Tissue-Engineered Vascular Grafts Created From Human Induced Pluripotent Stem Cells

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Key Words. Tissue engineering • Blood vessel graft • Induced pluripotent stem cells • Smooth muscle cells

ABSTRACT

The utility of human induced pluripotent stem cells (hiPSCs) to create tissue-engineered vascular grafts was evaluated in this study. hiPSC lines were first induced to a mesenchymal lineage via a neural crest intermediate using a serum-free, chemically defined differentiation scheme. Derived cells exhibited commonly known mesenchymal markers (CD90, CD105, and CD73 and negative marker CD45) and were shown to differentiate into several mesenchymal lineages (osteogenic, chondrogenic, and adipogenic). Functional vascular grafts were then engineered by culturing hiPSC-derived mesenchymal progenitor cells in a pulsatile bioreactor system over 8 weeks to induce smooth muscle cell differentiation and collagenous matrix generation. Histological analyses confirmed layers of calponin-positive smooth muscle cells in a collagen-rich matrix. Mechanical tests revealed that grafts had an average burst pressure of 700 mmHg, which is approximately half that of native veins. Additionally, studies revealed that karyotypically normal mesenchymal stem cell clones led to generation of grafts with predicted features of engineered vascular grafts, whereas derived clones having chromosomal abnormalities generated calcified vessel constructs, possibly because of cell apoptosis during culture. Overall, these results provide significant insight into the utility of hiPSC cells for vascular graft generation. They pave the way for creating personalized, patient-specific vascular grafts for surgical applications, as well as for creating experimental models of vascular development and disease.

STEM CELLS TRANSLATIONAL MEDICINE 2014;3:1535–1543

INTRODUCTION

Cardiovascular disease is the leading cause of death in the Western world. On average, there are 500,000 arterial bypasses each year in the U.S. alone [1]. To date, the surgical mainstays for peripheral vascular disease remain synthetic grafts made of polytetrafluoroethylene (Dacron) or Teflon or autologous grafts from the saphenous or mammary blood vessels. These synthetic grafts pose problems such as thrombosis, intimal hyperplasia, or compliance mismatch at the small diameter applications of less than 6 mm.

Various tissue-engineering approaches have emerged to fill this clinical void. Cell types such as smooth muscle cells [2] or fibroblasts [3] are cultured for several weeks on synthetic materials or natural materials such as fibrin [3, 4] or silk [5] to create tubular conduits resembling blood vessels. Alternatively, cell sheets [6] are rolled on mandrels to create blood vessel-like tissues. Various bioreactor arrangements have also been designed to promote a physiological-like environment for culture of better vessel grafts [7]. Besides these factors, one key component that still remains critical to the success of vascular engineering is the cell source.

Both donor age and species of origin affects the quality of the engineered grafts [8]. Human smooth muscle cells can display senescence after several weeks of in vitro culture. This poses challenges for creating autologous grafts for older or diseased patients, who probably are in the most critical need of such interventions. More recently, it has been shown that engineered vascular grafts can be stripped of the immunogenic cellular component by the use of detergents [9]. These decellularized grafts make it possible to use cells from any donor to create a cell-free off-the-shelf product that can then be transplanted into any recipient [10]. Hence, a renewable and superior cell source that can be expanded indefinitely to create a human cell bank for vascular engineering would be highly valuable for the creation of grafts with uniform properties.

Mesenchymal progenitors, as well as functional smooth muscle cells, have been derived from various diverse sources such as human hair follicle [11], amniotic fluid [12], bone marrow, adipose tissue [13], as well as human embryonic cells [14], and more recently from human induced pluripotent stem cells [15]. Several groups have used a method based on the spontaneous
differentiation into embryoid bodies [16] from which smooth muscle cells are selected [17, 18]. More recently, smooth muscle cells of different embryological origins such as neural crest, paraxial mesoderm, and lateral plate mesoderm have been generated from human embryonic stem (hES) and human induced pluripotent stem (hiPS) cells in a chemically defined, serum-free medium [15]. However, to date, hiPS cells have not been used for the creation of engineered vascular grafts.

