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ABSTRACT

Recent advances in the understanding of pluripotent stem cell biology and emerging technologies to reprogram somatic cells to a stem cell–like state are helping bring stem cell therapies for a range of human disorders closer to clinical reality. Human pluripotent stem cells (hPSCs) have become a promising resource for regenerative medicine and research into early development because these cells are able to self-renew indefinitely and are capable of differentiation into specialized cell types of all 3 germ layers and trophoctoderm. Human PSCs include embryonic stem cells (hESCs) derived from the inner cell mass of blastocyst-stage embryos and induced pluripotent stem cells (hiPSCs) generated *via* the reprogramming of somatic cells by the overexpression of key transcription factors. The application of hiPSCs and the finding that somatic cells can be directly reprogrammed into different cell types will likely have a significant impact on regenerative medicine. However, a major limitation for successful therapeutic application of hPSCs and their derivatives is the potential xenogeneic contamination and instability of current culture conditions. This review summarizes recent advances in hPSC culture and methods to induce controlled lineage differentiation through regulation of cell-signaling pathways and manipulation of gene expression as well as new trends in direct reprogramming of somatic cells.

KEY WORDS: regenerative medicine, nuclear reprogramming, feeder cells, guided tissue regeneration, polymers.

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Advances in Culture and Manipulation of Human Pluripotent Stem Cells

INTRODUCTION

The capacity for indefinite self-renewal and differentiation into specialized cell types of all 3 germ layers and trophoctoderm makes human pluripotent stem cells (hPSCs) a promising resource for regenerative medicine, tissue engineering, disease modeling, drug screening, and understanding early events in human development. Human induced pluripotent stem cells (hiPSCs), which are generated by the reprogramming of somatic cells into embryonic-like stem cells, have expanded the potential of its natural counterparts, human embryonic stem cells (hESCs), for use in regenerative medicine. Furthermore, the finding that the fate of somatic cells can be reprogrammed into a different cell type has opened a new avenue to explore direct cell-lineage conversion without passing through a pluripotent state.

However, a key prerequisite for the successful therapeutic application of hPSCs and their derivatives is the ability to develop strategies for large-scale production of clinical-grade cells. Currently, this large-scale production is limited by potential xenogeneic contamination and instability of current culture conditions. To overcome these limitations, a variety of novel culture formulations has been developed to achieve defined and xenogeneic-free microenvironments (Villa-Diaz *et al.*, 2013). Similarly, although not the focus of this review, the development of safe methods to induce genetic manipulations in cells has contributed to the same goal. Thus, the purpose of this review is to summarize recent advances in hPSC culture and methods to induce controlled lineage differentiation by regulating cell-signaling pathways, altering gene expression, and manipulating the extracellular environment that stem cells experience.

PROGRESS IN HUMAN PSC CULTURE: FROM DEPENDENCE ON FEEDER CELLS TO CULTURE ON SYNTHETIC MATRICES

The derivation and culture of hESC lines were originally described by the same procedure described for mouse ESCs. These methods included gamma-irradiated mouse embryonic fibroblasts (MEF) as feeder cells and culture medium supplemented with fetal bovine serum (FBS) (Thomson *et al.*, 1998). However, since the natures of mouse and human ESCs are not identical (Nichols and Smith, 2009), the culture conditions to support their growth have proved to be different as well. Because of its undefined conditions and variability, the above-mentioned medium was replaced with knockout serum replacer and fibroblast growth factor 2 (FGF2) for hESCs. As feeder cells, MEFs provide attachment sites for hPSCs and secrete multiple, but largely uncharacterized, factors that induce signaling networks to regulate hPSC fate. However, the interactions among hESCs and murine feeder cells highlight xenogeneic contamination as a major concern and thus limit the use of MEFs in therapeutic applications for humans.

Micro-organisms and non-human bioactive molecules are 2 general sources of xenogeneic contamination of hPSCs when co-cultured on MEFs. Viruses may be transferred from feeder cells to contaminate the cell derivatives of hPSCs. Compared to other micro-organisms, viral contamination is difficult to detect and address during cell culture. Viruses such as lymphocytic choriomeningitis virus (LCMV), first isolated from mouse colonies, have been reported to infect cell lines (Mahy *et al.*, 1991). Certainly, the possible presence of viruses in MEFs and serum would increase the risk for culturing clinical-grade hPSCs. Furthermore, non-human bioactive molecules such as Neu5Gc have been identified on the surfaces of hESCs when co-cultured with MEFs (Martin *et al.*, 2005). These bioactive molecules may induce an immune response in recipients of transplanted hPSC derivatives. In addition, gamma irradiation, which is used to inhibit MEF proliferation, induces apoptosis in feeder cells and leads to instability of the culture microenvironment (Villa-Diaz *et al.*, 2008), affecting both mechanistically driven research and the clinical application of hPSCs.

