the brains of rats with middle cerebral artery occlusion, immunofluorescence staining was used to evaluate survival, integrate, and differentiation of grafted cells, the functional recovery of the animals was tested by the Longa scores and spontaneous motor activity. RESULTS: Salvia miltiorrhiza (5mug/ml) significantly increased the gene and protein expression of Nestin compared with that in other groups. Microtubule-associated protein 2 (MAP2) expression in induction medium with 5mug/ml Salvia miltiorrhiza was significantly higher than that in the control group. After cells transplantation into the ischemic brain, more grafted MAP2(+) cells were found in Salvia miltiorrhiza-treated rats than others at 7 days. Salvia miltiorrhiza-treated rats showed the most remarkable functional recovery at 7 and 14 days. CONCLUSION: Salvia miltiorrhiza induces differentiation of induced pluripotent stem cells to differentiate into neurons efficiently. The plant provides neuroprotection to implanted cells and improves functional recovery after their transplantation to the ischemic brain tissues.

Song, R. S., J. M. Carroll, et al. "Generation, Expansion, and Differentiation of Human Induced Pluripotent Stem Cells (hiPSCs) Derived From the Umbilical Cords of Newborns." Curr Protoc Stem Cell Biol. 2014 May 16;29:1C.16.1-1C.16.13. doi: 10.1002/9780470151808.sc01c16s29.

The umbilical cord is tissue that is normally discarded after the delivery of the infant, but it has been shown to be a rich source of stem cells from the cord blood, Wharton's jelly, and umbilical endothelial cells. Patient-specific human induced pluripotent stem cells (hiPSCs) reprogrammed from patient specific human umbilical vein endothelial cells in the neonatal intensive care unit (NICU) population (specifically, premature neonates) have not been shown in the literature. This unit describes a protocol for the generation and expansion of hiPSCs originating from umbilical cords collected from patients in the NICU. Curr. Protoc. Stem Cell Biol. 29:1C.16.1-1C.16.13. (c) 2014 by John Wiley & Sons, Inc.

Song, Z., Q. Ji, et al. "Generation of CD44 gene-deficient mouse derived induced pluripotent stem cells : CD44 gene-deficient iPSCs." In Vitro Cell Dev Biol Anim. 2014 Jun 21.

Induced pluripotent stem cells (iPSCs) show good promise for the treatment of defects caused by numerous genetic diseases. Herein, we successfully generated CD44 gene-deficient iPSCs using Oct4, Sox2, Klf4, and vitamin C. The generated iPSCs displayed a characteristic morphology similar to the well-characterized embryonic stem cells. Alkaline phosphatase, cell surface (SSEA1, NANOG, and OCT4), and pluripotency markers were expressed at high levels in these cells. The iPSCs formed teratomas in vivo and supported full-term development of constructed porcine embryos by inter-species nuclear transplantation. Importantly, incubation with trichostatin A increased the efficiency of iPSCs generation by increasing the histone acetylation levels. Moreover, more iPSCs colonies appeared following cell passaging during colony picking, thus increasing the effectiveness of iPSCs selection. Thus, our work provides essential stem cell materials for the treatment of genetic diseases and proposes a novel strategy to enhance the efficiency of induced reprogramming.

Srikanth, P. and T. L. Young-Pearse "Stem cells on the brain: modeling neurodevelopmental and neurodegenerative diseases using human induced pluripotent stem cells." J Neurogenet. 2014 Mar-Jun;28(1-2):5-29. doi: 10.3109/01677063.2014.881358. Epub 2014 Mar 17.

Seven years have passed since the initial report of the generation of induced pluripotent stem cells (iPSCs) from adult human somatic cells, and in the intervening time the field of neuroscience has developed numerous disease models using this technology. Here, we review progress in the field and describe both the advantages and potential pitfalls of modeling neurodegenerative and neurodevelopmental diseases using this technology. We include tables with information on neural differentiation protocols and studies that developed human iPSC lines to model neurological diseases. We also discuss how one can: investigate effects of genetic mutations with iPSCs, examine cell fate-specific phenotypes, best determine the specificity of a phenotype, and bring in vivo relevance to this in vitro technique.

Stanslowsky, N., A. Haase, et al. "Functional differentiation of midbrain neurons from human cord blood-derived induced pluripotent stem cells." Stem Cell Res Ther. 2014 Mar 17;5(2):35. doi: 10.1186/scrt423.

INTRODUCTION: Human induced pluripotent stem cells (hiPSCs) offer great promise for regenerative therapies or in vitro modelling of neurodegenerative disorders like Parkinson's disease. Currently, widely used cell sources for the generation of hiPSCs are somatic cells obtained from aged individuals. However, a critical issue concerning the potential clinical use of these iPSCs is mutations that accumulate over lifetime and are transferred onto iPSCs during reprogramming which may influence the functionality of cells differentiated from them. The aim of our study was to establish a differentiation strategy to efficiently generate neurons including dopaminergic cells from human cord blood-derived iPSCs (hCBiPSCs) as a juvenescent cell source and prove their functional maturation in vitro. METHODS: The differentiation of hCBiPSCs was initiated by inhibition of transforming growth factor-beta and bone morphogenetic protein signaling using the small molecules dorsomorphin and SB 431542 before final maturation was carried out. hCBiPSCs and differentiated neurons were characterized by immunocytochemistry and quantitative real time-polymerase chain reaction. Since functional investigations of hCBiPSC-derived neurons are indispensable prior to clinical applications, we performed detailed analysis of essential ion channel properties using whole-cell patch-clamp recordings and calcium imaging. RESULTS: A Sox1 and Pax6 positive neuronal progenitor cell population was efficiently induced from hCBiPSCs using a newly established differentiation protocol. Neuronal progenitor cells could be further maturated into dopaminergic neurons expressing tyrosine hydroxylase, the dopamine transporter and engrailed 1. Differentiated hCBiPSCs exhibited voltage-gated ion currents, were able to fire action potentials and displayed synaptic activity indicating synapse formation. Application of the neurotransmitters GABA, glutamate and acetylcholine induced depolarizing calcium signal changes in neuronal cells providing evidence for the excitatory effects of these ligand-gated ion channels during maturation in vitro. CONCLUSIONS: This study demonstrates for the first time that hCBiPSCs can be used as a juvenescent cell source to generate a large number of functional neurons including dopaminergic cells which may serve for the development of novel regenerative treatment strategies.