Taking all these factors into consideration, we had two main goals: first to use the developmental aspects of smooth muscle cells (SMCs) to derive cells from iPSCs and second to use hiPS-derived cells to engineer vascular grafts. Hence, we developed a feeder-free chemically defined protocol based on relevant growth factors [19] to generate mesenchymal progenitors via a neural crest intermediate step. These iPSC-derived mesenchymal-like cells display properties similar to narrow stromal cells in terms of surface marker expression and multilineage capabilities (chondrogenic, osteogenic, and adipogenic). We then developed a protocol to culture these cells in a bioreactor under pulsatile flow conditions. At the end of 8 weeks of culture, cells successfully organized into a matrix-rich, tubular construct similar to native veins. When grown from karyotypically normal iPSC derived precursors, these tissue-engineered vascular grafts (TEVGs) had mechanical strength properties such as burst pressure and suture retention strength that measured approximately half that of saphenous veins. To our knowledge, this is the first report on growing small-diameter vascular grafts using hiPS cells.

**MATERIALS AND METHODS**

Detailed methods are provided in the supplemental online data.

**hiPSC Culture**

hiPSC lines IMR90-1 (C1) and foreskin (C2) that were used in this study were provided by Dr. James Thomson (University of Wisconsin) [20]. hiPSC lines C1 and C2 were cultured on Matrigel-coated (BD Biosciences, CA, http://www.bdbiosciences.com) plates in a commercially available mTeSR culture medium. Cells were routinely passaged every 5–6 days using dispase as previously described [21]. All cell culture products were obtained from Stemcell Technologies (Vancouver, BC, Canada, http://www.stemcell.com) unless stated otherwise.

**hiPSC Differentiation**

Cells were grown in a defined medium [22] containing SB431542 (10 mM; Tocris Bioscience, Bristol, U.K., http://www.tocris.com) and fibroblast growth factor 2 (FGF2; 10 ng/ml; R&D Systems Inc., Minneapolis, MN, http://www.rndsystems.com) in the presence of Wnt3a (25–50 ng/ml; R&D Systems Inc.) for 10–12 days for neural crest induction. For further induction into a mesenchymal lineage, neural crest cells were trypsinized and cultured in MesenCult growth medium (Stemcell Technologies) for 4–7 days. Subsequently, cells were maintained in MesenCult growth medium.

**Confirmation of hiPS-MSC Phenotype**

Cells were tested for their mesenchymal identity by flow cytometry for mesenchymal stem cell (MSC) markers (positive markers CD73, CD90, and CD105 and negative marker CD45). MSCs were also tested for their ability to differentiate into adipogenic, osteogenic, and chondrogenic lineages using a commercial kit (R&D Systems Inc.) as previously described [23]. All antibodies used are listed in supplemental online Table 1.

**Telomere Length Using Real-Time Polymerase Chain Reaction**

Relative telomere length was determined using quantitative reverse transcription (RT)-polymerase chain reaction (PCR) on genomic DNA as described previously [24]. The primer sequences are listed in supplemental online Table 2.

**Cell Doubling Time**

The cell doubling time was calculated using an online algorithm at http://www.doubling-time.com. Cells were seeded at an initial density of 100,000 cells per well of a 6-well plate. Cell density was calculated at 24, 48, and 72 hours postseeding. The assay was performed in triplicate.

**Small-Diameter Vessel Culture**

Bioreactors were set up for the growth of TEVGs as previously described [25, 26]. Briefly, cells were cultured on a polyglycolic acid (PGA) mesh over silicone tubing in a bioreactor under pulsatile conditions for 8 weeks. A detailed description of the vessel culture is provided in the supplemental online data.

**Statistical Analysis**

All experiments were performed in triplicate, and the data are expressed as the means ± SEM. Statistical analysis was evaluated by standard t test using the GraphPad Prism program. A p value < 0.05 was considered to be statistically significant.

