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Vascular diseases await translation of blood vessels engineered from stem cells

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Abstract

The discovery of human induced pluripotent stem cells (hiPSCs) might pave the way toward a long-sought solution for obtaining sufficient numbers of autologous cells for tissue engineering. Several methods exist for generating endothelial cells or perivascular cells from hiPSCs in vitro for use in the building of vascular tissue. We discuss current developments in the generation of vascular progenitor cells from hiPSCs and the assessment of their functional capacity in vivo, opportunities and challenges for the clinical translation of engineered vascular tissue, and modeling of vascular diseases using hiPSC-derived vascular progenitor cells.

The vascular system is vital to the care and feeding of every organ in the body, and accordingly, vascular complications give rise to widespread diseases—from retinopathies to coronary heart disease to peripheral vascular disease. Vascular pathologies theoretically could be treated by replacing the diseased vasculature with newly engineered functional blood vessels. The cellular components required to build stable blood vessels include endothelial cells (ECs)—which line the inner surface of blood and lymphatic vessels—and perivascular cells (PVCs) or mural cells—which include pericytes and vascular smooth muscle cells (vSMCs) (1). Pericytes are periendothelial in location, share a basement membrane with the ECs, and are present in capillaries. Larger-caliber and contractile vessels are surrounded by vSMCs (2, 3), which exhibit different phenotypes depending on vessel specifications and organ site (1).

Stem cells have the potential to provide a limitless supply of proliferative progenitor cells that can be used to differentiate into vascular cells in vivo and form a mature and durable

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SUPPLEMENTARY MATERIALS

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Table S1. Key developments in functional evaluation of hiPSC-ECs and PVCs in vivo

Table S2. Generation of hiPSC-ECs and PVCs.

Table S3. Modeling diseases using hiPSC-ECs and PVCs.

Movie S1. Multiphoton laser scanning image of iPSC-engineered blood vessels in vivo in day 12 cranial window of SCID mouse: Green eGFP HS27-iPSC ECs co-implanted with DsRed 10T1/2 cells.

Movie S2. Multiphoton laser scanning image of iPSC-engineered blood vessels in vivo in day 12 cranial window of SCID mouse: Green eGFP HS27-iPSC ECs co-implanted with DsRed 10T1/2 cells.

network of blood vessels. However, adult stem cells have comparatively more limited proliferative potential, diminished differentiation capacity, and increased senescence. Human induced pluripotent stem cells (hiPSCs) offer a promising alternative (4). Indeed, retroviral transduction of only four transcription factors reprograms terminally differentiated cells of adult origin into a pluripotent (embryonic stem cell–like) state. hiPSCs can, in principle, provide a rich source of nonimmunogenic allogeneic stem or progenitor cells capable of differentiation and organization into functional tissue in a biologically relevant microenvironment.

In addition to repairing diseased vasculature, the ability to create stable and functional blood vessels from hiPSCs offers tremendous regenerative potential (Fig. 1). This includes the engineering of other tissues that require a blood supply; the development of organs for transplantation; and the ability to examine—in vivo, in a culture dish, or in three-dimensional (3D) cultures—mechanisms that promote vascular pathologies and to test new strategies for correcting abnormal vasculature. However, the key challenge for all of these applications is the ability to derive adequate numbers of healthy progeny from hiPSC-derived vascular progenitor cells that can form functional vessels in vivo. Here, we discuss new developments in the generation of vascular progenitor cells from hiPSCs, their functional capacity in vivo, and opportunities and challenges for clinical translation in diseases with a vascular component, such as type 2 diabetes (T2D).

BRIEF HISTORY OF VASCULAR ENGINEERING

The pre-hiPSC era

Blood vessel formation occurs either from existing vessels (angiogenesis) or de novo by the differentiation of endothelial stem/progenitor cells (vasculogenesis) (1, 5). Vascular tissue engineering involves an intricately orchestrated series of cellular and molecular events that engage vascular progenitor cells and nonvascular cells in a specialized micro-environment (1). Two applications of vascular tissue engineering include (i) the generation of a vascular graft or bypass using scaffolds seeded with bone marrow–derived mononuclear cells to treat cardiovascular disease (6) and (ii) revascularization by stimulating angiogenesis using cell transplantation in the setting of chronic limb ischemia (7). Important developments in vascular tissue engineering include the exploration of various sources of vascular cells, identification of biologically relevant animal models of human disease, development of sophisticated techniques for in vivo visualization of engineered blood vessels, and the deciphering of mechanistic insights into blood vessel development and pathophysiology (3, 8–10).

Factors activating signaling pathways that orchestrate vasculo-genesis and angiogenesis include vascular endothelial growth factor (VEGF) family members, basic fibroblast growth factor (bFGF), platelet-derived growth factor–BB (PDGF-BB), semaphorin and Hedgehog family ligands, the Notch family of cell surface receptors, and the Tie-2 receptor and its angiopoietin ligands (11, 12). Thus, one approach is using the controlled release of angiogenic growth factors in combination with extracellular matrix (ECM), biochemical, and mechanical cues to stimulate the generation of new blood vessels from existing ones (13).

Another approach to generate new blood vessels is the implantation of tissue-engineered vessel constructs that consist of ECs, either alone or with PVCs embedded in scaffolds made of ECM components or synthetic polymers. Elegant mechanistic work has demonstrated that PVCs facilitate the stability of blood vessels via crosstalk with ECs, a process that appears to be mediated by PDGF-B and transforming growth factor–β (TGF-β) (14). However, the precise mechanisms that facilitate stabilization of blood vessels and their integration into the host circulation remain elusive. We have successfully generated durable vascular networks in immunodeficient mice using type 1 collagen–fibronectin matrices that contain human umbilical vein ECs (HUVECs) along with 10T1/2 mesenchymal stem/progenitor cells derived from mouse embryo (15). This approach led to the formation of functional microvascular networks that lasted for 1 year. To examine the formation and function of engineered blood vessels in vivo, we imaged engineered vessels by intravital multiphoton laser-scanning microscopy through chronic “transparent” windows in immunodeficient mice (8, 9, 15, 16). Detailed observations of the interface between an engineered vessel construct and the host tissue revealed a new anastomosis mechanism, in which implanted ECs wrap around nearby host vessels and cause reorganization of the vessel structure; this leads to perfusion of the engineered vessels and displacement of the host endothelium (a process termed “wrapping and tapping”) (17).

In an attempt to make the clinical translation of this vascular engineering method more feasible, researchers used endothelial progenitor cells (EPCs) isolated from adult peripheral blood in place of HUVECs. However, EPC-derived blood vessels regressed within 3 weeks, whereas the functional vasculature formed by HUVECs lasted for more than 4 months, suggesting that “stemness” may play a key role in the generation of robust, functional vessels. This observation could be attributed to a higher proliferative capacity or a greater resistance to stress-induced apoptosis (enhanced survival capacity) of cord blood–derived EPCs compared to peripheral blood EPCs (16). Replacement of murine 10T1/2 cells with either bone marrow–derived or adipose tissue–derived human mesenchymal stem cells (hMSCs) (18, 19) results in the MSCs differentiating into PVCs with a pericyte-like phenotype that stabilizes vessels in the mouse models (18). Last, the derivation of vascular progenitor cells from a potentially limitless source such as human embryonic stem cells (hESCs) generated tremendous excitement for vascular tissue engineering (20, 21). Functional blood vessels were created in mice by co-implanting hESC-ECs with 10T1/2 cells, and the resulting vascular networks lasted for at least 150 days (22). Although these experiments provided a proof of principle for stem cell–derived tissue-engineered blood vessels and hESCs remain the gold standard for pluripotency, the use of hESCs in the clinic remains controversial.