Takenaka-Ninagawa, N., Y. Kawabata, et al. "Generation of rat-induced pluripotent stem cells from a new model of metabolic syndrome." PLoS One. 2014 Aug 11;9(8):e104462. doi: 10.1371/journal.pone.0104462. eCollection 2014.

We recently characterized DahlS.Z-Leprfa/Leprfa (DS/obese) rats, derived from a cross between Dahl salt-sensitive rats and Zucker rats, as a new animal model of metabolic syndrome (MetS). Although the phenotype of DS/obese rats is similar to that of humans with MetS, the pathophysiological and metabolic characteristics in each cell type remain to be clarified. Hence, the establishment of induced pluripotent stem cells (iPSCs) derived from MetS rats is essential for investigations of MetS in vitro. Reports of rat iPSCs (riPSCs), however, are few because of the difficulty of comparing to other rodents such as mouse. Recently, the advantage of using mesenchymal stromal cells (MSCs) as a cell source for generating iPSCs was described. We aimed to establish riPSCs from MSCs in adipose tissues of both DS/obese rats and their lean littermates, DahlS.Z-Lepr+/Lepr+ (DS/lean) rats using lentivirus vectors with only three factors Oct4, Klf4, and Sox2 without c-Myc. The morphology, gene expression profiles, and protein expression of established colonies showed embryonic stem cell (ESCs)-like properties, and the differentiation potential into cells from all three germ layers both in vitro and in vivo (teratomas). Both riPSCs became adipocytes after induction of adipogenesis by insulin, T3, and dexamethasone. Real-time PCR analysis also revealed that both riPSCs and the adipose tissue from DS/obese and DS/lean rats possess similar expression patterns of adipocyte differentiation-related genes. We succeeded in generating riPSCs effectively from MSCs of both DS/obese and DS/lean rats. These riPSCs may well serve as highly effective tools for the investigation of MetS pathophysiology in vitro.

Talluri, T. R., D. Kumar, et al. "Non-viral reprogramming of fibroblasts into induced pluripotent stem cells by Sleeping Beauty and piggyBac transposons." Biochem Biophys Res Commun. 2014 Jul 18;450(1):581-7. doi: 10.1016/j.bbrc.2014.06.014. Epub 2014 Jun 10.

The generation of induced pluripotent stem (iPS) cells represents a promising approach for innovative cell therapies. The original method requires viral transduction of several reprogramming factors, which may be associated with an increased risk of tumorigenicity. Transposition of reprogramming cassettes represents a recent alternative to viral approaches. Since binary transposons can be produced as common plasmids they provide a safe and cost-efficient alternative to viral delivery methods. Here, we compared the efficiency of two different transposon systems, Sleeping Beauty (SB) and piggyBac (PB), for the generation of murine iPS. Murine fibroblasts derived from an inbred BL/6 mouse line carrying a pluripotency reporter, Oct4-EGFP, and fibroblasts derived from outbred NMRI mice were employed for reprogramming. Both transposon systems resulted in the successful isolation of murine iPS cell lines. The reduction of the core reprogramming factors to omit the proto-oncogene c-Myc was compatible with iPS cell line derivation, albeit with reduced reprogramming efficiencies. The transposon-derived iPS cells featured typical hallmarks of pluripotency, including teratoma growth in immunodeficient mice. Thus SB and PB transposons represent a promising non-viral approach for iPS cell derivation.

Tamaki, S. and Y. Tokumoto "Overexpression of cyclin dependent kinase inhibitor P27/Kip1 increases oligodendrocyte differentiation from induced pluripotent stem cells." In Vitro Cell Dev Biol Anim. 2014 Apr 25.

Cell transplantation therapy with oligodendrocyte precursor cells (OPCs) is a promising and effective treatment for diseases involving demyelination in the central nervous system (CNS). In previous studies, we succeeded in producing O4+ oligodendrocytes (OLs) from mouse- and human-induced pluripotent stem cells (iPSCs) in vitro; however, the efficiency of differentiation into OLs was lower for iPSCs than that for embryonic stem cells (ESCs). To clarify the cause of this difference, we compared the expression of proteins that contribute to OL differentiation in mouse iPSC-derived cells and in mouse ESC-derived cells. The results showed that the expression levels of cyclin dependent kinase inhibitor P27/Kip1, mitogen-activated protein kinase (MAPK) JNK3, and transcription factor Mash1 were lower in iPSC-derived cells. In contrast, the expression levels of MAPK P38alpha, P38gamma, and thyroid hormone receptor beta1 were higher in iPSC-derived cells. We attempted to compensate for the expression changes in P27/Kip1 protein and Mash1 protein in iPSC-derived cells through retrovirus vector-mediated gene expression. Although the overexpression of Mash1 had no effect, the overexpression of P27/Kip1 increased the differentiation efficiency of iPSC-derived cells into O4+ OLs.

Tan, G., L. Cheng, et al. "Foxm1 mediates LIF/Stat3-dependent self-renewal in mouse embryonic stem cells and is essential for the generation of induced pluripotent stem cells." PLoS One. 2014 Apr 17;9(4):e92304. doi: 10.1371/journal.pone.0092304. eCollection 2014.