**RESULTS**

**Derivation of Mesenchymal Cells From Human Pluripotent Cells**

First, we devised a protocol to efficiently derive relatively pure populations of mesenchymal progenitor cells from hiPS cells in a serum-free, defined manner (Fig. 1A). The IPS cell lines C1 and C2 used in the study express known markers of pluripotency such as Oct4, SSEA4, and Tra-1-60 by immunostaining and were also confirmed to be karyotypically normal (supplemental online Fig. 1). To initiate differentiation, hiPS cells were seeded on Matrigel-coated plates in the presence of differentiation medium containing a growth factor cocktail of FGF2, Wnt3a, and SB431542 that has been previously reported to induce neural crest differentiation [27]. After 10–12 days, a uniform population of neural crest cells appeared on the culture dish. As shown in supplemental online Figure 2A, we followed the dynamics of the induction of the neural crest. Initially, iPSC cells express pluripotency markers such as Oct4. Once differentiation is initiated, there is accumulation of neural crest markers such as HNK1 and SLUG over 10–12 days (supplemental online Fig. 2A). These cells had a distinct morphology (Fig. 1B) similar to stem cell-derived neural crest cells reported by other groups [27, 28]. They were confirmed positive for classic neural crest markers p75, HNK1, and AP2a by both RT-PCR (supplemental online Fig. 2B) and immunostaining (supplemental online Fig. 2C). These cells also express dorsal neural epithelial markers such as PAX3 and SOX9, from which the neural crest is known to emerge.
Additionally, they were found to be negative for the pluripotent marker Oct4. At this stage, neural crest cells could be frozen down and thawed as well as maintained in the neural crest stage for several passages.

For further differentiation into mesenchymal progenitors, the differentiation medium was switched to MesenCult, a mesenchymal stem cell medium. After 4–7 days, cells displayed distinct spindle-shaped morphology (Fig. 1B). When tested by flow cytometry, these cells were found positive for markers CD90 (98.8% positive), CD105 (99% positive), and CD73 (99.1% positive) and were negative for CD45 (Fig. 1C). Additionally, these cells could also be differentiated into other lineages such as osteogenic, adipogenic, and chondrogenic as indicated by positive immunostaining for markers osteocalcin, fatty acid-binding protein 4, aggrecan, respectively, thus confirming their multilineage potential (Fig. 1D). We confirmed this approach by testing another iPSC cell line C2 (supplemental online Fig. 1E–1H). Cells derived from line C2 also showed an intermediate neural crest.
population that had similar gene expression profile to cells derived from line C1. These neural crest cells derived from line C2 were negative for pluripotent marker OCT4, neural marker Pax6, and additionally expressed several neural crest markers such as p75, AP2a, Pax3, Sox10, and Slug (supplemental online Fig. 3A). However, these cells did not express HNK1. The exact impact of the lack of HNK1 expression on the functionality of these cells remains unknown at the current time. Additionally, mesenchymal cells derived from line C2 (clone C2 E1) had a uniform stromal cell surface marker expression (positive for markers CD73, CD90, and CD105 and negative for CD45) (supplemental online Fig. 3B). This confirms that we can derive mesenchymal progenitor cells, in general, from various hiPS cell lines.

Creating a Vessel Construct From hiPS-Derived Cells

The bioreactor conditions used to culture the vessel walls are represented in Figure 2A. In order to determine optimal medium conditions for the growth of vessel in the bioreactor, we performed a series of pilot experiments to determine the effect of various growth factors on the vascular smooth muscle differentiation of iPSC-MSCs seeded on small pieces of PGA polymer mesh. For C1 E8 cells, after 3 weeks of culture, we found that addition of transforming growth factor β1 (TGFβ1; 1 ng/ml) to the reactor medium not only increased expression of the smooth muscle marker calponin but also increased expression of chondrogenic marker gene collagen 2A (Col2A) and osteogenic marker gene osteocalcin (OCN) as determined by PCR analysis (Fig. 2B). Similar trends were observed when a combination of TGFβ1 (1 ng/ml) and platelet-derived growth factor BB (PDGFBB; 10 ng/ml) was added to the reactor medium. Addition of PDGF-BB alone seemed to have the minimal increase of undesired Col2A and OCN, while also increasing the smooth muscle differentiation gene calponin. We performed similar experiments to determine optimal bioreactor growth conditions for cells derived from hiPSC cell line C2 (i.e., C2 E1 clone). In this case, no statistically significant differences were found in expression levels for osteogenic or chondrogenic genes on addition of TGFβ1 (1 ng/ml) or PDGFBB (10 ng/ml) (supplemental online Fig. 4B). However, when cells were exposed to a combination of TGFβ1 and PDGFBB, we observed statistically significant increases in gene expression levels of chondrogenic and osteogenic markers. Hence, as an overall strategy to minimize differentiation toward undesired lineages, we chose to add PDGFBB (10 ng/ml) while avoiding the addition of TGFβ1 to the reactor medium during the 8-week bioreactor culture period.