To avoid the risks of animal contamination from MEFs, investigators have used human feeder cells for hPSC culture (Richards *et al.*, 2003). Multiple types of human cells—including fibroblasts derived from fetal muscle, fetal skin, adult fallopian tubal epithelium, placenta, uterine endometrium, foreskin, and mesenchymal cells—have been validated for their capability to support hPSC self-renewal. Among human feeder cells, fibroblast-like cells derived from hPSCs offer a potential autogenic system to support self-renewal of hPSCs. In addition to the diversity of human feeder cell lines, other innovations have been introduced, such as the use of FGF2-secreting human fibroblasts as feeder cells. This strategy reduced the need for exogenous supplementation of FGF2 to the culture medium (Unger *et al.*, 2009). Another innovation in hPSC culture is the use of an indirect co-culture system based on a microporous polymer membrane that allows for real-time conditioning of the culture medium by human fibroblasts, while maintaining complete separation between hPSCs and feeder cells (Abraham *et al.*, 2010). Nevertheless, the risk of contamination by human pathogens remains a concern when human feeder cells are used. Therefore, comprehensive screening and tests are required before feeder cells can be used in the culture of hPSCs intended for clinical applications.

To reduce the instability and potential contamination brought on by feeder cells, investigators have developed feeder-free culture systems for hPSCs. Matrigel™, the trade name for an extracellular matrix (ECM) extracted from Engelbreth-Holm-Swarm (EHS) mouse sarcomas, was the first example of a feeder-free substrate for hPSC culture (Xu *et al.*, 2001). To determine which components of Matrigel™, as well as other ECM molecules, may support hPSCs, researchers have examined a variety of specific ECM proteins. It has been reported that human recombinant (hr) laminin isoforms -111, -332, and 511, and hr vitronectin support the growth of undifferentiated hPSCs (Braam *et al.*, 2008; Miyazaki *et al.*, 2008). Human recombinant E-cadherin, another cell-cell interaction mediator, has also been reported to support hPSC self-renewal (Nagaoka *et al.*, 2010). These purified proteins are examples of defined and xenogeneic-free substrates being used for hPSC culture. Because these

proteins are generated as recombinant factors, they may not be subjected to the lot-to-lot variations observed in Matrigel™ extracts and other potential contaminations. However, human recombinant proteins are labor-intensive and costly to produce, which complicates their large-scale use. Therefore, the development of fully synthetic substrates for hPSC culture represents a new milestone in hPSC culture by overcoming many of the obstacles presented by biological substrates. Synthetic substrates have the following superiorities: They are defined, reproducible, and stable, show minimal lot-to-lot variability, demonstrate better scalability, and are cost-effective, easily prepared, and compatible with standard sterilization techniques (Villa-Diaz *et al.*, 2013). All these advantages make synthetic substrates promising for large-scale expansion of clinical-grade hPSCs and their derivatives for therapeutic use.

Peptide-based systems with surface arrays of self-assembled monolayers have been used to identify peptide surfaces that support hPSC self-renewal. Arrays of laminin-derived peptides, heparin-binding peptides, and high-affinity cyclic RGD peptides have all been shown to support hPSC culture (Derda *et al.*, 2007; Klim *et al.*, 2010; Kolhar *et al.*, 2010). The composition of surface array elements, specifically the density and sequence of peptides, has been reported to affect the ability of substrates to support hPSC growth (Derda *et al.*, 2007).

Numerous polymer-based substrates have been developed by different methodologies. For example, poly [2-(methacryloyloxy) ethyl dimethyl-(3-sulfopropyl) ammonium hydroxide] (PMEDSAH) is a fully defined synthetic polymer substrate developed through a surface-initiated graft polymerization technique (Villa-Diaz *et al.*, 2010). Hit 9 and the aminopropylmethacrylamide, APMAAm, are both fabricated by photopolymerization (Mei *et al.*, 2010; Irwin *et al.*, 2011). Both the semi-interpenetrating polymer hydrogel poly (N-isopropylacrylamide-co-acrylic acid) and the poly (methyl vinyl ether-alt-maleic anhydride) (PMVE-alt-MA), an anhydride-containing polymer substrate, are generated by radical polymerization (Li *et al.*, 2006; Brafman *et al.*, 2010). In addition, combinatorial approaches for polymer substrates have been implemented by using polymers as base substrates that are modified with biomolecules such as vitronectin and amino-containing peptides (Mei *et al.*, 2010; Melkounian *et al.*, 2010). All these substrates have demonstrated effective capacity to support hPSC growth.