Activation of signal transducer and activator of transcription 3 (Stat3) by leukemia inhibitory factor (LIF) is required for maintaining self-renewal and pluripotency of mouse embryonic stem cells (mESCs). Here, we have confirmed transcription factor Forkhead Box m1 (Foxm1) as a LIF/Stat3 downstream target that mediates LIF/Stat3-dependent mESC self-renewal. The expression of Foxm1 relies on LIF signaling and is stimulated by Stat3 directly in mESCs. The knockdown of Foxm1 results in the loss of mESC pluripotency in the presence of LIF, and the overexpression of Foxm1 alone maintains mESC pluripotency in the absence of LIF and feeder layers, indicating that Foxm1 is a mediator of LIF/Stat3-dependent maintenance of pluripotency in mESCs. Furthermore, the inhibition of Foxm1 expression prevents the reprogramming of mouse embryonic fibroblasts to induced pluripotent stem cells (iPSCs), suggesting that Foxm1 is essential for the reprogramming of somatic cells into iPSCs. Our results reveal an essential function of Foxm1 in the LIF/Stat3-mediated mESC self-renewal and the generation of iPSCs.

Tan, H. K., C. X. Toh, et al. "Human finger-prick induced pluripotent stem cells facilitate the development of stem cell banking." Stem Cells Transl Med. 2014 May;3(5):586-98. doi: 10.5966/sctm.2013-0195. Epub 2014 Mar 19.

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.

Tani, H., Y. Onuma, et al. "Long Non-Coding RNAs as Surrogate Indicators for Chemical Stress Responses in Human-Induced Pluripotent Stem Cells." PLoS One. 2014 Aug 29;9(8):e106282. doi: 10.1371/journal.pone.0106282. eCollection 2014.

In this study, we focused on two biological products as ideal tools for toxicological assessment: long non-coding RNAs (lncRNAs) and human-induced pluripotent stem cells (hiPSCs). lncRNAs are an important class of pervasive non-protein-coding transcripts involved in the molecular mechanisms associated with responses to cellular stresses. hiPSCs possess the capabilities of self-renewal and differentiation into multiple cell types, and they are free of the ethical issues associated with human embryonic stem cells. Here, we identified six novel lncRNAs (CDKN2B-AS1, MIR22HG, GABPB1-AS1, FLJ33630, LINC00152, and LINC0541471_v2) that respond to model chemical stresses (cycloheximide, hydrogen peroxide, cadmium, or arsenic) in hiPSCs. Our results indicated that the lncRNAs responded to general and specific chemical stresses. Compared with typical mRNAs such as p53-related mRNAs, the lncRNAs highly and rapidly responded to chemical stresses. We propose that these lncRNAs have the potential to be surrogate indicators of chemical stress responses in hiPSCs.

Tatarishvili, J., K. Oki, et al. "Human induced pluripotent stem cells improve recovery in stroke-injured aged rats." Restor Neurol Neurosci. 2014 Jan 1;32(4):547-58. doi: 10.3233/RNN-140404.

PURPOSE: Induced pluripotent stem cells (iPSCs) improve behavior and form neurons after implantation into the stroke-injured adult rodent brain. How the aged brain responds to grafted iPSCs is unknown. We determined survival and differentiation of grafted human fibroblast-derived iPSCs and their ability to improve recovery in aged rats after stroke. METHODS: Twenty-four months old rats were subjected to 30 min distal middle cerebral artery occlusion causing neocortical damage. After 48 h, animals were transplanted intracortically with human iPSC-derived long-term neuroepithelial-like stem (hiPSC-lt-NES) cells. Controls were subjected to stroke and were vehicle-injected. RESULTS: Cell-grafted animals performed better than vehicle-injected recipients in cylinder test at 4 and 7 weeks. At 8 weeks, cell proliferation was low (0.7 %) and number of hiPSC-lt-NES cells corresponded to 49.2% of that of implanted cells. Transplanted cells expressed markers of neuroblasts and mature and GABAergic neurons. Cell-grafted rats exhibited less activated microglia/macrophages in injured cortex and neuronal loss was mitigated. CONCLUSIONS: Our study provides the first evidence that grafted human iPSCs survive, differentiate to neurons and ameliorate functional deficits in stroke-injured aged brain.

Tomizawa, M., F. Shinozaki, et al. "Dual gene expression in embryoid bodies derived from human induced pluripotent stem cells using episomal vectors." Tissue Eng Part A. 2014 Jul 1.

Transcription factors are essential for the differentiation of human induced pluripotent stem cells (iPS) into specialized cell types. Embryoid body (EB) formation promotes the differentiation of iPS cells. We sought to establish an efficient method of transfection and rotary culture to generate EBs that stably express two genes. The pMetLuc2-Reporter vector was transfected using FuGENE HD (FuGENE), Lipofectamine LTX (LTX), X-tremeGENE, or TransIT-2020 transfection reagents. The media were analyzed using a Metridia luciferase (MetLuc) assay. Transfections were

performed on cells attached to plates/dishes (attached method) or suspended in media (suspension method). The 201B7 cells transfected with episomal vectors were selected using G418 (200 mug/mL) or hygromycin B (300 mug/mL). Rotary culture was performed at 2.5 or 9.9 rpm. Efficiency of EB formation was compared among plates and dishes. Cell density was compared at 1.6 x 103, x 104, and x 105 cells/mL. The suspended method of transfection using the FuGENE HD reagent was the most efficient. The expression of pEBMulti/Met-Hyg was detected 11 d post-transfection. Double transformants were selected 6 d post-transfection with pEBNK/EGFP-Neo and pEBNK/Cherry-Hyg. Both EGFP and CherryPicker were expressed in all of the surviving cells. EBs were formed most efficiently from cells cultured at a density of 1.6 x 105 cells/mL in 6-well plates or 6 cm dishes. The selected cells formed EBs. FuGENE-mediated transfection of plasmids using the suspension method was effective in transforming iPS cells. Furthermore, the episomal vectors enabled us to perform a stable double transfection of EB-forming iPS cells.