Two vessel constructs were engineered from each hiPSC-MSC clone in a single bioreactor. Vessel walls created using line C1 E8 are presented in Figure 2C and 2D. Histological analysis indicates that cells are distributed throughout the thickness of the vessel wall (Fig. 2E). Cell morphology appeared stretched and elongated similar to cells of the connective tissue. Little to no polymer fragments remain visible. Vessel wall thickness was approximately 250 μm, which is close to that of human saphenous vein [29]. Masson’s trichrome staining indicated blue staining of collagen strands (Fig. 2F). No elastin fibers were detected by Verhoff-Van Gieson staining (Fig. 2G). Additionally, staining with Alcian blue revealed deposition of glycosaminoglycans in the matrix (Fig. 2H). Taken together, these results indicate that iPSC-MSCs cultured under pulsatile conditions in the bioreactor were able to infiltrate the polymer scaffold, while also laying down collagenous matrix to enable creation of a tubular, vessel-like construct.

Cellular Phenotype in Engineered Vessel Wall

In order to confirm the phenotype of the cells present in the engineered vessel, we stained the vessel sections for known markers of smooth muscle cells. Immunostaining revealed that cells stained positive for α-smooth muscle actin (α-SMA; early marker) and SM22α and calponin (mid-differentiation markers) (Fig. 3A–3C). Mature marker smooth muscle myosin heavy chain was not detected. Most of the positive staining appeared to be localized to the outer layers of the vessel wall. This is similar to what we have observed in the past in vessels generated using human smooth muscle cells using a bioreactor culture approach. The inner layers of the cells had similar morphology to the outer cells but remained negative for SMC markers tested.

Additionally, we stained for other mesenchymal lineages and found that the vessel walls were negative for osteochondrogenic markers collagen 2 and aggrecan (Fig. 3G, 3H). The cells in the vessel wall also did not stain positive for apoptosis marker caspase 3 (Fig. 3I). To further confirm the presence of SMC markers, we performed Western blotting to check for SMC protein expression. As seen in Figure 3J, positive bands are visible for both α-SMA and calponin similar to those obtained from human aortic SMCs cultured in vitro (positive control). Taken together, these results confirm the SMC-like identity of the cellular layers in the vessel walls engineered using iPSC-MSCs.

Matrix Production and Strength of the Vessel

To analyze the vessel wall for matrix contributions, we performed immunostaining for known markers of vessel matrix molecules. We were able to detect positive staining for collagen I, collagen III, and fibronectin as seen in Figure 3D–3F. There is no overlap of the red (matrix staining) and the green (β-actin staining), indicating that the matrix staining is predominantly extracellular. Total collagen content, as determined by hydroxyproline assay (Table 1), revealed 45% collagen by dry weight (n = 1). Additionally, vessel walls were also stained for elastin; however, the staining was mostly negative.

Mechanical properties that were evaluated included suture strength for handling characteristics and burst pressure to assess vessel strength (Table 1). Burst pressure of the vessels was 700 mmHg (n = 1), half of that reported for saphenous vein (1,600 mmHg) [30]. Suture strength was approximately 30 g (n = 1). The results indicate that mechanically strong vessel constructs (comprising collagen I, collagen III, fibronectin, and glycosaminoglycans, as well as cells expressing multiple smooth muscle markers) can be engineered using hiPSC-derived MSCs. However, further optimization of the culture conditions may be required to enhance the mechanical characteristics of the vessels and make them more suitable for arterial implantation.