Thus, despite substantial advances in vascular tissue engineering, important challenges remain that have precluded successful application in the clinic. Cell therapy using EPCs has not been successful in generating robust vascular networks in humans (23). Therapeutic strategies that use proangiogenic cells for peripheral vascular disease (24) or ischemic heart disease patients (25) have led to only modest functional improvement. Vascular cells that are exposed to a hypoxic environment are known to be vulnerable to cell death in vivo. Given the heterogeneity of ECs (26), functionally viable engineered blood vessels might need a full complement, or specific types, of ECs to form different kinds of blood vessels,

including capillaries, arteries, and venules. Also, the “one size fits all” approach might not be appropriate when using PVCs, which could display different phenotypes depending on anatomical location. For example, PVC coverage of blood vessels in the brain is very tight but is scant in skeletal muscle. Besides engineering the cellular components, it is equally relevant to pay attention to the biological composition of the scaffold that forms the microenvironment for ECPVC interaction and stability. Inherent EC dysfunction and senescence of vascular cells with advanced aging is also likely to have an effect on the quality of the new vessels (27, 28).

The hiPSC era

Since the development of Yamanaka and colleagues’ groundbreaking method for reprogramming adult human fibroblasts into pluripotent stem cells (4), other methods have been devised for producing hiPSC derivatives that are potentially safer for use in the clinic (29–33). However, poor reprogramming efficiencies and an incomplete mechanistic understanding of the barriers to reprogramming have impeded clinical translation. It was recently demonstrated that totipotent mouse iPSCs generated in vivo are biologically more similar to ESCs than are in vitro–generated iPSCs (34). The selected inhibition of a single factor, Mbd3 (an epigenetic repressor), radically amplifies the reversion efficiency of primed (toward a lineage specification) mouse pluripotent epiblasts (derived from the late-blastocyst stage of embryo-genesis) back to naïve (ground-state) pluripotency. Further, more than 98% of mouse embryonic fibroblasts can be reprogrammed in a week's time when the traditional four “Yamanaka” factors are combined with the genetic depletion of Mbd3 (35). These reports have enhanced our understanding of reprogramming mechanisms in mouse and human iPSCs (hiPSCs).

A crucial outstanding issue for the field is what conditions most favorably influence the subsequent differentiation potential of hiPSCs—the cell of origin for hiPSCs (source), the efficiency of the reprogramming protocol, or both. The memory of hiPSCs—that is, the gene expression profile that reflects the cell's origin—diminishes over passages of the cells in culture. However, Ohi *et al*. investigated the genome-wide gene expression profiles of a variety of hiPSC lines and found that their somatic memory—even in the hiPSC lines derived with nonviral, nonintegrating techniques—suggests that the hiPSCs retain the demethylated (that is, activated) status of genes resident in the cells of origin (36). This is in contrast to ESCs, in which such genes are typically methylated (that is, inactivated). Of greater concern is that the active expression of certain genes in hiPSCs is also characteristic of cancer cells; thus, clinical translation cannot proceed before a careful examination of the genome and epigenome of hiPSC-derived cell lines.

These concerns notwithstanding, the first human clinical study using hiPSC-derived retinal pigment epithelial cells was approved in July 2013 for injection into the eyes of six patients diagnosed with age-related macular degeneration, a condition that leads to blindness (37). This trial, which officially began in September 2014, is the first study in which human patients were implanted with genetically matched skin cells that had been reprogrammed to hiPSCs and then differentiated into retinal cells (37, 38). The expectation of such a study is that the hiPSC-derived retinal cells will prevent ongoing destruction of eye photoreceptors.

However, this approach is unlikely to repair vision that has already been lost. If successful, the landscape for hiPSC derivatives for human translation will widen dramatically (37). The "proof of concept" for retinal sheet transplantation therapy for advanced retinal degenerative diseases has recently been demonstrated in mice (39).

The hope for hiPSC-derived tissue in regenerative medicine has been the ability to replace tissues or organs that would otherwise require transplantation. Indeed, hiPSC-derived hepatocytes, HUVECs, and MSCs have been used to engineer a functional vascularized liver in mice (40). Crosstalk among a combination of cell types is required for generating a functioning organoid that can recapitulate physiological function in vivo. In the liver organoids, the HUVECs formed blood vessels and MSCs likely differentiated into PVCs and stabilized the nascent vasculature. Cerebral organoids ("mini-brains") derived from hiPSCs have been created in a culture dish and grow to their maximum size in 2 months (41). The lack of a vasculature to deliver oxygen and nutrients to the core of the cerebral organoids likely prevents further development.

MAKING VASCULAR ECs FROM hiPSCs

ECs have been derived from hiPSCs that originated from a variety of cell types; skin fibroblasts (8, 42, 43), umbilical cord blood ECs (44, 45), bone marrow–derived $CD34^+$ mononuclear cells (44, 46), neonatal lung fibroblasts (44), dental pulp–derived cells (47), MSCs (43, 48), vSMCs (49), and lipoaspirates (50) have all been reprogrammed to hiPSCs and subsequently differentiated into ECs. Diverse methods have been used to produce ECs from hiPSCs in either 2D (8) or 3D (42, 43, 46, 49, 51) cultures under varying conditions, such as with the transfection of various combinations of transcription factors and growth on feeder or without feeder cells. Derivation of hiPSC-ECs involves the addition of cytokines or pharmacological agents to the cell culture medium, such as bone morphogenetic protein–4 (BMP-4), VEGF-A, bFGF, activin A, stem cell factor (SCF), Flt3, Flt3L, interleukin-3 (IL-3), IL-6, or a mitogen-activated protein kinase kinase (MEK) inhibitor to promote EC differentiation. hiPSC-ECs are characterized with the use of flow cytometry and a variety of cell surface selection markers [CD34, CD31, CD144/vascular endothelial–cadherin (VE-cadherin), CD146, Tie-2 (an angiopoietin receptor), fms-related tyrosine kinase-1 (FLT1), kinase insert domain receptor (KDR), and von Willebrand factor (vWF)] (tables S1 and S2).