Tsukiyama, T., M. Kato-Itoh, et al. "A comprehensive system for generation and evaluation of induced pluripotent stem cells using piggyBac transposition." PLoS One. 2014 Mar 25;9(3):e92973. doi: 10.1371/journal.pone.0092973. eCollection 2014.

The most stringent criterion for evaluating pluripotency is generation of chimeric animals with germline transmission ability. Because the quality of induced pluripotent stem cell (iPSC) lines is heterogeneous, an easy and accurate system to evaluate these abilities would be useful. In this study, we describe a simple but comprehensive system for generating and evaluating iPSCs by single transfection of multiple piggyBac (PB) plasmid vectors encoding Tet-inducible polycistronic reprogramming factors, a pluripotent-cell-specific reporter, a constitutively active reporter, and a sperm-specific reporter. Using this system, we reprogrammed 129 and NOD mouse embryonic fibroblasts into iPSCs, and then evaluated the molecular and functional properties of the resultant iPSCs by quantitative RT-PCR analysis and chimera formation assays. The iPSCs contributed extensively to chimeras, as indicated by the constitutively active TagRFP reporter, and also differentiated into sperm, as indicated by the late-spermatogenesis-specific Acr (acrosin)-EGFP reporter. Next, we established secondary MEFs from E13.5 chimeric embryos and efficiently generated secondary iPSCs by simple addition of doxycycline. Finally, we applied this system to establishment and evaluation of rat iPSCs and production of rat sperm in mouse-rat interspecific chimeras. By monitoring the fluorescence of Acr-EGFP reporter, we could easily detect seminiferous tubules containing rat iPSC-derived spermatids and sperm. And, we succeeded to obtain viable offspring by intracytoplasmic sperm injection (ICSI) using these haploid male germ cells. We propose that this system will enable robust strategies for induction and evaluation of iPSCs, not only in rodents but also in other mammals. Such strategies will be especially valuable in non-rodent species, in which verification of germline transmission by mating is inefficient and time-consuming.

Varga, E., C. Nemes, et al. "Generation of transgene-free mouse induced pluripotent stem cells using an excisable lentiviral system." Exp Cell Res. 2014 Apr 1;322(2):335-44. doi: 10.1016/j.yexcr.2014.02.006. Epub 2014 Feb 18.

One goal of research using induced pluripotent stem cell (iPSC) is to generate patient-specific cells which can be used to obtain multiple types of differentiated cells as disease models. Minimally or non-integrating methods to deliver the reprogramming genes are considered to be the best but they may be inefficient. Lentiviral delivery is currently among the most efficient methods but it integrates transgenes into the genome, which may affect the behavior of the iPSC if integration occurs into an important locus. Here we designed a polycistronic lentiviral construct containing four pluripotency genes with an EGFP selection marker. The cassette was excisable with the Cre-loxP system making possible the removal of the integrated transgenes from the genome. Mouse embryonic fibroblasts were reprogrammed using this viral system, rapidly resulting in large number of iPSC colonies. Based on the lowest EGFP expression level, one parental line was chosen for excision. Introduction of the Cre recombinase resulted in transgene-free iPSC subclones. The effect of the transgenes was assessed by comparing the parental iPSC with two of its transgene-free subclones. Both excised and non-excised iPSCs expressed standard pluripotency markers. The subclones obtained after Cre recombination were capable of differentiation in vitro, in contrast to the parental, non-excised cells and formed germ-line competent chimeras in vivo.

Wahlin, K. J., J. Maruotti, et al. "Modeling retinal dystrophies using patient-derived induced pluripotent stem cells." Adv Exp Med Biol. 2014;801:157-64. doi: 10.1007/978-1-4614-3209-8_20.

Retinal degenerative disease involving photoreceptor (PR) cell loss results in permanent vision loss and often blindness. Generation of induced pluripotent stem cell (iPSC)-derived retinal cells and tissues from individuals with retinal dystrophies is a relatively new and promising method for studying retinal degeneration mechanisms in vitro. Recent advancements in strategies to differentiate human iPSCs (hiPSCs) into 3D retinal eyecups with a strong resemblance to the mature retina raise the possibility that this system could offer a reliable model for translational drug studies. However, despite the potential benefits, there are challenges that remain to be overcome before stem-cell-derived retinal eyecups can be routinely used to model human retinal diseases. This chapter will discuss both the potential of these 3D eyecup approaches and the nature of some of the challenges that remain.

Wang, X., J. Qin, et al. "Reduced immunogenicity of induced pluripotent stem cells derived from sertoli cells." PLoS One. 2014 Aug 28;9(8):e106110. doi: 10.1371/journal.pone.0106110. eCollection 2014.

Sertoli cells constitute the structural framework in testis and provide an immune-privileged environment for germ cells. Induced pluripotent stem cells (iPS cells) resemble embryonic stem cells (ES cells) and are generated from somatic cells by expression of specific reprogramming transcription factors. Here, we used C57BL/6 (B6) Sertoli cells to generate iPS cells (Ser-iPS cells) and compared the immunogenicity of Ser-iPS cells with iPS cells derived from mouse embryonic fibroblast (MEF-iPS cells). Ser-iPS cells were injected into syngeneic mice to test for their in vivo immunogenicity in teratoma assay. Teratoma assay allows assessing in vivo immunogenicity of iPS cells and of their differentiated progeny simultaneously. We observed that early-passage Ser-iPS cells formed more teratomas with less immune cell infiltration and tissue damage and necrosis than MEF-iPS cells. Differentiating Ser-iPS cells in embryoid bodies (EBs) showed reduced T cell activation potential compared to MEF-iPS cells, which was similar to syngeneic ES cells. However, Ser-iPS cells lost their reduced immunogenicity in vivo after extended passaging in vitro and late-passage Ser-iPS cells exhibited an immunogenicity similar to MEF-iPS cells. These findings indicate that early-passage Ser-iPS cells retain some somatic memory of Sertoli cells that impacts on immunogenicity of iPS cells and iPS cell-derived cells in vivo and in vitro. Our data suggest that immune-privileged Sertoli cells might represent a preferred source for iPS cell generation, if it comes to the use of iPS cell-derived cells for transplantation.