Characteristics of Vessel Structures Created From Various iPSC-MSC Clones

We repeated the differentiation protocol described above using hiPSC cell line C1 and created an additional clone of iPSC-MSCs (e.g., C1 E6). Typically, hiPS cells were plated on Matrigel in the presence of growth factors Wnt3a, FGF2, and SB431542 to induce a neural crest intermediate and then further cultured in mesenchymal growth medium MesenCult to obtain a uniform mesenchymal cell population over 10–12 days. Karyotyping analyses revealed that, although some clones (C1 E8) were chromosomally normal, certain others (C1 E6) had abnormal
karyotypes (Fig. 4A, 4F). For clone C1 E6, chromosomal analysis revealed trisomy of chromosome 7 and 8. We also observed chromosomal aberrations in MSCs cells derived from iPS C2. Biclonal abnormalities were found for C2 E1 cells; some cells had a normal karyotype, whereas some others displayed abnormalities. We proceeded to generate vessels in the bioreactor using the abnormal clones. After 8 weeks of culture, we found striking differences in the nature of vessel walls created using karyotypically abnormal clone (i.e., C1 E6) in comparison with those we had created using the normal cells (i.e., C1 E8), despite the fact that the cells were derived from the same hiPS cell line C1.

For vessels generated from karyotypically abnormal populations, we observed a mostly acellular tissue under hematoxylin and eosin staining (Fig. 4A) as compared with vessels grown from chromosomally normal cells (Fig. 4B). Von Kossa staining revealed extensive calcification (black staining in Fig. 4H). In comparison, vessels made with normal cells showed no calcification (Fig. 4C).

Figure 2. Vessels engineered from human induced pluripotent stem cells. (A): Schematic of vessel culture protocol. (B): Determining conditions for bioreactor vessel cultures. Polymerase chain reaction analysis of C1 E8 cells seeded on pieces of polyglycolic acid polymer for 3 weeks in the presence of various growth factors TGFβ, PDGF, or TGFβ + PDGF reveals differences in the expression of various genes: CNN1 (for smooth muscle lineage), OCN (for osteogenic lineage), and Col2A (for chondrogenic lineage). The data were normalized to gene expression levels of C1 E8 cells on day 0 (n = 3). **, p > .05; *, p < .05. (C): Reactor setup. (D): Gross picture of vessels created using C1 E8 cells at the end of the 8-week culture. (E): Hematoxylin and eosin. (F): Trichrome collagen. (G): Verhoff-Van Gieson elastin. (H): Alcian blue for glycosaminoglycans. Scale bars = 50 μm (E–H). Abbreviations: CNN1, calponin; Col2A, collagen 2A; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; OCN, osteocalcin; PDGF, platelet-derived growth factor; TGF, transforming growth factor; T+P, TGFβ + PDGF.
Additionally, when karyotypically abnormal iPS-MSCs (i.e., C2 E1) derived from another iPS cell line C2 were used to create vessel grafts, we found the grafts to have a crumbly, paper-like appearance (indicative of extensive calcification in the grafts) (supplemental online Fig. 4A).

To summarize, we grew two vessels each from a normal clone (C1 E8) and an abnormal clone (C1 E6) that were derived from a single iPS cell line C1. Additionally, we also grew two vessels from an abnormal clone (C2 E1) that was derived from a different iPS cell line C2. Overall, the normal clone led to generation of vessel walls that were similar to native vessel walls, whereas abnormal clones led to highly calcified structures.

To further understand the reason for the observed differences between karyotypically normal and abnormal cells, we stained the cell populations for the proliferative marker Ki67 and senescent marker p21 (prior to culturing them in the bioreactor). For iPS cell line C1, both clones C1 E6 (karyotypically abnormal) and C1 E8 (karyotypically normal) had similar percentage of Ki67+ cells (Fig. 4D, 4I). However, the karyotypically abnormal clone C1 E6 had greater number of cells that stained positive for senescent marker p21 (Fig. 4E, 4J). Similar trends are observed when iPS-MSCs C2 E1 (karyotypically abnormal) derived from iPS cell line C2 are stained for Ki67 and p21 (supplemental online Fig. 3C).

Reduced telomere length is another measure of senescence. We measured the relative telomere length of the derived clones (prior to culturing them in the bioreactor) using real time PCR. Briefly, relative telomere length is obtained from the T/S ratio, which compares the telomere length of the clone to the telomere length of a normal cell line.

