Title
Induced Pluripotent Stem Cells for Regenerative Medicine

Journal
Annual Review of Biomedical Engineering, 16(1)

ISSN
1523-9829

Authors
Hirschi, Karen K
Li, Song
Roy, Krishnendu

Publication Date
2014-07-11

DOI
10.1146/annurev-bioeng-071813-105108

Peer reviewed

Published in final edited form as:
Annu Rev Biomed Eng. 2014 July 11; 16: 277–294. doi:10.1146/annurev-bioeng-071813-105108.

Induced Pluripotent Stem Cells for Regenerative Medicine

Karen K. Hirschi[1], **Song Li**[2], and **Krishnendu Roy**[3]

Karen K. Hirschi: karen.hirschi@yale.edu; Song Li: song_li@berkeley.edu

[1]Yale Cardiovascular Research Center and Yale Stem Cell Center, Yale University School of Medicine, New Haven, Connecticut 06511

[2]Department of Bioengineering, University of California, Berkeley, Berkeley, California 94720

[3]The Wallace H. Coulter Department of Biomedical Engineering at Georgia Tech and Emory University, Georgia Institute of Technology, Atlanta, Georgia 30332

Abstract

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.

Keywords

iPS cells; cell engineering; directed differentiation; tissue regeneration

INTRODUCTION

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.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

Definition and Generation of Induced Pluripotent Stem Cells

iPS cells are generated via genetic reprogramming of adult somatic cells that have limited differentiation potential but, upon reprogramming, express genes that enable them to regain plasticity and give rise to all cell types (1). Human iPS (hiPS) cells were initially derived from fibroblasts by transduction of genes encoding transcriptional regulators of stem cells: Oct4, Sox2, Lin28, and Nanog (OSLN) (2) or Oct4, Sox2, Klf4, and c-Myc (OSKM) (3). Reprogrammed hiPS cells are similar to human embryonic stem (hES) cells in morphology, proliferation rate, surface antigen expression, epigenetic status of pluripotent genes, and telomerase activity. In addition, hiPS cells can differentiate into cell types of all three germ layers in vitro and in vivo (3). However, there is some evidence to suggest that hiPS and hES cells are not identical at the transcriptional level (3–7). There are also reported differences in gene expression among iPS cell lines (6) that may reflect differences in the somatic cell source or even genetic variability among similar cells (8), in the reprogramming methodology, and/or in the degree to which the cells are genetically reprogrammed (recently reviewed in 9). Although the various methodologies established to reprogram iPS cells are all thought to achieve some degree of pluripotency, each has advantages and disadvantages with respect to future clinical use.

The process of generating iPS cells initially began with the use of retroviruses and/or lentiviruses to transduce regulatory genes either separately or in a single expression vector. However, the use of cells containing viruses that can integrate into host chromosomes and cause insertional mutagenesis and potentially malignant transformations (10) is not ideal for clinical studies. In addition, the presence of viruses may evoke an immunogenic response (11). Thus, new methodologies to generate iPS cells have been rapidly and continuously evolving. Plasmids (12, 13), synthesized RNAs (14), and proteins (15) have all been used to induce a pluripotent state in somatic cell types, and all of these methods appear to be more tolerable for clinical studies, relative to viral transduction. Regardless of the technology used, continued threat of having such cells become uncontrolled and induce genetic damage and malignant cell growth is ever-present, and the potential and fate of these cells in vivo are under intense investigation.

Advantages for Clinical Use

Despite potential issues with the use of hiPS cells for clinical therapy, they have a distinct advantage over other human pluripotent stem cells, such as hES cells; that is, they can be patient specific, thus theoretically reducing the need for immune suppression post transplantation. However, as mentioned above, this may be dependent upon the manner in which the cells are reprogrammed (i.e., the use of viruses for reprogramming may evoke an adverse immune response). Because of this promise of autologous cell therapy for genetic diseases and degenerative disorders, there remains tremendous interest in further optimizing the derivation of hiPS cells and directing their differentiation toward cells needed for tissue repair. New technologies and quantitative bioengineering approaches are being developed to enable improved generation, isolation, propagation, and differentiation of hiPS cells. This review focuses primarily on these recent developments and provides a concise overview of our understanding of iPS cell biology, as well as engineering approaches to enable the use of such cells in human therapies.

ENABLING TECHNOLOGIES

New Approaches for Improved Reprogramming

To enhance reprogramming efficiency or replace reprogramming genes, microRNAs (miRNAs) and small-molecule compounds have also been explored for cell reprogramming. MiRNAs are an integral part of the gene network and can be regulated by pluripotent genes and vice versa. Therefore, (*a*) the expression of pluripotent stem cell–specific miRNAs or reprogramming gene-related miRNAs or (*b*) the inhibition of tissue-specific miRNAs may promote cell reprogramming into hiPS cells. For example, miR-291-3p, miR-294, and miR-295 can replace *c-myc* and generate homogeneous populations of hiPS cell colonies (16), and the inhibition of *let-7* miRNA enhances the expression of target genes *c-myc* and *Lin-28* to promote cell reprogramming (17). There is also evidence that the miRNA302/367 cluster can reprogram somatic cells into hiPS cells without the requirement for exogenous transcription factors (18), although the reprogramming efficiency is lower.

Small-molecule compounds can replace some of the reprogramming genes or modulate epigenetic state to enable or improve reprogramming efficiency (19–22). Via high-throughput screening, an inhibitor of transforming growth factor beta (TGF-β) signaling was identified, which can replace Sox2 and induce Nanog expression (20). Inhibitors of the TGF-β and MEK pathways also facilitate mesenchymal-to-epithelial transition—a required step in iPS cell reprogramming (23). A combination of chemical compounds can replace Sox2 and c-myc (24), and Oct4-activating compounds were recently identified (21). Histone modifications, including acetylation and methylation, play an important role in epigenetic changes in cell reprogramming (25), and the small molecules that regulate histone modifications have been shown to significantly enhance reprogramming efficiency. Valproic acid (VPA), a histone deacetylase (HDAC) inhibitor, increases the percentage of $Oct4^+$ cells generated during reprogramming (19). Tranylcypromine hydrochloride (TCP), an inhibitor of lysine-specific demethylase, also improves reprogramming efficiency (20). A recent study demonstrated that it is feasible to generate iPS cells by using small molecules alone (26), which represents significant progress in cell reprogramming technology.

Biophysical factors such as the mechanical properties and micro/nanostructure of cell-adhesion substrates may also play a role in cell reprogramming. For example, micro/nanotopography can regulate cell and nucleus shape, modulate the epigenetic state, and thus replace biochemical factors (i.e., VPA, TCP) to enhance cell reprogramming into iPS cells (27). Interestingly, cell reprogramming with OSKM factors can be performed in suspension culture under adherence- and matrix-free conditions (28), which suggests that OSKM factors are sufficient to reprogram cells without the input of cell adhesion–induced signaling. How cell reprogramming efficiency is modulated by cell adhesion awaits further studies.

Label-Free Isolation of Reprogrammed hiPS Cells

Regardless of the reprogramming method, one of the key limitations of reprogramming somatic cells into iPS cells is the inherent low efficiency of complete reprogramming (~1% of cells get fully reprogrammed) (29, 30). As a result, reprogramming cultures contain non- or partially reprogrammed cells, as well as partially differentiated cells. The pure, fully

reprogrammed iPS cell population must then be isolated for further experiments. This process requires dissociation of cell aggregates, often manually, followed by labeling and sorting steps, all of which are time consuming, and it involves significant cell handling and manipulation, which leads to inefficiency and cell death. Although the recent work by Rais et al. (31) shows that depleting Mbd3 during reprogramming tremendously increases the efficiency of reprogramming (to nearly 100%) and synchronizes the reprogrammed cells, it remains to be seen how this method works across different platforms. Recently, a microfluidic approach was developed for label-free cell isolation based on the different adhesion strengths of fully reprogrammed hiPS cells compared with non- or partially reprogrammed cells, as well as other differentiated cells present in the culture (30). It was found that as fibroblast cells are reprogramed, they undergo a change in their integrin composition, leading to a decrease in adhesive strength with fibronectin. Specifically, fully reprogrammed iPS cells have lower adhesion strength compared with partially reprogrammed cells, which, in turn, have lower adhesion strength than undifferentiated cells. There are also differences in the adhesion properties of cells differentiated into the neuronal or cardiac lineages. Based on these findings, fibronectin-functionalized microfluidic channels were constructed and used to show that under certain shear force (i.e., flow rates), fully reprogrammed iPS cells can be detached and isolated from other, more adhesive cells in culture. The detached cells had an unaltered karyotype and were able to form embryoid bodies and differentiate into multiple lineages similarly to hiPS cells isolated in a conventional manner. Although more work needs to be done in validating this technique across all the different iPS cell lines and to scale it up for larger cultures, it represents a significant step forward in enabling wider usage of hiPS cells, both in research and in clinical applications.