Wang, Y., P. Liang, et al. "Genome Editing of Isogenic Human Induced Pluripotent Stem Cells Recapitulates Long QT Phenotype for Drug Testing." J Am Coll Cardiol. 2014 Aug 5;64(5):451-9. doi: 10.1016/j.jacc.2014.04.057.

BACKGROUND: Human induced pluripotent stem cells (iPSCs) play an important role in disease modeling and drug testing. However, the current methods are time-consuming and lack an isogenic control. OBJECTIVES: This study sought to establish an efficient technology to generate human PSC-based disease models with isogenic control. METHODS: The ion channel genes KCNQ1 and KCNH2 with dominant negative mutations causing long QT syndrome types 1 and 2, respectively, were stably integrated into a safe harbor AAVS1 locus using zinc finger nuclease technology. RESULTS: Patch-clamp recording revealed that the edited iPSC-derived cardiomyocytes (iPSC-CMs) displayed characteristic long QT syndrome phenotype and significant prolongation of the action potential duration compared with the unedited control cells. Finally, addition of nifedipine (L-type calcium channel blocker) or pinacidil (KATP-channel opener) shortened the action potential duration of iPSC-CMs, confirming the validity of isogenic iPSC lines for drug testing in the future. CONCLUSIONS: Our study demonstrates that iPSC-CM-based disease models can be rapidly generated by overexpression of dominant negative gene mutants.

Warlich, E., A. Schambach, et al. "FAS-based cell depletion facilitates the selective isolation of mouse induced pluripotent stem cells." PLoS One. 2014 Jul 16;9(7):e102171. doi: 10.1371/journal.pone.0102171. eCollection 2014.

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.

Weltner, J., R. Trokovic, et al. "[Induced pluripotent stem cells (iPS) in medical research]." Duodecim. 2014;130(8):785-92.

Pluripotent stem cells are capable of differentiating into cells of any tissue. The fact that iPS cell lines can be produced from skin cells or blood cells and directed to differentiate into a desired direction makes it possible to investigate e.g. myocardial or nerve cells having a disease-associated genotype. This will enable the development of experimental models of disease mechanisms and also apply them to drug screening, which may allow the development of novel types of treatment. In the future it may become possible to replace injured cells of a patient with autologous iPS cell derived transplants.

Whitworth, D. J., J. E. Frith, et al. "Derivation of Mesenchymal Stromal Cells from Canine Induced Pluripotent Stem Cells by Inhibition of the TGFbeta/Activin Signaling Pathway." Stem Cells Dev. 2014 Sep 4.

In this study we have generated canine mesenchymal stromal cells (MSCs), also known as mesenchymal stem cells, from canine induced pluripotent stem cells (ciPSCs) by small-molecule inhibition of the transforming growth factor beta (TGFbeta)/activin signaling pathway. These ciPSC-derived MSCs (ciPSC-MSCs) express the MSC markers CD73, CD90, CD105, STRO1, cPDGFRbeta and cKDR, in addition to the pluripotency factors OCT4, NANOG and REX1. ciPSC-MSCs lack immunostaining for H3K27me3, suggesting that they possess two active X chromosomes. ciPSC-MSCs are highly proliferative and undergo robust differentiation along the osteo-, chondro- and adipogenic pathways, but do not form teratoma-like tissues in vitro. Of further significance for the translational potential of ciPSC-MSCs, we show that these cells can be encapsulated and maintained within injectable hydrogel matrices that, when functionalized with bound pentosan polysulfate, dramatically enhance chondrogenesis and inhibit osteogenesis. The ability to efficiently derive large numbers of highly proliferative canine MSCs from ciPSCs that can be incorporated into injectable, functionalized hydrogels that enhance their differentiation along a desired lineage constitutes an important milestone towards developing an effective MSC-based therapy for osteoarthritis in dogs, but equally provides a model system for assessing the efficacy and safety of analogous approaches for treating human degenerative joint diseases.

Willard, V. P., B. O. Diekman, et al. "Cartilage derived from mouse induced pluripotent stem cells for osteoarthritis drug screening in vitro." Arthritis Rheumatol. 2014 Jul 21. doi: 10.1002/art.38780.

Objective: The discovery of novel disease-modifying drugs for osteoarthritis (OA) is limited by the lack of adequate genetically-defined cartilage tissues for application in high-throughput screening systems. We addressed this need by synthesizing cartilage from induced pluripotent stem cells (iPSCs) to establish and validate an in vitro model of OA. Methods: iPSC-derived or native mouse cartilage samples were treated with the cytokine interleukin-1alpha (IL-1alpha) for 3 days to model the inflammatory environment of OA. Biochemical content, mechanical properties, and gene expression of the resulting tissues were assayed. In addition, the inflammatory and catabolic environment of the media was assessed. To establish high-throughput capability, we utilized a 96-well plate format and conducted a screen of previously identified candidate OA drugs. Glycosaminoglycan release into the media was used as the primary output for screening. Results: Treatment of iPSC-derived or native cartilage with IL-1alpha induced characteristic features of OA in a rapid and dose-dependent manner. In addition to the loss of glycosaminoglycans and tissue mechanical properties, IL-1alpha treatment induced expression of matrix metalloproteinases and increased production of the inflammatory mediators nitric oxide and prostaglandin E2. In the high-throughput screen validation, all candidate OA therapeutics provided some benefit, but only the NF-kappaB inhibitor SC-514 effectively reduced cartilage loss in response to IL-1alpha. Conclusions: This work demonstrates the utility of iPSCs for studying cartilage pathology, and provides a platform for identifying novel, patient-specific therapeutics that prevent cartilage degradation and modify the course of OA development. (c) 2014 American College of Rheumatology.