### Table 1. Morphometric and mechanical properties of vessel walls created using human induced pluripotent stem cell-derived mesenchymal stem cell-like cells (C1 E8)

| Vessel type   | Average wall thickness (µm) | Collagen (% dry weight) | Suture retention (g) | Burst pressure (mmHg) | DNA (% dry weight) |
|---------------|-----------------------------|-------------------------|----------------------|-----------------------|-------------------|
| V468-1        | 250                         | 48.1                    | 30                   | 672                   | 0.1407            |
| V468-2        | 245                         | 43.6                    | 30                   | 827                   | 0.1422            |
| Human aorta   |                             |                         |                      |                       | 54.9              |

Figure 3. Characterization of vessel wall. (A–J): Immunofluorescent staining of vessel sections shows that vessel walls created with human induced pluripotent stem-derived cells (C1 E8 clone) stain positive for smooth muscle cells such as α-SMA (A), calponin (B), and SM22α (C) and matrix markers such as collagen I (D), collagen III (E), and fibronectin (F) while remaining negative for chondrogenic markers such as collagen II (G) and aggrecan (H) and apoptosis marker caspase (I). Additionally, Western analysis of protein content of the vessel walls (J) confirms presence of SMC markers showing the presence of α-SMA and calponin protein in engineered vessels. Scale bars = 25 µm (A–I). Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HUVECs, human umbilical vein endothelial cells; SMA, smooth muscle actin; SMCs, smooth muscle cells.
which is the ratio of the mean telomere repeat gene sequence (T) to the reference single copy gene (S). As seen in Figure 4K, an older adult (age 89 years) has a diminished T/S ratio when compared with a younger adult (age 21 years). When compared against these positive control samples, the T/S ratio was dramatically shortened for the abnormal clone C1 E6 in comparison with the normal clone C1 E8. These results further confirm that the abnormal clone displayed a senescent phenotype. Further analyses of the doubling time of the various clones C1 E6, C1 E8, and C2 E1, prior to reactor culture, however, showed no statistically significant differences (Fig. 4L; supplemental online Fig. 4D). For karyotypically normal cells C1 E8, we were able to maintain cells in culture for up to 20 weeks. Other clones such as C1 E6 and C2 E1 reached senescence over six or seven passages. Overall, these results demonstrate that the karyotypically abnormal cells had a senescent phenotype (prior to bio reactor culture).

**DISCUSSION**

In this proof-of-concept study, we demonstrate the utility of hiPS cells for creating tissue-engineered vessels made from iPSC-derived cells. First, we derived functional mesenchymal progenitor cells using a serum-free, chemically defined approach, using growth factors previously shown to induce neural crest [22, 27]. Cells derived exhibited commonly known surface markers of MSCs and exhibited multilineage potential. We then devised a protocol to culture these hiPS-MSCs for the creation of TEVGs. Compared with previously reported studies, our grafts exhibited multiple layers of smooth muscle-like cells [18, 23]. Additionally, the vessel walls showed matrix composition in terms of collagen and fibronectin similar to vessels engineered from human SMCs [25, 31]. Finally, these vessel walls had mechanical properties that approach those of native veins. We were further able to test these protocols on two hiPS clones, suggesting that this approach can be extended to other hES and hiPS cell lines.

Several reports in the literature have shown that properties of engineered vessel grafts can be substantially improved by optimizing the scheduling of growth factors that cells were exposed to over the culture period [26, 32]. It is well known that TGF-β1 and PDGFβB are two of the most critical factors known to play a role in differentiation of mesenchymal progenitors into functional smooth muscle cells [33, 34]. Although Gong and Niklason [26] found substantial improvement in the cellularity, as well as collagen content, when TGFβ1 was administered to the reactor media, others reports suggest that TGFβ1 acts more promiscuously [35, 36]. In our own previous work [23], we found that although mesenchymal cells (derived from human embryonic stem cells) were able to lay down dense collagenous matrix in the presence of TGFβ1, we also detected some undesirable collagen II and osteocalcin. In order to circumvent these issues, we performed experiments in which cells were tested for the effect of various growth factors in the bioreactor microenvironment without the pulsatile stretch (i.e., cells were seeded on the polymer mesh in the reactor medium) over a 3-week static culture period. Our results clearly showed that TGFβ1 not only upregulates smooth muscle markers but additionally upregulates markers of osteo-chondrogenic differentiation. PDGFβB on the other hand did not lead to substantial upregulation of non-smooth muscle cell markers. Using information gleaned from such experiments, we eliminated the use of TGFβ1 to improve the differentiation outcomes of MSC-like cells in the presence of the reactor culture.
conditions for the creation of vessel grafts. Taken together, our studies indicate that PDGFβR results in a more specific SMC specification.