Expansion of iPS Cells and Their Differentiated Progeny

In addition to directed differentiation of iPS cells into various lineages (discussed below), one of the fundamental bioengineering problems in iPS cell research is the development of technologies that enable large-scale expansion of undifferentiated iPS cells as well as expansion of their differentiated progenies. This is not only critical for research and preclinical studies, especially to conduct rapid and parallel experiments without the constraint of cell numbers, but also essential for eventual translation of iPS cells into clinical practice. Although the issue is not unique to iPS cells and applies also to embryonic and adult stem cells, iPS cells pose a new set of challenges in this domain, and little work has been done to specifically address their large-scale expansion. Recently, Lei & Schaffer (31a) introduced a hydrogel-based, defined 3D culture method that could be GMP compatible, free of animal- and human-derived factors, and scalable. Both ES and iPS cells were used for these studies, demonstrating versatility. Although the broad applicability of this method in other iPS cells and in different laboratories needs to be shown, the process described is certainly highly promising. Nevertheless, the lessons learned from ES cell expansion, as well as large-scale culture of other progenitor and differentiated cells, can be applied to iPS cells. Two recent reviews have provided detailed description of available technologies that have been evaluated for human progenitor cell expansion (32–34). As the majority of these studies were conducted on human ES cells and not on iPS cells, it remains to be seen

whether the methods translate to iPS cell processing. Nevertheless, the broad engineering concepts involved in stem cell expansion are worth discussing in this context.

As outlined in Figure 1, similar to stem cell differentiation, expansion of undifferentiated cells while maintaining their pluripotency—as well as expansion of differentiated, multipotent, and terminal cells—could be influenced by three niche-specific factors: (*a*) interactions of these cells with extracellular matrix components, (*b*) cell–cell communication, and (*c*) soluble factors. Mimicking the physiological stem cell niche for in vitro proliferation, self-renewal, and maintenance of stemness are particularly relevant for iPS cell–derived multipotent progenitors. For example, iPS cell–derived cardiomyocytes or hematopoietic stem cells can be expanded by creating a microenvironment that mimics the cardiac or bone-marrow niche. However, as iPS cells, per se, are not present physiologically, it is difficult to engineer a biologically relevant niche for their expansion. In this context, studies on expansion of ES cells could provide relevant baseline conditions under which iPS cell expansion can be further evaluated.

Well-mixed soluble factors and nutrients: bioreactor-based cultures—Among all technologies, bioreactor-based culture approaches have been the most widely explored for expansion of both animal and human stem cells, including hiPS cells (34–41). Bioreactors provide a well-mixed (dynamic) microenvironment for suspension cultures, thereby allowing efficient nutrient transport. They also provide a method for high-density, large-scale culture of stem cells while maintaining a small equipment footprint. Compared with traditional two-dimensional (2D) cultures, which are generally performed at low cell densities and require parallel handling of tens and hundreds of petri dishes to achieve scaled-up production, a three-dimensional (3D) bioreactor environment is readily amenable to scaling up in a single reactor vessel. Interestingly, most bioreactors also expose stem cells to shear forces as a result of stirring or perfusion. Although shear could be a relevant niche-specific variable to study in certain contexts (i.e., endothelial or cardiac differentiation), its effect on expansion of undifferentiated iPS cells and other progeny remains to be thoroughly studied. It is worth noting that the enabling technologies in this field come from the chemical engineering and bioprocessing industry and have been widely used for large-scale production of recombinant proteins, biofuels, etc., from relevant cells.

Generally speaking, there are four types of bioreactors widely studied in stem cell expansion: (*a*) stirred tank bioreactors, (*b*) perfusion bioreactors, (*c*) rotary vessel bioreactors, and (*d*) packed bed bioreactors. Stirred tank bioreactors, commonly referred to as spinner flasks, are impeller-driven systems that are characterized by a turbulent flow regime. These reactors have high working volume (typically 50 mL to hundreds of liters) and may not be suitable for small-volume process developmental studies and high-throughput parallel experiments, especially when expensive cytokines and other biofactors need to be added to the culture. The effect of high shear could also be a concern. However, stirred tanked bioreactors provide excellent nutrient and gas transport and are easily amenable to 3D scaffolds, microcarriers, and encapsulated stem cell cultures. Rotary vessel culture systems, for example the Synthecon™ bioreactors, allow for smaller-volume cultures (as low as 10 mL) with low shear on the cells and could also be amenable to 3D scaffold– and microcarrier-based cultures. However, large-volume production capabilities are limited

as compared with stirred tank–type systems. Perfusion and packed bed bioreactors have also been widely used for stem cell cultures (42–44), especially in the context of progenitor cell expansion and differentiation. The flow regime is generally laminar with low shear and allows fresh nutrients to flow continuously, thus more closely mimicking in vivo conditions. These types of systems are amenable to 3D scaffold–based cultures, as well as microencapsulated and microcarrier-based approaches.

Cell–cell and cell–matrix interactions: biomaterials and scaffolds—Although polymer-based 3D scaffolds and biomaterials have been widely explored in stem cell research, most of the work has focused on directed differentiation into specific lineages. In terms of cell expansion, simple cell aggregates, as well as microcarriers and microencapsulation, of stem cells into polymeric capsules have garnered the most interest (33, 45, 46). Although cell aggregates and microcarriers are attractive choices for 3D culture and can be readily interfaced with bioreactors, they do not allow rational design of the stem cell microenvironment. Scaffolds or material-directed (through either cell seeding or microencapsulation) cell expansion strategies not only allow high-density culture of pluripotent stem cells but also provide a 3D niche of synthetic materials or extracellular matrix components. These materials could be designed to affect specific cell-signaling pathways, leading to efficient expansion or differentiation. In addition, they could provide efficient cell–cell contact between the stem cells, as well as contact between stem cells and relevant stromal cells in a 3D environment, which is otherwise difficult to achieve in suspension cultures or 2D systems.

A critical aspect in choosing biomaterials and scaffold structures for iPS cell expansion is to ensure that self-renewal and proliferation occurs without the presence of feeder cells and that the process maintains the complete functionality of iPS cells. It is also essential to develop techniques that allow a defined, serum-free culture medium to be used to ensure reproducibility and scale-up. Although much work has been done on ES cells to achieve these goals, only recently have several reports shown efficient expansion of iPS cells and successful long-term expansion of hiPS and hES cells in a defined medium (47–50). Encapsulation into negatively charged hydrogels of poly(2-acrylamido-2-methyl-propane sulfonic acid) (PNaAMPS) was recently shown to maintain mouse iPS cell pluripotency and long-term self-renewal in a feeder-free culture (51). Matrigel-coated polystyrene microcarriers in a stirred tank bioreactor have also been used to successfully expand hiPS cells (52). Further research is needed to identify appropriate matrix materials and coculture or feeder-free conditions to successfully expand iPS cells while maintaining pluripotency and functionality. Work like that of Lei & Schaffer (31a), discussed above, is a step in the right direction.