Studies have detected similar gene expression profiles in hESC-ECs and hiPSC-ECs (52). A high level of concordance of gene expression was also noted between gene expression profiles of hiPSC-ECs derived from a single hiPSC line but with various differentiation protocols (52). However, hiPSC-ECs derived from a variety of hiPSC cell lines using distinct differentiation protocols display variability, in both in vivo and in vitro experiments, in the functionality of vascular networks derived from these cells (8).

hiPSC-ECs derived using 2D culture techniques demonstrated in vivo formation of blood vessels in mice when co-implanted with PVCs. However, hiPSC-ECs derived from 3D embryoid bodies, which are aggregates of pluripotent stem cells, were unable to form blood vessels in vivo. The mechanisms that underlie these differences in 2D and 3D procedures have yet to be elucidated (8). Reprogramming techniques that have generated partial iPSCs

that were then differentiated to ECs have demonstrated improved blood flow in mouse models of hindlimb ischemia (53).

A full complement of EC types can be derived from hiPSCs, which suggests that hiPSC-ECs are functionally diverse and presumably similar to human ECs (26). Rufaihah *et al*. have demonstrated that varying the exposure to soluble growth factors in differentiation protocols for hiPSC leads to differentiation into ECs with arterial, venous, or lymphatic EC characteristics (54). Culturing of cells in medium with high concentrations of VEGF yields an arterial-EC phenotype, whereas low VEGF levels promote venous specification, and a combination of high VEGF-C and angiopoietin-1 levels favors lymphatic differentiation. hiPSC-ECs with an arterial phenotype form capillary networks more robustly in vivo as compared with a heterogeneous hiPSC-EC population when seeded in subcutaneous Matrigel plugs and implanted in severe combined immunodeficient (SCID) mice. These results suggest that the arterial differentiation/specification of hiPSC-ECs might be beneficial for therapeutic angiogenesis, whereas hiPSC–lymphatic ECs might be more advantageous for the treatment of lymphedema (54). Indeed, immature EPCs also show plasticity between arterial and venous phenotypes. An arterial phenotype in ECs is believed to be induced by NOTCH signaling and shear stress and is facilitated by a high concentration of VEGF in medium used for endothelial differentiation (55). Identification of early vascular precursor cell surface markers such as CD87 (urokinase-type plasminogen activator receptor) (56) might provide clues into endothelial specification during development and offer access to vasculogenic clones of hiPSC-ECs (8).

MAKING PVCs FROM hiPSCs

PVCs such as pericytes and vSMCs are necessary for the stabilization and maturation of blood vessels engineered from hiPSCs. To facilitate translation to the clinic, ECs and PVCs could be derived from the same autologous hiPSCs. Related transition states in the form of MSC intermediate cells exist between the different types of mural/PVC progenitor cells and functional vSMCs and pericytes engineered from hiPSCs (57). Pericytes are the microvascular counterparts of vSMCs. Whereas pericytes exist only in a perivascular location and attached to the longitudinal axis of capillaries with finger-like extensions, vSMCs are positioned perpendicularly to the entire endothelial surface. The two cell types are also differentiated by morphology and the expression of cytosolic and membrane-bound markers (1, 2).

hiPSC-derived MSCs have been generated in vitro from neonatal or adult skin fibroblasts, human vSMCs, neonatal lung fibroblasts, bone marrow–derived $CD34^+$ progenitor cells, and hair keratinocytes (57, 58). With varying efficiency, the derivation and characterization of hiPSC–mural cells make use of the addition of growth factors to cultures and flow cytometry and a variety of selection markers, respectively. Selection markers used to characterize hiPSC-derived MSCs include CD73, CD146, CD105, CD90, CD24, CD44, CD54, NG2, PDGFR-β, HSP90, and human leukocyte antigen (HLA) class I, whereas hiPSC-derived SMCs and vSMCs are characterized with a-smooth muscle actin, calponin, SM22, TCF15, MYH11, ACTA2, CNN1, and CSPG4. Magnetic bead sorting and the

addition of cytokines to culture medium (BMP-4, DKK3, PD98059, Flt3, SCF, VEGF, IL-3, and IL-6) promote the differentiation of hiPSC-derived mural cells (tables S1 and S2).

Several reports describe hiPSC-to-MSC derivation and successful inoculation of these hiPSC-MSCs in rodent models of ischemia (59, 60). However, results obtained from human bone marrow–derived MSCs indicate that the mechanisms by which MSCs participate in revascularization remain unclear; it is possible that the therapeutic properties of MSCs result at least in part from the secretion of paracrine factors (61, 62). More recently, exosomes from hiPSC-derived MSCs were shown to have paracrine effects and to promote angiogenesis and improve vascularization in a mouse hindlimb ischemia model (63).

Dar *et al*. demonstrated that $CD105^{+}CD90^{+}CD73^{+}CD31^{-}$ multipotent clonogenic mesodermal progenitor cells that arise spontaneously from embryoid bodies are positive for pericyte markers CD146, NG2, and PDGFR-β but are negative for α-smooth muscle actin. When cultured, these mesodermal precursor cells form cartilage, bone, fat, and skeletal muscle cells and show vasculogenic potential in Matrigel plugs when co-implanted with hESC-ECs or HUVECs. The embryoid body–derived PVCs promote both vascular and muscle regeneration in mice (59). These findings have implications for the use of both ECs and pericytes derived from hiPSCs in vascular tissue engineering and also suggest that the hiPSC-PVCs derived from embryoid bodies might shed light on these elusive and often neglected perivascular support cells.

Another key finding was the generation of vascularized liver buds formed by the co-implantation of hiPSC-derived hepatocytes with (non-hiPSC) HUVECs and MSCs. The development of the vascular network within the liver organoids within 2 days was attributed to the subset of MSCs behaving like pericytes—that is, surrounding the HUVECs and stabilizing blood vessels—as has been demonstrated in previous experiments (15, 40).

Comparative studies suggest that hiPSC-derived SMCs are similar to the human SMC, sharing gene expression patterns, epigenetic modifications, and functional properties in vitro. hiPSC-SMCs from human aortic vascular SMCs have been redifferentiated back to SMCs and shown to be remarkably similar to the parenteral cells (64). hiPSC-SMCs derived from three progenitor subtypes (lateral plate mesoderm, paraxial mesoderm, and neuroectoderm) suggest that organ-specific SMCs exist and are an important resource for understanding human vascular disease and development (65). Quicker generation of hiPSC-PVCs has been enabled with transient reprogramming techniques (66). Furthermore, the limited proliferative potential of human adult MSCs seems to be offset by a stepwise approach to generate functional hiPSC-SMCs through a highly proliferative MSC progenitor cell population (57). The hiPSC-SMCs can be directed to adopt either a synthetic or contractile phenotype; the former displays high proliferation, migration, and ECM-protein production, whereas the latter displays low proliferation, synthetic activity, expression of myogenic proteins (such as α-smooth muscle actin and calponin), and a fibrillar organization (67).

FUNCTIONAL EVALUATION OF hiPSC-DERIVED VASCULAR CELLS

Although several methods exist to generate hiPSC-ECs in vitro, only a few studies have performed functional evaluations of hiPSC-ECs in vivo, with follow-up typically not extending beyond 1 month. hiPSC-ECs have promoted neovascularization in vivo in murine models of hindlimb ischemia (68), myocardial infarction (47, 50), and retinal ischemia (69). hiPSC-ECs have also been shown to reduce collagen deposition and, in combination with hiPSC-derived SMCs, to enhance vascularization in a mouse model of scleroderma (70). The functional response of hiPSC-ECs to proinflammatory stimuli has also been demonstrated in vivo. hiPSC-ECs respond to biomechanical cues, thereby directing differentiation into cells of the circulatory system and exhibiting an activated phenotype in response to proinflammatory stimuli (43). The ability to modulate hiPSC-ECs to develop athero-protective or athero-prone phenotypes has shown potential for drug screening to identify compounds that prevent atherosclerotic plaques.