Synthetic substrates for sustained hPSC culture are generally more stable and reproducible when compared with the first-generation culture systems. Clearly, an understanding of the molecular mechanisms that maintain self-renewal of hPSCs will be critical for the modification and improvement of the currently available technologies. Although not yet completely understood, the following physico-chemical properties may affect the capability of substrates to support hPSC growth: hydrophilicity, surface roughness, and stiffness (Villa-Diaz *et al.*, 2013), as well as the application of cell adhesion elements such as heparin-binding peptides (Klim *et al.*, 2010) and laminin-derived peptides (Derda *et al.*, 2007). Additionally, the compatibility of synthetic substrates with common sterilization techniques such as ultraviolet (UV) light radiation is another characteristic that may have an impact on the effectiveness of substrate preparations. For example, substrates with biological components such

as proteins and peptides are usually incompatible with common sterilization methods because biological components may undergo denaturation or degradation during the sterilization process. From this point of view, the pure polymer-based synthetic substrates may be superior to other substrates for hPSC culture.

CULTURE MEDIA, SUPPLEMENTS, AND CELL SIGNALING

In addition to feeder cells and feeder-free substrates, another key component of an effective hPSC culture system is the culture medium. Accompanied by the evolution of other components of the culture system, the development of new culture medium has undergone the following evolution: from medium-containing serum to serum-free medium, and from feeder-cell-conditioned medium to chemically defined and xenogeneic-free medium. As mentioned above, the culture of hPSCs was originally performed with fetal bovine serum (FBS) to supplement the culture medium (Thomson *et al.*, 1998) and later was replaced by knockout serum replacer and fibroblast growth factor 2 (FGF2) (Amit *et al.*, 2000). Human serum has also been used to replace animal-derived serum for the purpose of pursuing xenogeneic-free conditions. However, it has been reported that human serum may support hPSC for up to only 10 passages (Richards *et al.*, 2003). At the early stages of feeder-free culture system development, MEF-conditioned medium (CM) was commonly used (Xu *et al.*, 2001). However, to avoid drawbacks such as the multiple unknown and variable factors that CM contains, researchers have developed chemically defined media to work with xenogeneic-free substrates. This advancement not only paves the way for the future large-scale production of clinical-grade hPSCs, but also provides an ideal system for study of the molecular mechanisms of hPSC self-renewal (Villa-Diaz *et al.*, 2013). For example, mTesR medium was the first xenogeneic-free and serum-free commercial medium developed (Ludwig and Thomson, 2007), and further research has improved it into a chemically defined medium that contains only human proteins and 8 defined components (Chen *et al.*, 2011).

Numerous biological and chemical supplements for culture medium have been reported to facilitate hPSC self-renewal. Many of these discoveries and applications are the result of a greater understanding of hPSC pluripotency, which has been translated to the development of better additives and conditions. Some of these factors include FGF2, insulin, TGF β , BMP, Wnt, and IGF-II. In feeder cell culture systems, FGF2 has been shown to support hPSC self-renewal by promoting the expression of IGF-II, Activin A (a member of the TGF β superfamily), and Gremlin (a BMP antagonist) in feeder cells (Vallier *et al.*, 2005; Bendall *et al.*, 2007). In feeder-free cell culture systems, FGF2 has been reported to support undifferentiated hPSC growth without conditioned medium at a concentration of 100 ng/mL (C. Xu *et al.*, 2005). In other studies, dual activation of Smad3 and Erk by Activin A and FGF2 in serum-free and xenogeneic-free culture conditions was shown to support long-term maintenance of hESCs (Vallier *et al.*, 2005). Moreover, it has also been

reported that hESCs secrete a low-molecular-mass FGF2 (18-kDa) isoform and express its receptor, FGFR1. Blocking this receptor with a pharmaceutical inhibitor (SU5402) results in cell differentiation, suggesting a critical role for autocrine FGF signaling in maintaining hPSC self-renewal (Dvorak *et al.*, 2005). In addition, studies have indicated that Activin A is an effective medium supplement for hPSC self-renewal because it can enhance the expression of transcription factors, such as *Oct4* (also known as *POU5F1*), *Sox2*, and *Nanog* in hESCs (Vallier *et al.*, 2005). The observation of phosphorylation and localization of SMAD2/3 in the nucleus also indicates the activation of TGF β /Activin A/nodal pathways in undifferentiated hPSCs (James *et al.*, 2005).