MANIPULATION OF CELL FATE FOR CELL THERAPIES AND DISEASE MODELING

As summarized above, there are a number of ways in which iPS cells can be generated, and the methodologies are being continuously optimized to improve efficiency and enable clinical applications. There are also a number of ways in which the fate of iPS cells can be

directed or specific cell types can be derived from fibroblasts by direct reprogramming (Figure 2), as summarized in this section. The cells generated from iPS cells or direct reprogramming have demonstrated potential for in vivo therapies and in vitro disease modeling.

Directed Differentiation into Specific Lineages

An advantage of iPS cells is their potential for generating autologous cells for disease modeling, drug screening, and cell therapies. In general, the protocols that have been developed for ES cells can be used to differentiate iPS cells into specific cell types, including cardiomyocytes, vascular cells, neural cells, and hepatocytes. Many approaches have been explored to direct cell differentiation, including 3D cultures [i.e., embryoid bodies (EB), spheroids, rosettes]; coculture with supporting cells; monolayer cultures with specific growth factors, cytokines, and signaling inhibitors; and biophysical (electrical, mechanical) stimulation. In some cases, when cell development and differentiation pathways are well characterized, it is also feasible to isolate precursor cells at intermediate stages and direct their further differentiation in vitro.

Cardiovascular cells—EB culture results in a heterogeneous population of cells and is a common method for generating beating cardiomyocytes from pluripotent stem cells. iPS cells can be differentiated into functional cardiomyocytes in EB culture, although the efficiency of differentiation of some iPS cell lines into cardiomyocytes is lower than that of ES cells (53–55). Growth factors and cytokines such as granulocyte colony-stimulating factor receptor (G-CSFR) can boost the yield of cardiomyocytes (56), and biophysical factors can regulate cardiomyocyte maturation and function. There is evidence that embryonic cardiomyocytes beat best on a matrix with heart-like elasticity and the beating is inhibited by scar-like rigidity (57). In addition, 3D cell cultivation followed by electrical stimulation using biowires, collagen wires made on the polydimethylsiloxane (PDMS) microgrooves embedded with cells, has been shown to promote the maturation of cardiac tissues (58).

Mouse and human iPS cells can also differentiate into fetal liver kinase-1 (Flk1/KDR)-expressing cells and then be directed to specific lineages such as cardiomyocytes, endothelial cells (EC), and mural cells (59–61). A combination of activin A, bone morphogenetic protein-4 (BMP-4), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and Dickkopf homolog 1 (DKK1) can increase Flk1/KDR-expressing cell populations in EB culture, which, in turn, generate >50% contracting cardiomyocytes in 2D culture (59). Alternatively, Isl1-expressing multipotent cardiovascular progenitors can be generated from mouse iPS cells and have been shown to spontaneously differentiate into cardiomyocytes, EC, and mural cells (62).

In addition to Flk1/KDR- and Isl1-expressing progenitors, vascular cells can also be derived from $CD34^+$ progenitor cells. A higher proportion of $CD34^+$ cells (~20%) could be derived from hiPS cells through the inhibition of MEK/ERK signaling and the activation of BMP-4 signaling; these cells can further differentiate into EC and mural cells and contribute to neovasculogenesis in ischemic muscle (63). When cocultured with OP9 cells, hiPS cells

generate $CD31^+CD43^-$EC and $CD34^+CD43^+$ hematopoietic progenitors (64). Hematopoietic progenitors can also be generated from EB culture, followed by differentiation into blood cell lineages using VEGF and hematopoietic cytokines in a serum-free medium (65). It is worth noting that hematopoietic progenitors from hiPS cells exhibit limited expansion potential and early senescence (5).

Neural lineages—The differentiation of iPS cells into various neural cells has been widely studied. iPS cells can differentiate into neural stem cells (NSC) and neural crest stem cells (NCSC) and, subsequently, into specific neural lineages (66–68). In general, NSC can be isolated from the central region of EB-derived rosettes, whereas NCSC are found in the peripheral regions of rosettes. The conversion of iPS cells into neural lineages is significantly enhanced by the inhibition of TGF-β receptors and SMAD signaling (69, 70). To obviate the need for protocols based on EB culture, E-cadherin and N-cadherin can be immobilized on an engineered substratum to derive highly homogeneous populations of primitive ectoderm and NSC (71). A nerve growth factor (NGF)-coated porous polymer surface also enhances neural differentiation (72). In addition, the biophysical properties of the substrate regulate neural differentiation. Soft substrates promote neurogenic differentiation of pluripotent stem cells (73). For NSC differentiation, softer (~100–500-Pa) gels greatly favor neurons, whereas harder (~1,000–10,000-Pa) gels promote glial differentiation (74). There is also evidence that surface topography modulates the neural fate of pluripotent stem cells; the anisotropic patterns, which are like gratings, promote neuronal differentiation, whereas the isotropic patterns, which are like pillars and wells, promote glial differentiation (75). How to combine these approaches and optimize the culture conditions with biochemical and biophysical factors to enrich a specific neural lineage remains to be explored.

Neurons and glial cells derived from iPS cells have been tested in animal models to treat diseases and regenerate tissues in the central and peripheral nervous systems. For example, iPS cell–derived dopamine neurons improve the behavior of rats with Parkinson disease (76). In addition, iPS cells derived from patients with Parkinson disease can serve as an in vitro model for mechanistic studies (77). Similarly, motor neurons and their progenitors can be derived from iPS cells for the in vitro modeling of motor neuron diseases such as amyotrophic lateral sclerosis (ALS) (78, 79); functional neurons can be obtained from iPS cells generated from the fibroblasts of Rett syndrome patients as an in vitro model of autism spectrum disorders for drug screening (80); and neurons from schizophrenia iPS cells show diminished neuronal connectivity and offer insight into the genetic profile of this complex psychiatric disorder (81).

Multipotent NSC and NCSC have also been explored for neural tissue regeneration. iPS cell–derived NCSC can be used to treat peripheral neuropathy such as familial dysautonomia (82). NCSC, when transplanted into nerve conduits, can differentiate into Schwann cells to promote myelination and, thus, the regeneration of functional peripheral nerve regeneration (83). Neurospheres include a mixed cell population and have potential to differentiate into functional neurons, astrocytes, and oligodendrocytes. When iPS cell–derived neurospheres are transplanted into the spinal cord following contusive injury, they

differentiate into all three neural lineages, participate in remyelination, induce axon regrowth, and promote locomotor function recovery (84).

Hepatocytes—Hepatocytes have limited expansion potential, yet there is a great need to use hepatocytes to treat liver failure and to test drug toxicity. An efficient endoderm differentiation from iPS cells can be induced by using activin A or a combination of hepatocyte growth factor (HGF), activin A, and Wnt3a (85–87). Hepatocyte-like cells are then derived using BMP-2/bFGF and HGF under low oxygen tension. iPS cell–derived hepatocytes have a gene expression profile similar to that of mature hepatocytes and were able to rescue lethal fulminant hepatic failure in a mouse model (87).

Direct Reprogramming

Direct conversion of existing somatic cells into a different cell type would eliminate the need to revert cells to a pluripotent state and then direct cell differentiation. Direct reprogramming can be achieved by either expressing master transcriptional regulators for specific target cell types or partial reprogramming to direct the incomplete iPS cell reprogramming process to specific differentiation pathways. These approaches may also enable cell reprogramming in vivo for therapeutic treatment.

Direct lineage conversion—One example of in vivo cell reprogramming demonstrates that reexpression of key transcription factors (Ngn3, Pdx1, and Mafa) in pancreatic exocrine cells in adult mice enables reprogramming into insulin-secreted β-cells (88). Transcription factor expression has also been used to generate functional cardiomyocytes, neurons, and hepatocytes. A combination of three developmentally important transcription factors (Gata4, Mef2C, and Tbx5) rapidly and efficiently reprograms postnatal cardiac or dermal fibroblasts into differentiated cardiomyocyte-like cells (89). Forced expression of these transcription factors in ischemic heart reprograms cardiac fibroblasts into cardiomyocytes, decreases infarct size, and modestly attenuates cardiac dysfunction (90). An alternative combination of four transcription factors (Gata4, Mef2C, Tbx5, and Hand2) can also reprogram adult fibroblasts into beating cardiac-like myocytes in vitro and in vivo, improve cardiac function, and reduce adverse ventricular remodeling following myocardial infarction (91). However, the reprogramming efficiency of mature cardiomyocytes needs further improvement, and the optimal combination of transcriptional factors and chemical compounds for cardiomyocyte reprogramming awaits further investigation.