Yamada, R., K. Hattori, et al. "Control of adhesion of human induced pluripotent stem cells to plasma-patterned polydimethylsiloxane coated with vitronectin and gamma-globulin." J Biosci Bioeng. 2014 Sep;118(3):315-22. doi: 10.1016/j.jbiosc.2014.02.009. Epub 2014 Mar 18.

Human induced pluripotent stem cells (hiPSCs) are a promising source of cells for medical applications. Recently, the development of polydimethylsiloxane (PDMS) microdevices to control the microenvironment of hiPSCs has been extensively studied. PDMS surfaces are often treated with low-pressure air plasma to facilitate protein adsorption and cell adhesion. However, undefined molecules present in the serum and extracellular matrix used to culture cells complicate the study of cell adhesion. Here, we studied the effects of vitronectin and gamma-globulin on hiPSC adhesion to plasma-treated and untreated PDMS surfaces under defined culture conditions. We chose these proteins because they have opposite properties: vitronectin mediates hiPSC attachment to hydrophilic siliceous surfaces, whereas gamma-globulin is adsorbed by hydrophobic surfaces and does not mediate cell adhesion. Immunostaining showed that, when applied separately, vitronectin and gamma-globulin were adsorbed by both plasma-treated and untreated PDMS surfaces. In contrast, when PDMS surfaces were exposed to a mixture of the two proteins, vitronectin was preferentially adsorbed onto plasma-treated surfaces, whereas gamma-globulin was adsorbed onto untreated surfaces. Human iPSCs adhered to the vitronectin-rich plasma-treated surfaces but not to the gamma-globulin-rich untreated surfaces. On the basis of these results, we used perforated masks to prepare plasma-patterned PDMS substrates, which were then used to pattern hiPSCs. The patterned hiPSCs expressed undifferentiated-cell markers and did not escape from the patterned area for at least 7 days. The patterned PDMS could be stored for up to 6 days before hiPSCs were plated. We believe that our results will be useful for the development of hiPSC microdevices.

Yamaguchi, T., S. Hamanaka, et al. "The generation and maintenance of rat induced pluripotent stem cells." Methods Mol Biol. 2014;1210:143-50. doi: 10.1007/978-1-4939-1435-7_11.

This chapter describes a newly developed method for generating and maintaining rat induced pluripotent stem cells (riPSCs). We first provide a detailed protocol for the generation of lentiviral vector carrying three reprogramming factors to produce high-quality riPSCs. This technique allows reprogramming of rat somatic cells to ground state with germ-line competence. Subsequently, we elaborate a detailed protocol for the generation of riPSCs from rat embryonic fibroblast (REF). Finally, the protocols for the optimal culture conditions of riPSCs and preparation of frozen stock are described. We also outline the advantages of generating riPSCs.

Yan, T., A. Mizutani, et al. "Characterization of cancer stem-like cells derived from mouse induced pluripotent stem cells transformed by tumor-derived extracellular vesicles." J Cancer. 2014 Jul 5;5(7):572-84. doi: 10.7150/jca.8865. eCollection 2014.

Several studies have shown that cancer niche can perform an active role in the regulation of tumor cell maintenance and progression through extracellular vesicles-based intercellular communication. However, it has not been reported whether this vesicle-mediated communication affects the malignant transformation of normal stem cells/progenitors. We have previously reported that the conditioned medium derived from the mouse Lewis Lung Carcinoma (LLC) cell line can convert mouse induced pluripotent stem cells (miPSCs) into cancer stem cells (CSCs), indicating that normal stem cells when placed in an aberrant microenvironment can give rise to functionally active CSCs. Here, we focused on the contribution of tumor-derived extracellular vesicles (tEVs) that are secreted from LLC cells to induce the transformation of miPSCs into CSCs. We isolated tEVs from the conditioned medium of LLC cells, and then the differentiating miPSCs were exposed to tEVs for 4 weeks. The resultant tEV treated cells (miPS-LLCev) expressed Nanog and Oct3/4 proteins comparable to miPSCs. The frequency of sphere formation of the miPS-LLCev cells in suspension culture indicated that the self-renewal capacity of the miPS-LLCev cells was significant. When the miPS-LLCev cells were subcutaneously transplanted into Balb/c nude mice, malignant liposarcomas with extensive angiogenesis developed. miPS-LLCevPT and miPS-LLCevDT, the cells established from primary site and disseminated liposarcomas, respectively, showed their capacities to self-renew and differentiate into adipocytes and endothelial cells.

Yang, X., M. Rodriguez, et al. "Tri-iodo-l-thyronine promotes the maturation of human cardiomyocytes-derived from induced pluripotent stem cells." J Mol Cell Cardiol. 2014 Jul;72:296-304. doi: 10.1016/j.yjmcc.2014.04.005. Epub 2014 Apr 13.

BACKGROUND: Cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) have great potential as a cell source for therapeutic applications such as regenerative medicine, disease modeling, drug screening, and toxicity testing. This potential is limited, however, by the immature state of the cardiomyocytes acquired using current protocols. Tri-iodo-l-thyronine (T3) is a growth hormone that is essential for optimal heart growth. In this study, we investigated the effect of T3 on hiPSC-CM maturation. METHODS AND RESULTS: A one-week treatment with T3 increased cardiomyocyte size, anisotropy, and sarcomere length. T3 treatment was associated with reduced cell cycle activity, manifest as reduced DNA synthesis and increased expression of the cyclin-dependent kinase inhibitor p21. Contractile force analyses were performed on individual cardiomyocytes using arrays of microposts, revealing an almost two-fold higher force per-beat after T3 treatment and also an enhancement in contractile kinetics. This improvement in force generation was accompanied by an increase in rates of calcium release and reuptake, along with a significant increase in sarcoendoplasmic reticulum ATPase expression.