Similar to the TGF β signaling pathway, the activation of the Wnt signaling pathway by a GSK-3-specific inhibitor has been reported to support the self-renewal of hPSCs (Sato *et al.*, 2004). The effect of BMP is opposite those of TGF β and Wnt signaling pathways on hPSC self-renewal, since its inhibition supports self-renewal of these cells. For example, Noggin (500 ng/mL), a BMP antagonist, can support undifferentiated hESCs on Matrigel™ in combination with FGF2 (40 ng/mL) (R.H. Xu *et al.*, 2005). However, the studies of signaling pathways regulating hPSC self-renewal are still limited, and it remains unclear whether other signaling molecules or pathways may be involved.

CELL LINEAGE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

Just as culture conditions to expand hPSCs in the undifferentiated state have evolved to the point of using defined and xenogeneic-free conditions, the protocols to induce *in vitro* cell lineage differentiation have been improved significantly in the past few years. This advancement is essential for maximizing the potential of hPSC derivatives for therapeutic use and to improve our understanding of the molecular mechanisms of tissue and organ development. Initial protocols to induce differentiation of hPSCs involved the formation of embryoid bodies (EB) in serum-containing medium, followed by adherent culture of EBs on gelatin-coated plates. Subsequent outgrowth of a heterogeneous cell population can then be sorted or selected for the desired cell lineage. This methodology was implemented, for example, to derive mesenchymal stem cells from hESCs (Brown *et al.*, 2009).

In contrast, by recapitulating lessons from embryonic development, controlled cell lineage conversion toward progenitors and fully differentiated cells has been achieved by the treatment of monolayer cultures of hPSCs and derivatives with morphogens and chemical inhibitors in serum-free medium. The following are a few examples of controlled differentiation into cells representative of the 3 germ layers and trophoblasts (Fig.). The capacity of hPSCs to differentiate into trophoblasts has been demonstrated by treatment with several members of the TGF β superfamily, such as BMP4, BMP2, BMP7, and GDF5, but not with TGF β 1 or Activin A (Xu *et al.*, 2002). Conversely, the dual inhibition of BMP and TGF β by Noggin and SB431542, a specific chemical inhibitor, directs the differentiation of

hPSCs toward a neuronal lineage (PAX6⁺ cells). Once this initial differentiation is directed, neural crest stem cells can be isolated by flow cytometry with p75 and HNK1 antibodies (Chambers *et al.*, 2009). Further neuronal specification of PAX6⁺ cells toward motor neurons has been achieved by, first, promotion of the caudalization of induced neurons with retinoic acid, followed by treatment with sonic hedgehog (SHH) to induce neuronal ventralization. Maturation into motor neurons is finally promoted by treatment with BDNF, GDNF, and IGF-I (Li *et al.*, 2005).

The formation of definitive endoderm (DE) is a prerequisite for further differentiation into mature endoderm derivatives from anterior foregut, midgut, and posterior foregut endoderm. Thus, to induce formation of DE, undifferentiated hPSCs are treated with Activin A (D'Amour *et al.*, 2005). To specify their identity toward anterior foregut endoderm (AFE) progenitor cells, investigators have treated Activin A-induced DE cells with dual inhibition of TGF β and BMP signaling. AFE-induced cells can then be directed toward cells expressing ventral AFE markers such as *NKX2.1*, *PAX1*, and *NKX2.5* if treated with a combination of WNT3a, KGF, FGF10, BMP4, and EGF. In turn, ventral induced AFE cells can give rise to cells expressing high *SFTPC* mRNA levels, when exposed to a regimen of retinoic acid, WNT3a, FGF10, and FGF7, suggesting the derivation of lung cells. Treatment of ventral induced AFE cells with either FGF8 or sonic hedgehog (SHH) induces the up-regulation of the parathyroid-specific marker *GCM2* (Green *et al.*, 2011). Derivatives from the posterior foregut endoderm lineages have also been induced *in vitro* by treatment of DE cells derived from hPSCs. For example, to generate hepatic cells, Activin A-induced DE cells are stimulated with FGF4 and BMP2 (Cai *et al.*, 2007), while intestinal cell differentiation requires treatment with FGF4 and WNT3a in three-dimensional culture conditions (Spence *et al.*, 2011). Pancreatic cells have been derived by sequential treatment of DE cells with FGF10 and KAAD-cyclopamine, followed by retinoic acid (D'Amour *et al.*, 2006).