A combination of the transcription factors Ascl1, Brn2, and Myt1l is sufficient to efficiently convert mouse fibroblasts into functional neurons in vitro (92). In addition, replacement of Ascl1 with a microRNA (miR-124) can directly reprogram adult human primary dermal fibroblasts into functional neurons (93). Functional conversion of endogenous cells in the adult brain to induced neuronal fates is also possible. Brain pericytes can be reprogrammed into neuronal cells by retrovirus-mediated coexpression of transcription factors Sox2 and Ascl1/Mash1; these induced neuronal cells acquire the ability of repetitive action-potential firing and serve as synaptic targets for other neurons (94).

Direct conversion of differentiated cells into hepatocytes has been achieved as well. Forced expression of Gata4, Hnf1α, and Foxa3 combined with inactivation of p19(Arf) results in

induction of functional hepatocyte-like (iHep) cells from mouse fibroblasts, which are capable of restoring liver functions (95). Alternatively, specific combinations of two transcription factors (Hnf4α plus Foxa1, Foxa2, or Foxa3) can convert mouse fibroblasts into iHep cells in vitro and reconstitute damaged hepatic tissues after transplantation (96).

Partial iPS cell reprogramming—An alternative reprogramming strategy is to shortcut iPS cell reprogramming at the early stage and redirect cell fate by using growth factors and chemical compounds. This approach has been used to generate cardiomyocytes, NSC, and vascular cells. As early as 4 days post iPS cell reprogramming, partially reprogrammed iPS cells (PiPS cells) were switched to cardiogenic medium with BMP-4 and JAK-STAT inhibitor (preventing iPS cell generation) and converted into spontaneously contracting patches of differentiated cardiomyocytes (97). Similarly, constitutively inducing Sox2, Klf4, and c-Myc while strictly limiting Oct4 activity to the initial phase of reprogramming generated expandable NSC with the potential to differentiate into neurons, astrocytes, and oligodendrocytes (98). PiPS cells, when treated with VEGF, differentiated into EC that can improve neovascularization and blood flow recovery in a model of hind-limb ischemia (99). When PiPS cells are seeded on collagen IV and maintained in smooth muscle cell (SMC) differentiation media, SMC-like cells are derived, which can repopulate decellularized vessel grafts and ultimately give rise to functional tissue-engineered vessels (100).

CLINICAL APPLICATIONS

As reviewed above, bioengineering strategies can facilitate the reprogramming, expansion, isolation, and directed differentiation of iPS cells. Continuous improvement in these technologies will be required to harness the potential of iPS cells for clinical applications, including cell replacement, disease modeling, and drug screening, as discussed below.

Cell Replacement

iPS cells hold tremendous promise for regenerative medicine, especially for replacing diseased or injured cells in target organs. A key challenge in translating this promise into clinical reality is our ability to efficiently deliver iPS cells or iPS cell–derived progenitors and therapeutic cells to target tissues while maintaining high viability and functionality.

Cell delivery—Delivery of iPS cells and their progeny into internal organs can currently be achieved either through (*a*) intravenous injection of cells with the expectation that they will home to the site of disease or injury or (*b*) local administration of the cells via catheter placement or following open surgery. For local delivery, injectable and implantable biomaterial scaffolds are being used, similarly to strategies explored in tissue engineering for decades (e.g., in cardiac cell therapies as reviewed in 101). By contrast, for systemic delivery, cells are generally injected naked (i.e., without carrier cells) in a buffer, although this often results in high levels of cell death (102). Recent reports have suggested that delivering stem and therapeutic cells in polymeric hydrogels of specific mechanical modulus could significantly increase their viability during the injection process by reducing the membrane shear forces experienced by cells during injection and needle ejection (102). However, whether this strategy can be useful in systemic delivery to specific organs remains to be seen, particularly because only a small percentage of the surviving injected cells are

expected to home to, and engraft within, target organs. In this context, local delivery avoids the issue of homing and could provide significant improvement in engraftment. Specifically, using scaffold-based delivery and instructive materials that allow for cell survival and proliferation while affecting specific signaling pathways in a predesigned manner could provide the necessary niche in diseased tissues that allows efficient engraftment.

Cell survival and function—Strategies to achieve increased survival of iPS cell–derived cells upon transplantation could include local immune modulation to reduce the inflammatory response and thereby reduce stem cell apoptosis. Codelivery of growth factors, extracellular matrix components, and supporting cells (i.e., stromal cells) could also improve the survival and optimal function of iPS cell–derived cell types, especially lineage-specific stem and progenitor cells that are typically regulated by the cells within their surrounding microenvironment (niche). In addition, it may be possible to mimic the essential functions of niche cells via functionalization of the delivery scaffold with appropriate cell-signaling ligands. These areas have barely been explored, especially with respect to iPS cells, and need significant attention in order to translate iPS cell–based therapies to clinical reality.

Disease Modeling and High-Throughput Drug Screening

One major advantage of somatic cell reprogramming is the ability to generate pluripotent stem cells from patients with specific genetic and chronic disorders. These disease-specific iPS cells can then be differentiated into specific lineages, thereby providing a potentially unlimited source of cells to study the initiation and progression of the specific disorder, as well as to study how therapeutic interventions would affect the diseased cells (i.e., drug screening and selection).

This potential of iPS cells has opened up a whole new aspect of research in which modeling and in vitro high-throughput evaluation of complex disease models are becoming a reality. Recently, iPS cells have been widely applied to studying cardiac diseases (e.g., long QT syndrome), neurodegenerative diseases (e.g., ALS and Alzheimer disease), and other disorders (103–107). Reprogramming of cells isolated from patients with long QT syndrome or ALS and subsequently differentiating those cells to cardiomyocytes or neurons provides biologically relevant disease models that were previously inaccessible to the scientific community. The pathology of the diseases, such as arrhythmia and long action potentials in long QT syndrome and cytosolic aggregation and short neuritis in ALS, were represented in the patient-specific iPS cell–derived cardiomyocytes and motor neurons, respectively. The effects of various therapeutic agents have been evaluated using these models to identify compounds and strategies that rescue or alleviate the pathology. This concept has enormous implications for drug discovery and clinical practice, as it has been extremely difficult to generate animal models of many such diseases that faithfully represent the corresponding human disease and allow identification of drug targets and understanding of effects of treatment. In addition to long QT syndrome and ALS, significant progress has been made in developing models for autoinflammatory disorders (CINCA) (108), Alzheimer disease (107), sickle cell disease (109), and ataxia (110).

It should be noted that this concept of high-throughput drug screening using iPS cell–derived somatic cells can also be applied to normal human cells (e.g., liver cells, neurons, or cardiac cells) to assess drug side effects in the general population. The potential extends to studies on gender-and ethnicity-specific effects of drugs, as well for studying effects on infants and children, which have otherwise been extremely difficult to achieve.

SUMMARY AND FUTURE CHALLENGES

The exciting and rapid advancement of cell reprogramming technologies has opened new avenues for regenerative medicine, disease modeling, and drug screening. To harness these potentials, one needs to further understand the fundamental mechanisms of cell reprograming in order to manipulate the process and improve the quality, efficiency, accuracy, and consistency of reprogramming.