Yoshida, T., Y. Ozawa, et al. "The use of induced pluripotent stem cells to reveal pathogenic gene mutations and explore treatments for retinitis pigmentosa." Mol Brain. 2014 Jun 16;7:45. doi: 10.1186/1756-6606-7-45.

BACKGROUND: Retinitis pigmentosa (RP) is an inherited human retinal disorder that causes progressive photoreceptor cell loss, leading to severe vision impairment or blindness. However, no effective therapy has been established to date. Although genetic mutations have been identified, the available clinical data are not always sufficient to elucidate the roles of these mutations in disease pathogenesis, a situation that is partially due to differences in genetic backgrounds. RESULTS: We generated induced pluripotent stem cells (iPSCs) from an RP patient carrying a rhodopsin mutation (E181K). Using helper-dependent adenoviral vector (HDAdV) gene transfer, the mutation was corrected in the patient's iPSCs and also introduced into control iPSCs. The cells were then subjected to retinal differentiation; the resulting rod photoreceptor cells were labeled with an Nrl promoter-driven enhanced green fluorescent protein (EGFP)-carrying adenovirus and purified using flow cytometry after 5 weeks of culture. Using this approach, we found a reduced survival rate in the photoreceptor cells with the E181K mutation, which was correlated with the increased expression of endoplasmic reticulum (ER) stress and apoptotic markers. The screening of therapeutic reagents showed that rapamycin, PP242, AICAR, NQDI-1, and salubrinal promoted the survival of the patient's iPSC-derived photoreceptor cells, with a concomitant reduction in markers of ER stress and apoptosis. Additionally, autophagy markers were found to be correlated with ER stress, suggesting that autophagy was reduced by suppressing ER stress-induced apoptotic changes. CONCLUSION: The use of RP patient-derived iPSCs combined with genome editing provided a versatile cellular system with which to define the roles of genetic mutations in isogenic iPSCs with or without mutation and also provided a system that can be used to explore candidate therapeutic approaches.

Young, J. S., R. A. Morshed, et al. "Advances in stem cells, induced pluripotent stem cells, and engineered cells: delivery vehicles for anti-glioma therapy." Expert Opin Drug Deliv. 2014 Jul 8:1-14.

Introduction: A limitation of small molecule inhibitors, nanoparticles (NPs) and therapeutic adenoviruses is their incomplete distribution within the entirety of solid tumors such as malignant gliomas. Currently, cell-based carriers are making their way into the clinical setting as they offer the potential to selectively deliver many types of therapies to cancer cells. Areas covered: Here, we review the properties of stem cells, induced pluripotent stem cells and engineered cells that possess the tumor-tropic behavior necessary to serve as cell carriers. We also report on the different types of therapeutic agents that have been delivered to tumors by these cell carriers, including: i) therapeutic genes; ii) oncolytic viruses; iii) NPs; and iv) antibodies. The current challenges and future promises of cell-based drug delivery are also discussed. Expert opinion: While the emergence of stem cell-mediated therapy has resulted in promising preclinical results and a human clinical trial utilizing this approach is currently underway, there is still a need to optimize these delivery platforms. By improving the loading of therapeutic agents into stem cells and enhancing their migratory ability and persistence, significant improvements in targeted cancer therapy may be achieved.

Yu, P., Y. Lu, et al. "Nonviral Minicircle Generation of Induced Pluripotent Stem Cells Compatible with Production of Chimeric Chickens." Cell Reprogram. 2014 Aug 1.

Chickens are vitally important in numerous countries as a primary food source and a major component of economic development. Efforts have been made to produce transgenic birds through pluripotent stem cell [primordial germ cells and embryonic stem cells (ESCs)] approaches to create animals with improved traits, such as meat and egg production or even disease resistance. However, these cell types have significant limitations because they are hard to culture long term while maintaining developmental plasticity. Induced pluripotent stem cells (iPSCs) are a novel class of stem cells that have proven to be robust, leading to the successful development of transgenic mice, rats, quail, and pigs and may potentially overcome the limitations of previous pluripotent stem cell systems in chickens. In this study we generated chicken (c) iPSCs from fibroblast cells for the first time using a nonviral minicircle reprogramming approach. ciPSCs demonstrated stem cell morphology and expressed key stem cell markers, including alkaline phosphatase, POU5F1, SOX2, NANOG, and SSEA-1.

Zhang, R., L. Hao, et al. "Gene expression analysis of induced pluripotent stem cells from aneuploid chromosomal syndromes." BMC Genomics. 2013;14 Suppl 5:S8. doi: 10.1186/1471-2164-14-S5-S8. Epub 2013 Oct 16.

BACKGROUND: Human aneuploidy is the leading cause of early pregnancy loss, mental retardation, and multiple congenital anomalies. Due to the high mortality associated with aneuploidy, the pathophysiological mechanisms of aneuploidy syndrome remain largely unknown. Previous studies focused mostly on whether dosage compensation occurs, and the next generation transcriptomics sequencing technology RNA-seq is expected to eventually uncover the mechanisms of gene expression regulation and the related pathological phenotypes in human aneuploidy. RESULTS: Using next generation transcriptomics sequencing technology RNA-seq, we profiled the transcriptomes of four human aneuploid induced pluripotent stem cell (iPSC) lines generated from monosomy x (Turner syndrome), trisomy 8 (Warkany syndrome 2), trisomy 13 (Patau syndrome), and partial trisomy 11:22 (Emanuel syndrome) as well as two umbilical cord matrix iPSC lines as euploid controls to examine how phenotypic abnormalities develop with aberrant karyotype. A total of 466 M (50-bp) reads were obtained from the six iPSC lines, and over 13,000 mRNAs were identified by gene annotation. Global analysis of gene expression profiles and functional analysis of differentially expressed (DE) genes were implemented. Over 5000 DE genes are determined between aneuploidy and euploid iPSCs respectively while 9 KEGG pathways are overlapped enriched in four aneuploidy samples. CONCLUSIONS:

Zhang, R., T. Takebe, et al. "Identification of proliferating human hepatic cells from human induced pluripotent stem cells." Transplant Proc. 2014 May;46(4):1201-4. doi: 10.1016/j.transproceed.2013.12.021.