In order to create engineered vessel grafts, cells must have very high expansion capabilities, as well as lay down matrix such as collagen over long-term culture times [37]. One of the notable attractions of using hiPS-derived cells is the promise that cells derived from hiPS cells may be “younger” and have greater functionality compared with adult cells. In our hands, the cellular replicative abilities varied among the various MSC-like cell clones derived from iPS cells. In the best case, derived cells remained actively dividing in culture for up to 90 population doublings. In comparison, vascular SMC from elderly patients generally have 5–10 PDs when cultured in vitro [31]. Generally, vessel grafts can be created from cells that have 30–40 PDs [2, 6, 31]. In the current study, we were successful in creating mechanically strong vessels with cells that had the capability of expanding for 90 PDs.

Another important result of this study is the varied karyotype of the derived cells [38]. Although the karyotype alteration could possibly be concurrent with passaging in culture, we did not observe evidence for this in our experiments. In the clones that were karyotypically normal, no aberrant chromosomal alterations were observed even at high passage numbers, whereas abnormalities were observed at fairly low passage numbers in karyotypically abnormal cells. The alterations in karyotype appeared to be more clone-dependent. The reasons for the abnormalities in certain derived clones remain unclear. In fact, it has been previously reported that critically short telomeres are subject to recombination events that increase the risk of chromosomal abnormalities [39]. Hence, the abnormal karyotype (prior to vessel culture) may have been caused by the short telomere length. The reasons for the extensive calcification that we observed using cells with shortened telomeres and abnormal karyotype also remain unclear, but one of the hallmarks of replicative senescence (RS) is calcification. Studies have revealed increased alkaline phosphatase activity (a key protein involved in matrix calcification) during RS of cultured vascular SMCs [40, 41]. Another explanation might involve the neural crest origin of the derived cells, which is known to be prone to calcification [42]. Because calcification of vascular smooth muscle cells shares many similarities with that of bone formation [43], the involvement of the osteogenic differentiation potential of these mesenchymal cells cannot be discounted. Future studies will focus on testing these hypotheses to provide a more detailed understanding for our observations.

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CONCLUSION

In the present study, we provide evidence that human iPS cells can be used as a novel cell source to create vascular grafts with good mechanical handling properties. To our knowledge, this is the first study using hiPS cells to create small-diameter blood vessel mimics. In the future, the mechanical properties of these vessel grafts must be substantially improved to make them suitable for in vivo implantation studies [10, 25]. No doubt, a functional TEVG requires a functional endothelium lining. We will also work on approaches to coat the lumen of the engineered vessels with iPS-derived endothelial cells or other antithrombogenic coatings as we have reported previously [9]. With improved methods of differentiation and protocols for creating TEVGS with iPS-derived cells, one can envisage creating banks of such cells. Banked iPS-derived MSCs or SMCs could then be used for creating personalized patient-matched grafts for particular applications or more generally to create implantable off-the-shelf TEVGS with uniform biological and mechanical properties. This is a novel and translatable application of the hiPS technology.

ACKNOWLEDGMENTS

We thank the Yale Cytogenetics Laboratory for assistance with chromosomal analysis of cell lines. We also thank Jason Thomson from the Yale Stem Cell Center for assistance with hiPS cell culture. This work was supported by NIH Grant HL083895-06A1 (L.N.) and by Connecticut Innovations Stem Cell Grant 11SCY08-S01515 (S.S.).

AUTHOR CONTRIBUTIONS

S.S.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, financial support; J.O., J.S., S.T.: L.Z., and H.Q.: collection of data; S.D.: collection of data, data analysis; A.H.: data analysis; L.N.: conception and design, data interpretation, manuscript editing, financial support.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

L.N. has uncompensated employment, intellectual property rights, consultancy, and ownership interest in Humacyte, Inc.
logical origin-dependent disease susceptibility.

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