Understanding the Mechanisms of Cell Reprogramming

Cell identity and phenotype are defined by heritable epigenetic state, including DNA methylation and histone modifications. The genetic circuits for cell reprogramming also involve a myriad of biomolecules, such as transcriptional factors, enzymes, signaling molecules, and miRNAs. Recent molecular and cell biology studies have unveiled how a limited number of reprogramming factors can initiate global and specific genomic remodeling that results in the change in cell fate. However, many questions remain to be addressed. Genome-wide epigenetic analysis is needed to provide insight into the whole picture of the spatial and temporal reprogramming process. Synthetic biology and novel genetic editing tools may enable the dissection of signaling events. Systems biology approaches may help generate models of complicated molecular network involved in reprogramming. Whereas iPS cell reprogramming techniques have been widely studied, little is known about the mechanisms of direct cell reprogramming. In addition, the molecular profile and the functions of reprogrammed cells need to be defined for quality control and therapeutic safety.

Engineering the Reprogramming Microenvironment In Vitro and In Vivo

The findings that small-molecule compounds can replace transcriptional factors for cell reprogramming make it possible to manipulate cell fate in a controlled microenvironment. The current methods of transcriptional factor–free reprogramming have low efficiency and are not consistent. The timing and dosage of specific biochemicals need to be optimized. The accuracy and efficiency of direct lineage conversion are critical for the safety and efficacy of in vivo therapies. If such therapies are realized, one may turn fibroblasts into functional cardiomyocytes in vivo, which would not only suppress scar formation but also improve heart muscle regeneration; to cure Parkinson disease, one may reprogram brain cells into dopamine neurons. Engineered nanoparticles and smart biomaterials that allow the controlled release may be developed to enhance reprogramming efficiency in vivo. Besides biochemical factors, the role of biophysical factors in epigenetic modifications and cell reprogramming needs further investigation. The mechanical forces in the microenvironment and the stiffness, topography, and micro/nanostructure of biomaterials may facilitate cell reprogramming, together with biochemical factors.

Patient-Specific Microorgan Systems

Although we have discussed the challenges of deriving distinct cell types from patient-specific iPS cells, there is another greater challenge on the horizon: creating patient-specific microorgan systems that can mimic selected functions of complex organs. Functional somatic cells within distinct tissue microenvironments require proper cell–cell and cell–matrix interactions to regulate cell phenotype and function as well as modulate cell responses to microenvironmental factors and drugs. Therefore, to optimally simulate in vivo tissue functions and closely mimic responses to drugs, it is desirable to fabricate 3D microtissue constructs that can be used for ex vivo testing. If this is achieved, one can envision devising strategies to functionally integrate such microorgans through biological or artificial perfusion systems. Such integrated organ systems can then be used to understand how disease or drug metabolism in one organ affects the functioning of other organs.

LITERATURE CITED

1. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006; 126:663–76. [PubMed: 16904174]
2. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007; 318:1917–20. [PubMed: 18029452]
3. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007; 131:861–72. [PubMed: 18035408]
4. Chin MH, Mason MJ, Xie W, Volinia S, Singer M, et al. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. Cell Stem Cell. 2009; 5:111–23. [PubMed: 19570518]
5. Feng Q, Lu SJ, Klimanskaya I, Gomes I, Kim D, et al. Hemangioblastic derivatives from human induced pluripotent stem cells exhibit limited expansion and early senescence. Stem Cells. 2010; 28:704–12. [PubMed: 20155819]
6. Narsinh KH, Sun N, Sanchez-Freire V, Lee AS, Almeida P, et al. Single cell transcriptional profiling reveals heterogeneity of human induced pluripotent stem cells. J Clin Investig. 2011; 121:1217–21. [PubMed: 21317531]
7. Bilic J, Izpisua Belmonte JC. Concise review: Induced pluripotent stem cells versus embryonic stem cells: close enough or yet too far apart? Stem Cells. 2012; 30:33–41. [PubMed: 22213481]
8. Abyzov A, Mariani J, Palejev D, Zhang Y, Haney MS, et al. Somatic copy number mosaicism in human skin revealed by induced pluripotent stem cells. Nature. 2012; 492:438–42. [PubMed: 23160490]
9. Yamanaka S. Induced pluripotent stem cells: past, present and future. Cell Stem Cell. 2012; 10:678–84. [PubMed: 22704507]
10. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature. 2007; 448:313–17. [PubMed: 17554338]
11. Zhao T, Zhang ZN, Rong Z, Xu Y. Immunogenicity of induced pluripotent stem cells. Nature. 2011; 474:212–15. [PubMed: 21572395]
12. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. Science. 2008; 322:949–53. [PubMed: 18845712]
13. Okita K, Matsumura Y, Sato Y, Okada A, Morizane A, et al. A more eficient method to generate integration-free human iPS cells. Nat Methods. 2011; 8:409–12. [PubMed: 21460823]
14. Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell. 2010; 7:618–30. [PubMed: 20888316]

15. Kim D, Kim CH, Moon JI, Chung YG, Chang MY, et al. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell. 2009; 4:472–76. [PubMed: 19481515]
16. Judson RL, Babiarz JE, Venere M, Blelloch R. Embryonic stem cell–specific microRNAs promote induced pluripotency. Nat Biotechnol. 2009; 27:459–61. [PubMed: 19363475]
17. Melton C, Judson RL, Blelloch R. Opposing microRNA families regulate self-renewal in mouse embryonic stem cells. Nature. 2010; 463:621–26. [PubMed: 20054295]
18. Anokye-Danso F, Trivedi CM, Juhr D, Gupta M, Cui Z, et al. Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell. 2011; 8:376–88. [PubMed: 21474102]
19. Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, et al. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nat Biotechnol. 2008; 26:795–97. [PubMed: 18568017]
20. Li Y, Zhang Q, Yin X, Yang W, Du Y, et al. Generation of iPSCs from mouse fibroblasts with a single gene, *Oct4*, and small molecules. Cell Res. 2011; 21:196–204. [PubMed: 20956998]
21. Li W, Tian E, Chen ZX, Sun G, Ye P, et al. Identification of Oct4-activating compounds that enhance reprogramming efficiency. Proc Natl Acad Sci USA. 2012; 109:20853–58. [PubMed: 23213213]
22. Ichida JK, Blanchard J, Lam K, Son EY, Chung JE, et al. A small-molecule inhibitor of Tgf-β signaling replaces *Sox2* in reprogramming by inducing*Nanog*. Cell Stem Cell. 2009; 5:491–503. [PubMed: 19818703]
23. Lin T, Ambasudhan R, Yuan X, Li W, Hilcove S, et al. A chemical platform for improved induction of human iPSCs. Nat Methods. 2009; 6:805–8. [PubMed: 19838168]
24. Shi Y, Desponts C, Do JT, Hahm HS, Scholer HR, Ding S. Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. Cell Stem Cell. 2008; 3:568–74. [PubMed: 18983970]
25. Buganim Y, Faddah DA, Jaenisch R. Mechanisms and models of somatic cell reprogramming. Nat Rev Genet. 2013; 14:427–39. [PubMed: 23681063]
26. Hou P, Li Y, Zhang X, Liu C, Guan J, et al. Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. Science. 2013; 341:651–54. [PubMed: 23868920]
27. Downing TL, Soto J, Morez C, Houssin T, Fritz A, et al. Biophysical regulation of epigenetic state and cell reprogramming. Nat Mater. 2013; 12:1154–62. [PubMed: 24141451]
28. Fluri DA, Tonge PD, Song H, Baptista RP, Shakiba N, et al. Derivation, expansion and differentiation of induced pluripotent stem cells in continuous suspension cultures. Nat Methods. 2012; 9:509–16. [PubMed: 22447133]
29. Zhao Y, Yin X, Qin H, Zhu F, Liu H, et al. Two supporting factors greatly improve the efficiency of human iPSC generation. Cell Stem Cell. 2008; 3(5):475–79. [PubMed: 18983962]
30. Singh A, Suri S, Lee T, Chilton JM, Cooke MT, et al. Adhesion strength-based, label-free isolation of human pluripotent stem cells. Nat Methods. 2013; 10(5):438–44. [PubMed: 23563795]
31. Rais Y, Zviran A, Geula S, Gafni O, Chomsky E, et al. Deterministic direct reprogramming of somatic cells to pluripotency. Nature. 2013; 502:65–70. [PubMed: 24048479]
31a. Lei Y, Schaffer DV. A fully defined and scalable 3D culture system for human pluripotent stem cell expansion and differentiation. Proc Natl Acad Sci USA. 2013; 110(52):5039–48.
32. Serra M, Brito C, Correia C, Alves PM. Process engineering of human pluripotent stem cells for clinical application. Trends Biotechnol. 2012; 30(6):350–59. [PubMed: 22541338]
33. Wilson JL, McDevitt TC. Stem cell microencapsulation for phenotypic control, bioprocessing, and transplantation. Biotechnol Bioeng. 2013; 110(3):667–82. [PubMed: 23239279]
34. Villa-Diaz LG, Ross AM, Lahann J, Krebsbach PH. Concise review: The evolution of human pluripotent stem cell culture: from feeder cells to synthetic coatings. Stem Cells. 2013; 31(1):1–7. [PubMed: 23081828]
35. de Peppo GM, Sladkova M, Sjövall P, Palmquist A, Oudina K, et al. Human embryonic stem cell-derived mesodermal progenitors display substantially increased tissue formation compared to human mesenchymal stem cells under dynamic culture conditions in a packed bed/column bioreactor. Tissue Eng Part A. 2013; 19(1–2):175–87. [PubMed: 22924642]