The derivation of multipotent mesoderm progenitors has been achieved by treatment of hPSCs with BMP4 and Activin A (Yao *et al.*, 2006; Evseenko *et al.*, 2010). Interestingly, the

Figure. Controlled cell lineage induction by manipulation of signaling pathways and gene expression *in vitro*. This illustration shows how human pluripotent stem cells (hPSCs) – human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC) – can be maintained in the pluripotent state or directed into specific cell lineages by manipulating key signaling pathways and gene expression. hESCs are derived from the inner cell mass of the blastocyst, while hiPSCs are induced by overexpression of pluripotent genes in somatic cells (**top center**). Similarly, overexpression of other specific genes in somatic cells can induce their reprogramming into different cell identities (**top right**). Both hPSCs can be directed into derivatives of the 3 germ layers (ectoderm, endoderm, and mesoderm) and trophoectoderm by sequential stimulation and inhibition of signaling pathways with morphogens and chemical inhibitors (**bottom**). IGF, insulin-like growth factor; FGF, fibroblast growth factor; TGF β , transforming growth factor β ; BMP, bone morphogenetic protein; RA, retinoic acid; SHH, sonic hedgehog; BDNF, brain-derived neurotrophic factor; GDNF, glial-derived neurotrophic factor; EGF, epidermal growth factor; and GDF, growth differentiation factor.

progression toward defined differentiation conditions for mesoderm progenitors includes the use of synthetic substrates functionalized with peptide ligands for $\alpha 5 \beta 1$ and $\alpha 6 \beta 1$ integrins instead of MatrigelTM (Liu *et al.*, 2011). To induce differentiation toward primitive-streak mesendoderm, the precursor of mesoderm, hPSCs are treated with Activin A, Wnt3a, FGF2, and BMP4. Subsequently, to reduce the expression of endoderm genes in these populations, Activin A and Wnt3a are substituted

with follistatin. In turn, directed-mesoderm cells can be induced to chondrocytes by subsequent treatment with GFD5 instead of BMP4 (Oldershaw *et al.*, 2010). Effective cardiomyocyte differentiation of hPSCs has been achieved with temporal modulation of canonical Wnt signaling by treatment with Gsk3 inhibitors, followed by inhibitor of Wnt production-4 (IWP4) supplementation. Alternatively, similar results can be obtained when the later chemical inhibitor is replaced by shRNA inhibition of β -catenin (Lian *et al.*, 2012).

NEW TRENDS IN INDUCING SPECIFIC CELL LINEAGES

The observation that genetic manipulation can be used to induce specific cell lineage fate is reinforced by the recent development of induced pluripotent stem (iPS) cells by overexpressing key transcription factors (*Oct4*, *Sox2*, *Klf4*, and *c-Myc*) that are able to redirect the cell fate of somatic cells (Takahashi *et al.*, 2007). This has opened an exciting new avenue of research on directing the fate of somatic cells into specific cell types without passing through a pluripotent state. One early example of direct reprogramming, even before the derivation of iPSCs, was the generation of induced skeletal muscle cells by overexpression of *MyoD* in fibroblasts and other somatic cells, suggesting that this transcription factor is a master regulator of myogenesis (Weintraub *et al.*, 1989). Subsequently, the reprogramming of fibroblasts into other specific cell types has been reported. Cardiac myocytes, for example, have been induced from fibroblasts after the overexpression of 3 developmental transcription factors, *Gata4*, *Mef2C*, and *Tbx5* (Ieda *et al.*, 2010).