Mass-scale production of hepatocytes from human induced pluripotent stem cells (iPSCs) with functional properties of primary hepatocytes is of great value in clinical transplantation for liver failure as well as in facilitating drug development by predicting humanized drug metabolism profiles. In this report, we generated human hepatocyte-like cells from human iPSCs with the use of a stepwise protocol. Aiming at future clinical and industrial application, it is important to determine the suitable stage of iPSC-derived hepatic cells that possess high proliferative capacity to intensively expand the hepatic cells. Ki67 immunostaining showed that human iPSC-derived hepatic endoderm cells contained Ki67(+) cells at the highest level in the middle stage of hepatic differentiation, suggesting that the abundance of proliferating hepatic progenitor cells exists in this stage. Extensive expansion and differentiation of human iPSC-derived hepatic progenitors will provide future perspectives in transplantation therapy and drug development.

Zhang, R. R., T. Takebe, et al. "Efficient hepatic differentiation of human induced pluripotent stem cells in a three-dimensional microscale culture." Methods Mol Biol. 2014;1210:131-41. doi: 10.1007/978-1-4939-1435-7_10.

Human induced pluripotent stem cells (iPSCs) represent a novel source of hepatocytes for drug development, disease modeling studies, and regenerative therapy for the treatment of liver diseases. A number of protocols for generating functional hepatocytes have been reported worldwide; however, reproducible and efficient differentiation remained challenging under conventional two-dimensional (2D) culture. In this study, we describe an efficient differentiation protocol for generating functional hepatocyte-like cells from human iPSC-derived homogenous hepatic endoderm cells combined with three-dimensional (3D) microscale culture system. First, hepatic endoderm cells (iPSC-HEs) were directly differentiated using two-step approaches, and then cultured in the 3D micropattern plate. Human iPSC-HEs quickly reaggregated and formed hundreds of round-shaped spheroids at day 4 of cell plating. The size distribution of iPSC-HEs derived spheroids was relatively uniform around 100-200 mum in diameter. After 14 days, iPSC-HEs efficiently differentiated into hepatocyte-like cells in terms of hepatic maker gene expression compared with conventional 2D approach. We conclude that our scalable and three-dimensional culture system would be one promising approach to generate a huge number of hepatocyte-like cells from human iPSCs aiming at future industrial and clinical applications.

Zhang, Y., D. Wang, et al. "Rat induced pluripotent stem cells protect H9C2 cells from cellular senescence via a paracrine mechanism." Cardiology. 2014;128(1):43-50. doi: 10.1159/000357423. Epub 2014 Apr 12.

OBJECTIVES: Cellular senescence may play an important role in the pathology of heart aging. We aimed to explore whether induced pluripotent stem cells (iPSCs) could inhibit cardiac cellular senescence via a paracrine mechanism. METHODS: We collected iPSC culture supernatant, with or without oxidative stress, as conditioned medium (CM) for the rat cardiomyocyte-derived cell line H9C2. Then we treated H9C2 cells, cultured with or without CM, with hypoxia/reoxygenation to induce cellular senescence and measured senescence-associated beta-galactosidase (SA-beta-gal) activity, G1 cell proportion and expression of the cell cycle regulators p16(INK4a), p21(Waf1/Cip1) and p53 at mRNA and protein levels in H9C2 cells. In addition, we used Luminex-based analysis to measure concentrations of trophic factors in iPSC-derived CM. RESULTS: We found that iPSC-derived CM reduced SA-beta-gal activity, attenuated G1 cell cycle arrest and reduced the expression of p16(INK4a), p21(Waf1/Cip1) and p53 in H9C2 cells. Furthermore, the CM contained more trophic factors, e.g. tissue inhibitor of metalloproteinase-1 and vascular endothelial growth factor, than H9C2-derived CM.

Zhao, C., A. P. Farruggio, et al. "Recombinase-mediated reprogramming and dystrophin gene addition in mdx mouse induced pluripotent stem cells." PLoS One. 2014 Apr 29;9(4):e96279. doi: 10.1371/journal.pone.0096279. eCollection 2014.

A cell therapy strategy utilizing genetically-corrected induced pluripotent stem cells (iPSC) may be an attractive approach for genetic disorders such as muscular dystrophies. Methods for genetic engineering of iPSC that emphasize precision and minimize random integration would be beneficial. We demonstrate here an approach in the mdx mouse model of Duchenne muscular dystrophy that focuses on the use of site-specific recombinases to achieve genetic engineering. We employed non-viral, plasmid-mediated methods to reprogram mdx fibroblasts, using phiC31 integrase to insert a single copy of the reprogramming genes at a safe location in the genome. We next used Bxb1 integrase to add the therapeutic full-length dystrophin cDNA to the iPSC in a site-specific manner. Unwanted DNA sequences, including the reprogramming genes, were then precisely deleted with Cre resolvase. Pluripotency of the iPSC was analyzed before and after gene addition, and ability of the genetically corrected iPSC to differentiate into myogenic precursors was evaluated by morphology, immunohistochemistry, qRT-PCR, FACS analysis, and intramuscular engraftment. These data demonstrate a non-viral, reprogramming-plus-gene addition genetic engineering strategy utilizing site-specific recombinases that can be applied easily to mouse cells. This work introduces a significant level of precision in the genetic engineering of iPSC that can be built upon in future studies.

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