36. Serra M, Brito C, Correia C, Alves PM. Process engineering of human pluripotent stem cells for clinical application. Trends Biotechnol. 2012; 30(6):350–59. [PubMed: 22541338]
37. Nakagawa Y, Nakamura S, Nakajima M, Endo H, Dohda T, et al. Two differential flows in a bioreactor promoted platelet generation from human pluripotent stem cell-derived megakaryocytes. Exp Hematol. 2013; 41(8):742–48. [PubMed: 23618622]
38. Cimetta E, Sirabella D, Yeager K, Davidson K, Simon J, et al. Microfluidic bioreactor for dynamic regulation of early mesodermal commitment in human pluripotent stem cells. Lab Chip. 2013; 13(3):355–64. [PubMed: 23232509]
39. Shafa M, Day B, Yamashita A, Meng G, Liu S, et al. Derivation of iPSCs in stirred suspension bioreactors. Nat Methods. 2012; 9(5):465–66. [PubMed: 22484846]
40. Kinney MA, Sargent CY, McDevitt TC. The multiparametric effects of hydrodynamic environments on stem cell culture. Tissue Eng Part B Rev. 2011; 17(4):249–62. [PubMed: 21491967]
41. Fridley KM, Fernandez I, Li MT, Kettlewell RB, Roy K. Unique differentiation profile of mouse embryonic stem cells in rotary and stirred tank bioreactors. Tissue Eng Part A. 2010; 16(11):3285–98. [PubMed: 20528675]
42. de Peppo GM, Marcos-Campos I, Kahler DJ, Alsalman D, Shang L, et al. Engineering bone tissue substitutes from human induced pluripotent stem cells. Proc Natl Acad Sci USA. 2013; 110(21): 8680–85. [PubMed: 23653480]
43. Lu L, Mende M, Yang X, Körber HF, Schnittler HJ, et al. Design and validation of a bioreactor for simulating the cardiac niche: a system incorporating cyclic stretch, electrical stimulation, and constant perfusion. Tissue Eng Part A. 2012; 19(3–4):403–14. [PubMed: 22991978]
44. Liao J, Guo X, Grande-Allen KJ, Kasper FK, Mikos AG. Bioactive polymer/extracellular matrix scaffolds fabricated with a flow perfusion bioreactor for cartilage tissue engineering. Biomaterials. 2010; 31(34):8911–20. [PubMed: 20797784]
45. Want AJ, Nienow AW, Hewitt CJ, Coopman K. Large-scale expansion and exploitation of pluripotent stem cells for regenerative medicine purposes: beyond the T flask. Regen Med. 2012; 7(1):71–84. [PubMed: 22168499]
46. Sun LY, Lin SZ, Li YS, Harn HJ, Chiou TW. Functional cells cultured on microcarriers for use in regenerative medicine research. Cell Transplant. 2011; 20(1):49–62. [PubMed: 20887678]
47. Olmer R, Haase A, Merkert S, Cui W, Palecek J, et al. Long term expansion of undifferentiated human iPS and ES cells in suspension culture using a defined medium. Stem Cell Res. 2010; 5(1): 51–64. [PubMed: 20478754]
48. Cao N, Liang H, Huang J, Wang J, Chen Y, et al. Highly efficient induction and long-term maintenance of multipotent cardiovascular progenitors from human pluripotent stem cells under defined conditions. Cell Res. 2013; 23(9):1119–32. [PubMed: 23896987]
49. Wang Y, Chou BK, Dowey S, He C, Gerecht S, Cheng L. Scalable expansion of human induced pluripotent stem cells in the defined xeno-free E8 medium under adherent and suspension culture conditions. Stem Cell Res. 2013; 11(3):1103–16. [PubMed: 23973800]
50. Fontes A, Macarthur CC, Lieu PT, Vemuri MC. Generation of human-induced pluripotent stem cells (hiPSCs) using episomal vectors on defined Essential 8™ Medium conditions. Methods Mol Biol. 2013; 997:57–72. [PubMed: 23546748]
51. Yang JJ, Liu JF, Kurokawa T, Kitada K, Gong JP. Hydrogels as feeder-free scaffolds for long-term self-renewal of mouse induced pluripotent stem cells. J Tissue Eng Regen Med. 2012 In press. 10.1002/term.1640
52. Kehoe DE, Jing D, Lock LT, Tzanakakis ES. Scalable stirred-suspension bioreactor culture of human pluripotent stem cells. Tissue Eng Part A. 2010; 16(2):405–21. [PubMed: 19739936]
53. Mauritz C, Schwanke K, Reppel M, Neef S, Katsirntaki K, et al. Generation of functional murine cardiac myocytes from induced pluripotent stem cells. Circulation. 2008; 118:507–17. [PubMed: 18625890]
54. Zhang J, Wilson GF, Soerens AG, Koonce CH, Yu J, et al. Functional cardiomyocytes derived from human induced pluripotent stem cells. Circ Res. 2009; 104:e30–41. [PubMed: 19213953]
55. Zwi L, Caspi O, Arbel G, Huber I, Gepstein A, et al. Cardiomyocyte differentiation of human induced pluripotent stem cells. Circulation. 2009; 120:1513–23. [PubMed: 19786631]