The direct generation of proliferative neuronal precursor cells and functionally distinct neuronal subtypes from mouse and human fibroblasts has been described by forced expression of selected transcription factors. Neuronal precursor cells capable of self-renewal and differentiation into neurons, astrocytes, and oligodendrocytes have been induced by reprogramming fibroblasts with combinations of transcription factors that include *Sox2*, *FoxG1*, and *Brn2* (Lujan *et al.*, 2012) or *Brn4*/*Pou3f4*, *Sox2*, *Klf4*, *c-Myc*, and *E47/Tcf3* (Han *et al.*, 2012). Interestingly, *Sox2* seems to function as a master regulator in the neuronal stem cell fate, since its single overexpression is able to induce multipotent neural stem cells (Ring *et al.*, 2012). Conversely, overexpression of *Myt1l* and *Brn2* in combination with microRNA-124 induces the generation of functional neurons that exhibit typical neuronal morphology and marker gene expression, generate action potentials, and produce mutually functional synapses (Ambasudhan *et al.*, 2011). Induction of dopaminergic neurons has been reported by force expression of *Mash1* (also known as *Ascl1*), *Nurr1* (also known as *Nr4a2*), and *Lmx1a* (Caiazzo *et al.*, 2011), while the induction into spinal motor neurons required the use of 7 factors: *Ascl1*, *Brn2*, *Myt1l*, *Lhx3*, *Hb9*, *Isl1*, and *Ngn2* (Son *et al.*, 2011). In contrast, the induction of neural crest stem cells (NCSC) with the capacity to differentiate into multiple neural crest-derived mesenchymal and neuronal lineages has been achieved by the single ectopic expression of *Notch1* in melanocytes (Zabierowski *et al.*, 2011). NCSCs are precursors of melanocytes, which indicates that the later cells may already have NCSC-related pathways that

become activated by a single master regulator. Importantly, direct lineage reprogramming is possible between cell types with different germ layer origins. For example, it has been demonstrated that terminally differentiated hepatocytes (endoderm) can be induced into neurons (ectoderm) with the same neuronal transcription factors (*Ascl1*, *Brn2*, *Myt1l*) that will convert fibroblasts – which may have a mesoderm-neural-crest origin – into induced neurons (Marro *et al.*, 2011).

It has been observed that, during the reprogramming of fibroblasts to iPSCs, subpopulations of cellular intermediates emerge that co-express genes associated with differentiated lineages. Those subpopulations fail to establish a pluripotent state, but have the potential to be re-directed into specified cell lineages with appropriated signaling inputs. In this way, fibroblasts with forced expression of *Oct4* and treatment with Flt3 and SCF are directed into multi-lineage blood progenitors that give rise to granulocytic, monocytic, megakaryocytic, and erythroid lineages with *in vivo* engraftment capacity (Szabo *et al.*, 2010). Similarly, transient transduction of *Oct4*, *Sox2*, *Klf4*, and *c-Myc* into fibroblasts, followed by treatment with FGF2, EGF, and FGF4, induces transdifferentiation into neuronal progenitor cells (Kim *et al.*, 2011). In contrast, when followed by inhibition of the JAK-STAT pathway, cardiomyocyte formation is induced (Efe *et al.*, 2011).

CONCLUSIONS

Recent advances in culture and manipulation of hPSCs have improved the prospects for meaningful progress in regenerative medicine, disease modeling, and drug and toxicology screening. For example, the successful development of xenogeneic-free and defined microenvironments for hPSC culture will support the large-scale production of clinical-grade hPSCs and thus provide an alternative cell source for tissue regeneration strategies (Villa-Diaz *et al.*, 2013). Likewise, because hPSCs can be directed to differentiate toward cells from all 3 germ layers, this source of cells has more potential and versatility than any adult stem cell.

FUTURE DIRECTIONS

As the field progresses, the study of hPSCs, such as ESCs, will remain an important area of research because no other human cell type has as much capacity to reveal insights into early events in human development. Human ESCs also represent normal human cells that have not undergone genetic manipulation, and, since hESCs can be derived from embryos with naturally occurring genetic mutations, the study of disease-specific ESCs should lead to improved diagnosis and treatment for specific inherited diseases.

However, the study of hiPSCs, and the recent advances in direct cell reprogramming, will likely surpass hESCs in the potential for regenerative medicine as methods are developed to generate personalized cells safely and reproducibly. Recently, cells from dental tissues such as dental pulp have been used to generate iPSCs (Yan *et al.*, 2010). Because epigenetic modifications of DNA inherent in genetic reprogramming may be influenced by the character of the parental cell type (Kim *et al.*,

2010), iPSCs derived from oral or dental tissues may turn out to be more easily directed to differentiate toward specific oral tissues. Thus, iPSCs generated from cells of dental origin may have the potential to regenerate many of the unique structures in the oral cavity.

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