Ideally, hiPSC-engineered blood vessel precursors for use in the clinic should be HLA-matched (Fig. 2). This could be achieved by generating both the ECs and PVCs from fibroblasts isolated from the same individual and then using both of these cells to form blood vessels in vivo. Indeed, we and another group have demonstrated this approach using different techniques (8, 44). Kusuma *et al*. derived early vascular cells (EVCs) from hiPSCs that were capable of maturing into both ECs and PVCs and organizing into vascular networks in hyaluronic acid–based hydrogels (44). VE-cadherin–positive (VE-Cad$^+$) EVCs actively form tube-like structures in vitro, and on day 3 in culture, NG2$^+$ pericyte-like cells encircle the tubular structures. Vascular networks are identifiable within the hydrogel in vitro using confocal microscopy. Two weeks after subcutaneous implantation of these vessel constructs, survival and perfusion of these vessels in vivo were confirmed by histo-logical observation (44). However, functionality and sustainability of these vessels in vivo remain unknown.

Our recent work demonstrated the ability to form hiPSC-engineered blood vessels that remain healthy and stable in vivo for 280 days in a cranial window model and for 30 days in dorsal skinfold chambers in SCID mice (8). The EPCs were derived from hiPSCs using a triple combination of selection markers [CD34, neuropilin-1 (NP-1), and KDR] and an efficient 2D culture system for hiPSC-EPC expansion. Noninvasive longitudinal in vivo microscopy was used to measure parameters such as permeability to bovine serum albumin and velocity and flow of red blood cells (RBCs), as well as to confirm the function of blood vessels in vivo (Fig. 1 and movies S1 and S2). The RBC velocities in the engineered blood vessels are comparable to normal endogenous host vessels (1.36 ± 0.3 mm/s) and demonstrate a higher permeability compared to endogenous vessels, suggesting that the engineered vessel network is functional (delivers oxygen and nutrients) and thus might support engineered tissues. However, the vessel wall might not have matured fully in these constructs. Together, these experiments reveal that durable hiPSC-derived blood vessels could be formed from endothelial and mesenchymal precursor cell types isolated from the same hiPSC line (8). Recently, another report showed that when hiPSCs are differentiated to ECs, the VE-Cad$^+$ cells that are also CD31$^+$CD34$^+$CD14$^-$KDR^{high} exhibit high angiogenic and clonogenic proliferation abilities among the EC lineage (71).

CLINICAL TRANSLATION

Worldwide, there are currently 4857 clinical trials registered at clinical-trials.gov that involve stem cells, with more than half of these in the United States (Fig. 3). Of these, 420 clinical trials use stem cells for the treatment of peripheral vascular disease, and 144 use stem cells to treat diabetes. There are 21 clinical trials listed in clinicaltrials.gov that have used or are using hESC-derived products, primarily for eye disorders such as macular degeneration. Notably, currently, there are 32 trials listed in clinicaltrials.gov that involve hiPSCs, predominantly in the United States (n = 24); the rest are being carried out in France (n = 3), Israel (n = 2), Spain (n = 1), UK (n = 1), and Slovenia (n = 1). These studies include characterization of hiPSC-derived cells from cardiovascular disease, neurological disease, retinal degenerative diseases, and immunodeficiency disorders. Currently, there are no clinical trials that have used hiPSC-derived products for alleviating vascular disease such as peripheral limb ischemia or coronary heart disease. However, there is an increasing body of literature that describes basic research with hiPSC-derived ECs and PVCs being tested in vitro and in in vivo functional studies in mice (tables S2 and S3).

The potential applications of hiPSC-derived vascular cells are broad and include engineering blood vessels in coronary heart disease, alleviating critical limb ischemia in peripheral artery disease, and examining the basis of diabetic vascular disease such as peripheral ischemia and retinopathy. Heart disease and stroke remain outstanding causes of cardiovascular disease burden in the United States. Coronary heart disease accounted for one in six deaths in 2009. The total burden of cardiovascular disease and stroke cost $312.6 billion in 2011. This is startling when one considers that in 2008, the total cost of all benign and malignant neoplasms was estimated at $228 billion (72). In 2010, stroke was the fourth leading cause of death in the United States, and worldwide, stroke is the second common cause of death after heart disease and is responsible for 1/10 of all deaths (73). Thus, the potential benefit of engineering stable and functional vascular tissue is enormous.

MODELS AND MECHANISMS

Diabetic vascular disease

India is second to China in global prevalence of T2D, followed by the United States. In 2011, more than 371 million people worldwide suffered from T2D, and more than $471 billion was spent on health care for diabetic patients. By 2030, 552 million are expected to have T2D worldwide, and 0.7% of these will develop retinopathy and become blind (www.idf.org/diabetesatlas/5e/Update2012). Of the 63 million Indians with diabetes, it is alarming to note that a third of T2D patients already have microvascular complications at presentation, and in developing countries, T2D vascular complications commonly result in diabetic foot ulcers and nontraumatic limb amputations (74). hiPSC-derived vascular progenitor cells that assist with revascularization of ischemic tissue could save limbs (Fig. 2). However, in these vascular diseases, the host endothelium is often dysfunctional (75), and it is possible that the hiPSC-derived cells from a diseased patient carry such memory. Conversely, the resetting of the epigenetic landscape as a consequence of reprogramming might yield hiPSC-derived vascular progeny that differ from their parent cell—a property that has been demonstrated in mice (76).

Diabetic hiPSCs have been derived mainly for the differentiation or proposed differentiation to pancreatic β cells (77). From a developmental perspective, however, it is known that the endothelium is instructive in providing signals that determine morphogenesis and cell differentiation of the liver and pancreas (78). The value of obtaining diabetic hiPSC-derived vascular cells would be most robust in examining monogenic diabetes, such as type 1 diabetes (T1D) or maturity-onset diabetes of the young (MODY) (79). We have been successful in isolating hiPSC-derived ECs and PVCs from T1D and MODY cases (8). Whereas the T1D-iPS–engineered blood vessels remained functional in mice for 4 months, the MODY-iPS-ECs failed to form functional blood vessels in vivo, despite demonstrating bona fide endothelial tube–forming potential in vitro in Matrigel (8).

T2D-hiPSC lines derived from elderly patients are capable of generating insulin-producing β cells. In contrast, there are no published data, to date, that demonstrate the derivation of vascular progenitor cells from diabetic hiPSCs. The derivation of T2D-hiPSC-ECs and T2D-hiPSC-PVCs might reveal a robust phenotype, which, even in the absence of a well-defined genetic background, might lead to mechanistic insights into vascular dysfunction in T2D. It is reasonable to speculate that data from the evaluation of hiPSC-engineered blood vessels from healthy controls and T1D and MODY patients (8) could be applied to the investigation of T2D-hiPSC vasculature.