56. Shimoji K, Yuasa S, Onizuka T, Hattori F, Tanaka T, et al. G-CSF promotes the proliferation of developing cardiomyocytes in vivo and in derivation from ESCs and iPSCs. Cell Stem Cell. 2010; 6:227–37. [PubMed: 20207226]
57. Engler AJ, Carag-Krieger C, Johnson CP, Raab M, Tang HY, et al. Embryonic cardiomyocytes beat best on a matrix with heart-like elasticity: Scar-like rigidity inhibits beating. J Cell Sci. 2008; 121:3794–802. [PubMed: 18957515]
58. Nunes SS, Miklas JW, Liu J, Aschar-Sobbi R, Xiao Y, et al. Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes. Nat Methods. 2013; 10:781–87. [PubMed: 23793239]
59. Yang L, Soonpaa MH, Adler ED, Roepke TK, Kattman SJ, et al. Human cardiovascular progenitor cells develop from a KDR^{+} embryonic-stem-cell-derived population. Nature. 2008; 453:524–28. [PubMed: 18432194]
60. Narazaki G, Uosaki H, Teranishi M, Okita K, Kim B, et al. Directed and systematic differentiation of cardiovascular cells from mouse induced pluripotent stem cells. Circulation. 2008; 118:498–506. [PubMed: 18625891]
61. Taura D, Sone M, Homma K, Oyamada N, Takahashi K, et al. Induction and isolation of vascular cells from human induced pluripotent stem cells—brief report. Arterioscler Thromb Vasc Biol. 2009; 29:1100–3. [PubMed: 19423866]
62. Moretti A, Bellin M, Jung CB, Thies TM, Takashima Y, et al. Mouse and human induced pluripotent stem cells as a source for multipotent $Isl1^{+}$ cardiovascular progenitors. FASEB J. 2010; 24:700–11. [PubMed: 19850773]
63. Park SW, Koh YJ, Jeon J, Cho YH, Jang MJ, et al. Efficient differentiation of human pluripotent stem cells into functional $CD34^{+}$ progenitor cells by combined modulation of the MEK/ERK and BMP4 signaling pathways. Blood. 2010; 116:5762–72. [PubMed: 20884805]
64. Choi KD, Yu J, Smuga-Otto K, Salvagiotto G, Rehrauer W, et al. Hematopoietic and endothelial differentiation of human induced pluripotent stem cells. Stem Cells. 2009; 27:559–67. [PubMed: 19259936]
65. Grigoriadis AE, Kennedy M, Bozec A, Brunton F, Stenbeck G, et al. Directed differentiation of hematopoietic precursors and functional osteoclasts from human ES and iPS cells. Blood. 2010; 115:2769–76. [PubMed: 20065292]
66. Hu BY, Zhang SC. Differentiation of spinal motor neurons from pluripotent human stem cells. Nat Protoc. 2009; 4:1295–304. [PubMed: 19696748]
67. Hu BY, Du ZW, Zhang SC. Differentiation of human oligodendrocytes from pluripotent stem cells. Nat Protoc. 2009; 4:1614–22. [PubMed: 19834476]
68. Lee G, Chambers SM, Tomishima MJ, Studer L. Derivation of neural crest cells from human pluripotent stem cells. Nat Protoc. 2010; 5:688–701. [PubMed: 20360764]
69. Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat Biotechnol. 2009; 27:275–80. [PubMed: 19252484]
70. Zhou J, Su P, Li D, Tsang S, Duan E, Wang F. High-efficiency induction of neural conversion in human ESCs and human induced pluripotent stem cells with a single chemical inhibitor of transforming growth factor beta superfamily receptors. Stem Cells. 2010; 28:1741–50. [PubMed: 20734356]
71. Haque A, Yue XS, Motazedian A, Tagawa Y, Akaike T. Characterization and neural differentiation of mouse embryonic and induced pluripotent stem cells on cadherin-based substrata. Biomaterials. 2012; 33:5094–106. [PubMed: 22520296]
72. Kuo YC, Huang MJ. Material-driven differentiation of induced pluripotent stem cells in neuron growth factor-grafted poly(epsilon-caprolactone)-poly(beta-hydroxybutyrate) scaffolds. Biomaterials. 2012; 33:5672–82. [PubMed: 22591608]
73. Keung AJ, Asuri P, Kumar S, Schaffer DV. Soft microenvironments promote the early neurogenic differentiation but not self-renewal of human pluripotent stem cells. Integr Biol (Camb). 2012; 4:1049–58. [PubMed: 22854634]
74. Saha K, Keung AJ, Irwin EF, Li Y, Little L, et al. Substrate modulus directs neural stem cell behavior. Biophys J. 2008; 95:4426–38. [PubMed: 18658232]

75. Ankam S, Suryana M, Chan LY, Moe AA, Teo BK, et al. Substrate topography and size determine the fate of human embryonic stem cells to neuronal or glial lineage. Acta Biomater. 2013; 9:4535–45. [PubMed: 22906625]
76. Wernig M, Zhao JP, Pruszak J, Hedlund E, Fu D, et al. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc Natl Acad Sci USA. 2008; 105:5856–61. [PubMed: 18391196]
77. Soldner F, Hockemeyer D, Beard C, Gao Q, Bell GW, et al. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell. 2009; 136:964–77. [PubMed: 19269371]
78. Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science. 2008; 321:1218–21. [PubMed: 18669821]
79. Karumbayaram S, Novitch BG, Patterson M, Umbach JA, Richter L, et al. Directed differentiation of human-induced pluripotent stem cells generates active motor neurons. Stem Cells. 2009; 27:806–11. [PubMed: 19350680]
80. Marchetto MC, Carromeu C, Acab A, Yu D, Yeo GW, et al. A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. Cell. 2010; 143:527–39. [PubMed: 21074045]
81. Brennand KJ, Simone A, Jou J, Gelboin-Burkhart C, Tran N, et al. Modelling schizophrenia using human induced pluripotent stem cells. Nature. 2011; 473:221–25. [PubMed: 21490598]
82. Lee G, Papapetrou EP, Kim H, Chambers SM, Tomishima MJ, et al. Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. Nature. 2009; 461:402–6. [PubMed: 19693009]
83. Wang A, Tang Z, Park IH, Zhu Y, Patel S, et al. Induced pluripotent stem cells for neural tissue engineering. Biomaterials. 2011; 32:5023–32. [PubMed: 21514663]
84. Tsuji O, Miura K, Okada Y, Fujiyoshi K, Mukaino M, et al. Therapeutic potential of appropriately evaluated safe-induced pluripotent stem cells for spinal cord injury. Proc Natl Acad Sci USA. 2010; 107:12704–9. [PubMed: 20615974]
85. Si-Tayeb K, Noto FK, Sepac A, Sedlic F, Bosnjak ZJ, et al. Generation of human induced pluripotent stem cells by simple transient transfection of plasmid DNA encoding reprogramming factors. BMC Dev Biol. 2010; 10:81. [PubMed: 20682060]
86. Sullivan GJ, Hay DC, Park IH, Fletcher J, Hannoun Z, et al. Generation of functional human hepatic endoderm from human induced pluripotent stem cells. Hepatology. 2010; 51:329–35. [PubMed: 19877180]
87. Chen YF, Tseng CY, Wang HW, Kuo HC, Yang VW, Lee OK. Rapid generation of mature hepatocyte-like cells from human induced pluripotent stem cells by an efficient three-step protocol. Hepatology. 2012; 55:1193–203. [PubMed: 22095466]
88. Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. In vivo reprogramming of adult pancreatic exocrine cells to β-cells. Nature. 2008; 455:627–32. [PubMed: 18754011]
89. Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, et al. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell. 2010; 142:375–86. [PubMed: 20691899]
90. Qian L, Huang Y, Spencer CI, Foley A, Vedantham V, et al. In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. Nature. 2012; 485:593–98. [PubMed: 22522929]
91. Song K, Nam YJ, Luo X, Qi X, Tan W, et al. Heart repair by reprogramming non-myocytes with cardiac transcription factors. Nature. 2012; 485:599–604. [PubMed: 22660318]
92. Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Südhof TC, Wernig M. Direct conversion of fibroblasts to functional neurons by defined factors. Nature. 2010; 463:1035–41. [PubMed: 20107439]
93. Ambasudhan R, Talantova M, Coleman R, Yuan X, Zhu S, et al. Direct reprogramming of adult human fibroblasts to functional neurons under defined conditions. Cell Stem Cell. 2011; 9:113–18. [PubMed: 21802386]

94. Karow M, Sanchez R, Schichor C, Masserdotti G, Ortega F, et al. Reprogramming of pericyte-derived cells of the adult human brain into induced neuronal cells. Cell Stem Cell. 2012; 11:471–76. [PubMed: 23040476]

95. Huang P, He Z, Ji S, Sun H, Xiang D, et al. Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. Nature. 2011; 475:386–89. [PubMed: 21562492]

96. Sekiya S, Suzuki A. Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors. Nature. 2011; 475:390–93. [PubMed: 21716291]

97. Efe JA, Hilcove S, Kim J, Zhou H, Ouyang K, et al. Conversion of mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy. Nat Cell Biol. 2011; 13:215–22. [PubMed: 21278734]

98. Thier M, Worsdorfer P, Lakes YB, Gorris R, Herms S, et al. Direct conversion of fibroblasts into stably expandable neural stem cells. Cell Stem Cell. 2012; 10:473–79. [PubMed: 22445518]