Other vascular diseases

hiPSC-derived vascular cells have been investigated from rare vascular diseases with known genetic mutations (table S3). hiPSCs from the premature aging disease Hutchinson-Gilford progeria syndrome (HGPS) were differentiated into five lineages: neural progenitor, endothelial, fibroblasts, vSMCs, and MSCs (80). Survival of HGPS-hiPSC-vSMCs was reduced under hypoxic conditions, and HGPS-hiPSC-MSCs were unable to prevent necrosis in a murine hindlimb ischemia model, as compared with healthy MSCs. Fibroblasts derived from patients with Williams-Beuren syndrome (WBS) who displayed pulmonary and aortic stenosis were successfully differentiated to SMCs. These WBS-hiPSC-SMCs recapitulated the disease phenotype in vitro by demonstrating high proliferation and immature phenotype with low expression of differentiated SMC markers. The WBS-hiPSC-SMC phenotype was rescued with the use of rapamycin, which inhibits smooth muscle proliferation through the mammalian target of rapamycin (mTOR) pathway (80). This result supports the testing of rapamycin in treatment of individuals with WBS and represents an example of the use of hiPSCs for modeling of diseases associated with smooth muscle proliferation and for the discovery of treatment modalities to be tried in the clinic (81). In attempts to model disease in a dish, hiPSC-SMCs were derived from patients with supravalvular aortic stenosis (82); hiPSC-differentiated cardiomyocytes were derived from patients with the LEOPARD syndrome (83); and hiPSC-ECs were derived from patients with the moyamoya disease (idiopathic cerebrovascular disease) (84).

Besides using hiPSCs to model vascular disease, hiPSC-ECs have been useful in generating an in vitro blood-brain barrier (BBB) model (85). The BBB is composed of specialized ECs known as brain micro-vascular ECs as well as PVCs and astrocytes—star-shaped glial cells found in the brain and spinal cord. The inaccessibility of these cell types from humans has

resulted in studies of the BBB from rodents, cows, and pigs. A robust BBB of human origin was created for the first time with the use of hiPSC derivatives. When hiPSCs were co-differentiated into neural cell and EC lineages, the resulting hiPSC-ECs acquired BBB properties such as the expression of tight junction proteins, glucose transporter, and P-glycoprotein. These hiPSC-ECs demonstrate extremely high transendothelial electrical resistance, low molecular permeability, and polarized transporter activity in response to astrocytes in a coculture. This work offers a meaningful platform with which to examine developmental aspects of BBB formation and more clinically relevant issues such as opportunities to promote BBB repair after stroke or to restrain the recruitment of blood vessels by aggressive brain tumors. The in vitro BBB model may also permit the assessment of drug delivery.

TRANSLATIONAL CHALLENGES

Only 7 years have passed since Yamanaka and colleagues demonstrated, in a landmark study, that it is possible to reprogram adult somatic cells back to an embryonic stem cell–like state. This discovery inspired the hope that hiPSC-derived cells could be used in the clinic to treat myriad diseases (38, 40). However, many challenges remain, especially with respect to the engineering of 3D tissues using hiPSCs (Fig. 2). Providing a vascular supply to newly generated tissues remains one of the most challenging barriers in building solid organs.

The state of organ transplantation in the United States is dire: One patient is listed for transplant every 11 min, and 130 join this list every day. Although 75 patients receive a transplant every day, 19 die because organs are not available (www.thenationalnetworkoforgandonors.org/statistics.html). hiPSC-derived vascular cells have broad application for regenerative medicine, because blood vessels are essential for any functional organs—as recently shown in the generation of functional livers in mice by co-implanting hepatocytes, MSCs, and vascular ECs (83). The expectation is that newly created, genetically matched organs will reduce the need for organ transplants in the future. But first, scientists much deal with such challenges as the variability of hiPSC lines and the long-term safety issues involved in the use of these cells, which are being addressed by researchers around the world. Animal models using hiPSC-derived retinal pigment epithelial cells showed negligible tumorigenic potential (86). However, this issue remains an important one for current and future hiPSC clinical trial testing. Also needed are better ways of engineering organ-specific ECs, because these cells are heterogeneous, in terms of both cellular content (arterial, venular, or lymphatic) and function, depending on anatomical location (fenestrated versus nonfenestrated) (26). Therefore, engineering specific type of ECs or organ-specific ECs is biologically relevant and important for clinical application.

Several other outstanding issues must be addressed before hiPSC-derived vascular progenitor cells find application in the clinic. Pinpointing the appropriate vasculogenic clones in and deciphering the molecular mechanisms that confer vasculogenic potential on hiPSCs are necessary before we begin to develop methods for scaling up to large numbers of hiPSC vascular progeny. For example, the selection of triple-positive $CD34^{+}KDR^{+}NP1^{+}$ EC

precursors from a population of vascularized hiPSCs provides highly vasculogenic cells that form functional blood vessels in vivo when co-implanted with PVCs (Fig. 1) (8).

We also must consider the variability of hiPSC lines, such as the cell of origin and the reprogramming techniques used. These factors will influence the success of hiPSC vascular derivatives in regenerative medicine. Further, is the timing of vascular insult critical in vascular therapy using hiPSC-derived vascular cells (similar to spinal cord injury, in which immediate cell therapy is necessary to elicit a response)? When translating mouse systems to humans, efforts to scale up hiPSC-derived vascular cells will likely require bioreactors, facilities for clinical-grade production, biocompatible scaffolds, and release devices to track the immune response in vivo. Another issue is the ability to obtain homogeneous populations of cells derived from hiPSCs. Cost-effective therapy using hiPSC derivatives remains a critical issue, because manufacturing costs can be often prohibitive. The possibility that hiPSC derivatives might “drift” to an immature or cancerous phenotype in vivo also remains a cause for concern (86). Recent reports of tumors detected after treatment with stem cells reinforce the need for vigilant and prolonged follow-up (87).

Above all, in the rush to produce safe and biologically relevant hiPSC-derived vascular progeny, the concern is that the host endothelium is often dysfunctional in vascular disease, and it is possible that the hiPSC-derived cells carry such memory. To overcome this problem, researchers require a mechanistic understanding of healthy EC generation. As of today, the only clinical trial in progress uses retinal pigment epithelium to treat age-related macular degeneration. Other proposed clinical trials involving hiPSC derivatives include platelets and genetically corrected keratinocytes to treat epidermolysis bullosa. Other possible trials with hiPSCs include oligodendrocyte precursor cells, dopaminergic neurons, retinal ganglion cells, keratinocytes, and hematopoietic grafts (38). The hope is that hiPSC-derived vascular progenitor cells, too, will find a place in clinical trials to alleviate vascular insufficiency. Until this happens, hiPSC-derived vascular cells serve as an exceptional source to examine diseased human cells in a dish.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

REFERENCES AND NOTES

1. Jain RK. Molecular regulation of vessel maturation. Nat. Med. 2003; 9:685–693. [PubMed: 12778167]
2. Armulik A, Genové G, Betsholtz C. Pericytes: Developmental, physiological, and pathological perspectives, problems, and promises. Dev. Cell. 2011; 21:193–215. [PubMed: 21839917]
3. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature. 2011; 473:298–307. [PubMed: 21593862]
4. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007; 131:861–872. [PubMed: 18035408]
5. Patel-Hett S, D'Amore PA. Signal transduction in vasculogenesis and developmental angiogenesis. Int. J. Dev. Biol. 2011; 55:353–363. [PubMed: 21732275]

6. Udelsman BV, Maxfield MW, Breuer CK. Tissue engineering of blood vessels in cardiovascular disease: Moving towards clinical translation. Heart. 2013; 99:454–460. [PubMed: 23363931]
7. Jadlowiec C, Brenes RA, Li X, Lv W, Protack CD, Collins MJ, Dardik A. Stem cell therapy for critical limb ischemia: What can we learn from cell therapy for chronic wounds? Vascular. 2012; 20:284–289. [PubMed: 23086986]
8. Samuel R, Daheron L, Liao S, Vardam T, Kamoun WS, Batista A, Buecker C, Schäfer R, Han X, Au P, Scadden DT, Duda DG, Fukumura D, Jain RK. Generation of functionally competent and durable engineered blood vessels from human induced pluripotent stem cells. Proc. Natl. Acad. Sci. U.S.A. 2013; 110:12774–12779. [PubMed: 23861493]
9. Kamoun WS, Chae S-S, Lacorre DA, Tyrrell JA, Mitre M, Gillissen MA, Fukumura D, Jain RK, Munn LL. Simultaneous measurement of RBC velocity, flux, hematocrit and shear rate in vascular networks. Nat. Methods. 2010; 7:655–660. [PubMed: 20581828]
10. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature. 2000; 407:249–257. [PubMed: 11001068]
11. Park C, Kim TM, Malik AB. Transcriptional regulation of endothelial cell and vascular development. Circ. Res. 2013; 112:1380–1400. [PubMed: 23661712]
12. Tan KS, Tamura K, Lai MI, Veerakumarasivam A, Nakanishi Y, Ogawa M, Sugiyama D. Molecular pathways governing development of vascular endothelial cells from ES/iPS cells. Stem Cell Rev. 2013; 9:586–598. [PubMed: 23765563]
13. Discher DE, Mooney DJ, Zandstra PW. Growth factors, matrices, and forces combine and control stem cells. Science. 2009; 324:1673–1677. [PubMed: 19556500]
14. Hirschi KK, Rohovsky SA, Beck LH, Smith SR, D'Amore PA. Endothelial cells modulate the proliferation of mural cell precursors via platelet-derived growth factor-BB and heterotypic cell contact. Circ. Res. 1999; 84:298–305. [PubMed: 10024303]
15. Koike N, Fukumura D, Gralla O, Au P, Schechner JS, Jain RK. Tissue engineering: Creation of long-lasting blood vessels. Nature. 2004; 428:138–139. [PubMed: 15014486]
16. Au P, Daheron LM, Duda DG, Cohen KS, Tyrrell JA, Lanning RM, Fukumura D, Scadden DT, Jain RK. Differential in vivo potential of endothelial progenitor cells from human umbilical cord blood and adult peripheral blood to form functional long-lasting vessels. Blood. 2008; 111:1302–1305. [PubMed: 17993613]
17. Cheng G, Liao S, Kit Wong H, Lacorre DA, di Tomaso E, Au P, Fukumura D, Jain RK, Munn LL. Engineered blood vessel networks connect to host vasculature via wrapping-and-tapping anastomosis. Blood. 2011; 118:4740–4749. [PubMed: 21835951]
18. Au P, Tam J, Fukumura D, Jain RK. Bone marrow–derived mesenchymal stem cells facilitate engineering of long-lasting functional vasculature. Blood. 2008; 111:4551–4558. [PubMed: 18256324]
19. Grainger SJ, Carrion B, Ceccarelli J, Putnam AJ. Stromal cell identity influences the in vivo functionality of engineered capillary networks formed by co-delivery of endothelial cells and stromal cells. Tissue Eng. Part A. 2013; 19:1209–1222. [PubMed: 23216113]
20. Lu S-J, Kelley T, Feng Q, Chen A, Reuveny S, Lanza R, Oh SK. 3D microcarrier system for efficient differentiation of human pluripotent stem cells into hematopoietic cells without feeders and serum. Regen. Med. 2013; 8:413–424. [PubMed: 23826696]
21. Sun X, Cheng L, Duan H, Lin G, Lu G. Characterization and comparison of embryonic stem cell-derived KDR+ cells with endothelial cells. Microvasc. Res. 2012; 84:149–154. [PubMed: 22706170]
22. Wang ZZ, Au P, Chen T, Shao Y, Daheron LM, Bai H, Arzigian M, Fukumura D, Jain RK, Scadden DT. Endothelial cells derived from human embryonic stem cells form durable blood vessels in vivo. Nat. Biotechnol. 2007; 25:317–318. [PubMed: 17322871]
23. Park KM, Gerecht S. Harnessing developmental processes for vascular engineering and regeneration. Development. 2014; 141:2760–2769. [PubMed: 25005471]
24. Blum A, Balkan W, Hare JM. Advances in cell-based therapy for peripheral vascular disease. Atherosclerosis. 2012; 223:269–277. [PubMed: 22494624]
25. Perin EC, Silva GV. Cell-based therapy for chronic ischemic heart disease—A clinical perspective. Cardiovasc. Ther. 2011; 29:211–217. [PubMed: 20955540]