99. Margariti A, Winkler B, Karamariti E, Zampetaki A, Tsai TN, et al. Direct reprogramming of fibroblasts into endothelial cells capable of angiogenesis and reendothelialization in tissue-engineered vessels. Proc Natl Acad Sci USA. 2012; 109:13793–98. [PubMed: 22869753]

100. Karamariti E, Margariti A, Winkler B, Wang X, Hong X, et al. Smooth muscle cells differentiated from reprogrammed embryonic lung fibroblasts through DKK3 signaling are potent for tissue engineering of vascular grafts. Circ Res. 2013; 112:1433–43. [PubMed: 23529184]

101. Nunes SS, Song H, Chiang CK, Radisic M. Stem cell-based cardiac tissue engineering. J Cardiovasc Transl Res. 2011; 4(5):592–602. [PubMed: 21748529]

102. Aguado BA, Mulyasasmita W, Su J, Lampe KJ, Heilshorn SC. Improving viability of stem cells during syringe needle flow through the design of hydrogel cell carriers. Tissue Eng Part A. 2012; 18(7–8):806–15. [PubMed: 22011213]

103. Bellin M, Marchetto MC, Gage FH, Mummery CL. Induced pluripotent stem cells: the new patient? Nat Rev Mol Cell Biol. 2012; 13(11):713–26. [PubMed: 23034453]

104. Malan D, Friedrichs S, Fleischmann BK, Sasse P. Cardiomyocytes obtained from induced pluripotent stem cells with long-QT syndrome 3 recapitulate typical disease-specific features in vitro. Circ Res. 2011; 109(8):841–47. [PubMed: 21799153]

105. Sinnecker D, Goedel A, Dorn T, Dirschinger RJ, Moretti A, Laugwitz KL. Modeling long-QT syndromes with iPS cells. J Cardiovasc Transl Res. 2013; 6(1):31–36. [PubMed: 23076501]

106. Song B, Sun G, Herszfeld D, Sylvain A, Campanale NV, et al. Neural differentiation of patient specific iPS cells as a novel approach to study the pathophysiology of multiple sclerosis. Stem Cell Res. 2012; 8(2):259–73. [PubMed: 22265745]

107. Yahata N, Asai M, Kitaoka S, Takahashi K, Asaka I, et al. Anti-Aβ drug screening platform using human iPS cell-derived neurons for the treatment of Alzheimer's disease. PLoS ONE. 2011; 6(9):e25788. [PubMed: 21984949]

108. Tanaka T, Takahashi K, Yamane M, Tomida S, Nakamura S, et al. Induced pluripotent stem cells from CINCA syndrome patients as a model for dissecting somatic mosaicism and drug discovery. Blood. 2012; 120(6):1299–308. [PubMed: 22723549]

109. Zou J, Mali P, Huang X, Dowey SN, Cheng L. Site-specific gene correction of a point mutation in human iPS cells derived from an adult patient with sickle cell disease. Blood. 2011; 118(17): 4599–608. [PubMed: 21881051]

110. Liu J, Verma PJ, Evans-Galea MV, Delatycki MB, Michalska A, et al. Generation of induced pluripotent stem cell lines from Friedreich ataxia patients. Stem Cell Rev. 2011; 7(3):703–13. [PubMed: 21181307]

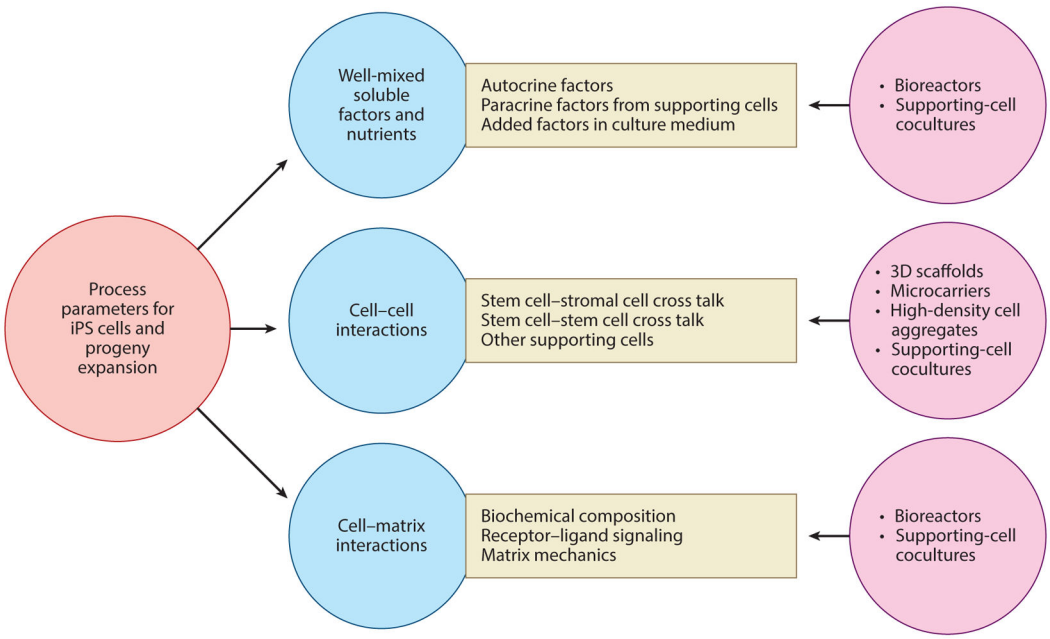

Figure 1.

Niche-specific factors that could influence iPS cell differentiation, expansion of undifferentiated cells while maintaining pluripotency, and expansion of iPS cell–derived progeny.

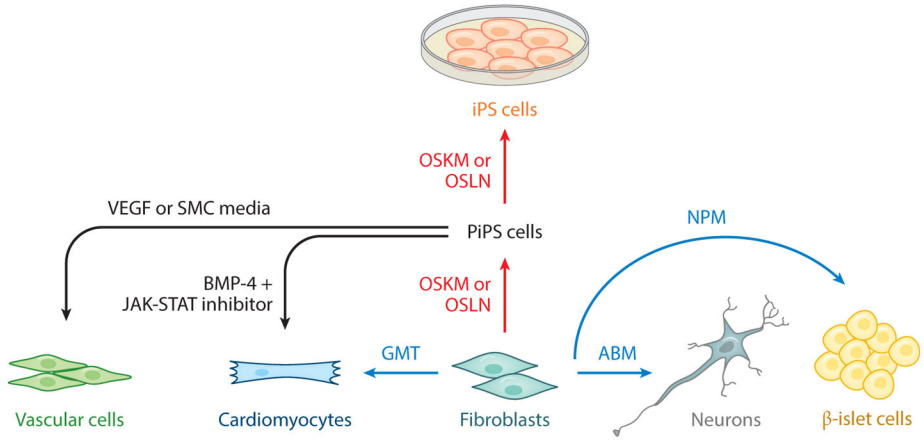

Figure 2.

Strategies to reprogram fibroblasts into iPS cells and other lineages. Cells can be reprogrammed into PiPS cells and then into iPS cells by using transcriptional factor OSKM or OSLN (*red arrows*). Cells can also be directly reprogrammed into specific cell types such as β-islet cells, cardiomyocytes, and neurons (*blue arrows*) by using NPM (Ngn3, Pdx1, and Mafa), GMT (GATA4, MEF2C, and TBX5), and ABM (Ascl1, Brn2, and Myt1l), respectively. Furthermore, cells can be induced into PiPS cells and differentiate into cardiomyocytes or vascular cells (EC or SMC). Abbreviations: BMP-4, bone morphogenetic protein-4, EC, endothelial cells; iPS cells, induced pluripotent stem cells; OSKM, Oct4, Sox2, Klf4, and c-Myc; OSLN, Oct4, Sox2, Lin28, and Nanog; PiPS cells, partially reprogrammed iPS cells; SMC, smooth muscle cells; VEGF, vascular endothelial growth factor.