26. Aird WC. Endothelial cell heterogeneity. Cold Spring Harb. Perspect. Med. 2012; 2:a006429.
27. Heeschen C, Lehmann R, Honold J, Assmus B, Aicher A, Walter DH, Martin H, Zeiher AM, Dimmeler S. Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. Circulation. 2004; 109:1615–1622. [PubMed: 15037527]
28. Fadini GP, Losordo D, Dimmeler S. Critical reevaluation of endothelial progenitor cell phenotypes for therapeutic and diagnostic use. Circ. Res. 2012; 110:624–637. [PubMed: 22343557]
29. Lin C, Yu C, Ding S. Toward directed reprogramming through exogenous factors. Curr. Opin. Genet. Dev. 2013; 23:519–525. [PubMed: 23932127]
30. Kaji K, Norrby K, Paca A, Mileikovsky M, Mohseni P, Woltjen K. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. Nature. 2009; 458:771–775. [PubMed: 19252477]
31. Kudva YC, Ohmine S, Greder LV, Dutton JR, Armstrong A, De Lamo JG, Khan YK, Thatava T, Hasegawa M, Fusaki N, Slack JMW, Ikeda Y. Transgene-free disease-specific induced pluripotent stem cells from patients with type 1 and type 2 diabetes. Stem Cells Transl. Med. 2012; 1:451–461. [PubMed: 23197849]
32. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. Science. 2008; 322:945–949. [PubMed: 18818365]
33. Warren L, Manos PD, Ahfeldt T, Loh Y-H, Li H, Lau F, Ebina W, Mandal PK, Smith ZD, Meissner A, Daley GQ, Brack AS, Collins JJ, Cowan C, Schlaeger TM, Rossi DJ. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell. 2010; 7:618–630. [PubMed: 20888316]
34. Abad M, Mosteiro L, Pantoja C, Cañamero M, Rayon T, Ors I, Graña O, Megías D, Domínguez O, Martínez D, Manzanares M, Ortega S, Serrano M. Reprogramming in vivo produces teratomas and iPS cells with totipotency features. Nature. 2013; 502:340–345. [PubMed: 24025773]
35. Rais Y, Zviran A, Geula S, Gafni O, Chomsky E, Viukov S, Mansour AA, Caspi I, Krupalnik V, Zerbib M, Maza I, Mor N, Baran D, Weinberger L, Jaitin DA, Lara-Astiaso D, Blecher-Gonen R, Shipony Z, Mukamel Z, Hagai T, Gilad S, Amann-Zalcenstein D, Tanay A, Amit I, Novershtern N, Hanna JH. Deterministic direct reprogramming of somatic cells to pluripotency. Nature. 2013; 502:65–70. [PubMed: 24048479]
36. Ohi Y, Qin H, Hong C, Blouin L, Polo JM, Guo T, Qi Z, Downey SL, Manos PD, Rossi DJ, Yu J, Hebrok M, Hochedlinger K, Costello JF, Song JS, Ramalho-Santos M. Incomplete DNA methylation underlies a transcriptional memory of somatic cells in human iPS cells. Nat. Cell Biol. 2011; 13:541–549. [PubMed: 21499256]
37. Reardon S, Cyranoski D. Japan stem-cell trial stirs envy. Nature. 2014; 513:287–288. [PubMed: 25230622]
38. Cyranoski D. Stem cells cruise to clinic. Nature. 2013; 494:413. [PubMed: 23446394]
39. Assawachananont J, Mandai M, Okamoto S, Yamada C, Eiraku M, Yonemura S, Sasai Y, Takahashi M. Transplantation of embryonic and induced pluripotent stem cell-derived 3D retinal sheets into retinal degenerative mice. Stem Cell Rep. 2014; 2:662–674.
40. Takebe T, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, Zhang R-R, Ueno Y, Zheng Y-W, Koike N, Aoyama S, Adachi Y, Taniguchi H. Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature. 2013; 499:481–484. [PubMed: 23823721]
41. Lancaster MA, Renner M, Martin CA, Wenzel D, Bicknell LS, Hurles ME, Homfray T, Penninger JM, Jackson AP, Knoblich JA. Cerebral organoids model human brain development and microcephaly. Nature. 2013; 501:373–379. [PubMed: 23995685]
42. Park TS, Zimmerlin L, Zambidis ET. Efficient and simultaneous generation of hematopoietic and vascular progenitors from human induced pluripotent stem cells. Cytometry A. 2013; 83:114–126. [PubMed: 22736485]
43. Adams WJ, Zhang Y, Cloutier J, Kuchimanchi P, Newton G, Sehrawat S, Aird WC, Mayadas TN, Luscinskas FW, García-Cardeña G. Functional vascular endothelium derived from human induced pluripotent stem cells. Stem Cell Rep. 2013; 1:105–113.

44. Kusuma S, Shen YI, Hanjaya-Putra D, Mali P, Cheng L, Gerecht S. Self-organized vascular networks from human pluripotent stem cells in a synthetic matrix. Proc. Natl. Acad. Sci. U.S.A. 2013; 110:12601–12606. [PubMed: 23858432]
45. Lagarkova MA, Shutova MV, Bogomazova AN, Vassina EM, Glazov EA, Zhang P, Rizvanov AA, Chestkov IV, Kiselev SL. Induction of pluripotency in human endothelial cells resets epigenetic profile on genome scale. Cell Cycle. 2010; 9:937–946. [PubMed: 20160486]
46. Bai H, Xie YL, Gao YX, Cheng T, Wang ZZ. The balance of positive and negative effects of TGF-b signaling regulates the development of hematopoietic and endothelial progenitors in human pluripotent stem cells. Stem Cells Dev. 2013; 22:2765–2776. [PubMed: 23758278]
47. Yoo CH, Na H-J, Lee D-S, Heo SC, An Y, Cha J, Choi C, Kim JH, Park J-C, Cho YS. Endothelial progenitor cells from human dental pulp-derived iPS cells as a therapeutic target for ischemic vascular diseases. Biomaterials. 2013; 34:8149–8160. [PubMed: 23896001]
48. Xu Y, Liu L, Zhang L, Fu S, Hu Y, Wang Y, Fu H, Wu K, Xiao H, Liu S, Yu X, Zheng W, Feng B, Huang H. Efficient commitment to functional CD34+ progenitor cells from human bone marrow mesenchymal stem-cell-derived induced pluripotent stem cells. PLOS One. 2012; 7:e34321. [PubMed: 22496789]
49. Kim KL, Song S-H, Choi K-S, Suh W. Cooperation of endothelial and smooth muscle cells derived from human induced pluripotent stem cells enhances neovascularization in dermal wounds. Tissue Eng. Part A. 2013; 19:2478–2485. [PubMed: 23790124]
50. Wang Y, Zhang WY, Hu S, Lan F, Lee AS, Huber B, Lisowski L, Liang P, Huang M, de Almeida PE, Won JH, Sun N, Robbins RC, Kay MA, Urnov FD, Wu JC. Genome editing of human embryonic stem cells and induced pluripotent stem cells with zinc finger nucleases for cellular imaging. Circ. Res. 2012; 111:1494–1503. [PubMed: 22967807]
51. Shao Y, Sang J, Fu J. On human pluripotent stem cell control: The rise of 3D bioengineering and mechanobiology. Biomaterials. 2015; 52:26–43. [PubMed: 25818411]
52. White MP, Rufaihah AJ, Liu L, Ghebremariam YT, Ivey KN, Cooke JP, Srivastava D. Limited gene expression variation in human embryonic stem cell and induced pluripotent stem cell-derived endothelial cells. Stem Cells. 2013; 31:92–103. [PubMed: 23079999]
53. Margariti A, Winkler B, Karamariti E, Zampetaki A, Tsai T.-n. Baban D, Ragoussis J, Huang Y, Han J-D, Zeng L, Hu Y, Xu Q. Direct reprogramming of fibroblasts into endothelial cells capable of angiogenesis and reendothelialization in tissue-engineered vessels. Proc. Natl. Acad. Sci. U.S.A. 2012; 109:13793–13798. [PubMed: 22869753]
54. Rufaihah AJ, Huang NF, Kim J, Herold J, Volz KS, Park TS, Lee JC, Zambidis ET, Reijo-Pera R, Cooke JP. Human induced pluripotent stem cell-derived endothelial cells exhibit functional heterogeneity. Am. J. Transl. Res. 2013; 5:21–35. [PubMed: 23390563]
55. Oliver G, Srinivasan RS. Endothelial cell plasticity: How to become and remain a lymphatic endothelial cell. Development. 2010; 137:363–372. [PubMed: 20081185]
56. Drukker M, Tang C, Ardehali R, Rinkevich Y, Seita J, Lee AS, Mosley AR, Weissman IL, Soen Y. Isolation of primitive endoderm, mesoderm, vascular endothelial and trophoblast progenitors from human pluripotent stem cells. Nat. Biotechnol. 2012; 30:531–542. [PubMed: 22634564]
57. Bajpai VK, Mistriotis P, Loh Y-H, Daley GQ, Andreadis ST. Functional vascular smooth muscle cells derived from human induced pluripotent stem cells via mesenchymal stem cell intermediates. Cardiovasc. Res. 2012; 96:391–400. [PubMed: 22941255]
58. Lin L, Bolund L, Luo Y. Towards personalized regenerative cell therapy: Mesenchymal stem cells derived from human induced pluripotent stem cells. Curr. Stem Cell Res. Ther. 2015 10.2174/1574888X10666150723150236.
59. Dar A, Domev H, Ben-Yosef O, Tzukerman M, Zeevi-Levin N, Novak A, Germanguz I, Amit M, Itskovitz-Eldor J. Multipotent vasculogenic pericytes from human pluripotent stem cells promote recovery of murine ischemic limb. Circulation. 2012; 125:87–99. [PubMed: 22095829]
60. Lian Q, Zhang Y, Zhang J, Zhang HK, Wu X, Zhang Y, Lam FF-Y, Kang S, Xia JC, Lai W-H, Au K-W, Chow YY, Siu C-W, Lee C-N, Tse H-F. Functional mesenchymal stem cells derived from human induced pluripotent stem cells attenuate limb ischemia in mice. Circulation. 2010; 121:1113–1123. [PubMed: 20176987]

61. Bailey AM, Mendicino M, Au P. An FDA perspective on preclinical development of cell-based regenerative medicine products. Nat. Biotechnol. 2014; 32:721–723. [PubMed: 25093890]
62. Park JH, Hwang I, Hwang SH, Han H, Ha H. Human umbilical cord blood-derived mesenchymal stem cells prevent diabetic renal injury through paracrine action. Diabetes Res. Clin. Pract. 2012; 98:465–473. [PubMed: 23026513]
63. Hu GW, Li Q, Niu X, Hu B, Liu J, Zhou SM, Guo SC, Lang HL, Zhang CQ, Wang Y, Deng ZF. Exosomes secreted by human-induced pluripotent stem cell-derived mesenchymal stem cells attenuate limb ischemia by promoting angiogenesis in mice. Stem Cell Res. Ther. 2015; 6:10. [PubMed: 26268554]
64. Lee T-H, Song S-H, Kim KL, Yi J-Y, Shin G-H, Kim JY, Kim J, Han Y-M, Lee SH, Lee S-H, Shim SH, Suh W. Functional recapitulation of smooth muscle cells via induced pluripotent stem cells from human aortic smooth muscle cells. Circ. Res. 2010; 106:120–128. [PubMed: 19959777]
65. Cheung C, Bernardo AS, Trotter MWB, Pedersen RA, Sinha S. Generation of human vascular smooth muscle subtypes provides insight into embryological origin–dependent disease susceptibility. Nat. Biotechnol. 2012; 30:165–173. [PubMed: 22252507]
66. Karamariti E, Margariti A, Winker B, Wang X, Hong X, Baban D, Ragoussis J, Huang Y, Han JD, Wong MM, Sag CM, Shah AM, Hu Y, Xu Q. Smooth muscle cells differentiated from reprogrammed embryonic lung fibroblasts through DKK3 signaling are potent for tissue engineering of vascular grafts. Circ. Res. 2013; 112:1433–1443. [PubMed: 23529184]
67. Wanjare M, Kuo F, Gerecht S. Derivation and maturation of synthetic and contractile vascular smooth muscle cells from human pluripotent stem cells. Cardiovasc. Res. 2013; 97:321–330. [PubMed: 23060134]
68. Rufaihah AJ, Huang NF, Jamé S, Lee JC, Nguyen HN, Byers B, De A, Okogbaa J, Rollins M, Reijo-Pera R, Gambhir SS, Cooke JP. Endothelial cells derived from human iPSCs increase capillary density and improve perfusion in a mouse model of peripheral arterial disease. Arterioscler. Thromb. Vasc. Biol. 2011; 31:e72–e79. [PubMed: 21836062]
69. Park TS, Bhutto I, Zimmerlin L, Huo JS, Nagaria P, Miller D, Rufaihah AJ, Talbot C, Aguilar J, Grebe R, Merges C, Reijo-Pera R, Feldman RA, Rassool F, Cooke J, Lutty G, Zambidis ET. Vascular progenitors from cord blood–derived induced pluripotent stem cells possess augmented capacity for regenerating ischemic retinal vasculature. Circulation. 2014; 129:359–372. [PubMed: 24163065]
70. Azhdari M, Baghaban-Eslaminejad M, Baharvand H, Aghdami N. Therapeutic potential of human-induced pluripotent stem cell-derived endothelial cells in a bleomycin-induced scleroderma mouse model. Stem Cell Res. 2013; 10:288–300. [PubMed: 23396195]
71. Sahara M, Hansson EM, Wernet O, Lui KO, Später D, Chien KR. Manipulation of a VEGF-Notch signaling circuit drives formation of functional vascular endothelial progenitors from human pluripotent stem cells. Cell Res. 2014; 24:820–841. [PubMed: 24810299]
72. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Magid D, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER, Moy CS, Mussolino ME, Nichol G, Paynter NP, Schreiner PJ, Sorlie PD, Stein J, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. American Heart Association Statistics Committee and Stroke Statistics Subcommittee, Executive summary: Heart disease and stroke statistics—2013 update: A report from the American Heart Association. Circulation. 2013; 127:143–152. [PubMed: 23283859]
73. Corbyn Z. Statistics: A growing global burden. Nature. 2014; 510:S2–S3. [PubMed: 24964023]
74. Cavanagh P, Attinger C, Abbas Z, Bal A, Rojas N, Xu Z-R. Cost of treating diabetic foot ulcers in five different countries. Diabetes Metab. Res. Rev. 2012; 28(Suppl. 1):107–111. [PubMed: 22271734]
75. Hill JM, Zalos G, Halcox JPJ, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N. Engl. J. Med. 2003; 348:593–600. [PubMed: 12584367]
76. Polo JM, Liu S, Figueroa ME, Kulalert W, Eminli S, Tan KY, Apostolou E, Stadtfeld M, Li Y, Shioda T, Natesan S, Wagers AJ, Melnick A, Evans T, Hochedlinger K. Cell type of origin

influences the molecular and functional properties of mouse induced pluripotent stem cells. Nat. Biotechnol. 2010; 28:848–855. [PubMed: 20644536]

77. Maehr R, Chen S, Snitow M, Ludwig T, Yagasaki L, Goland R, Leibel RL, Melton DA. Generation of pluripotent stem cells from patients with type 1 diabetes. Proc. Natl. Acad. Sci. U.S.A. 2009; 106:15768–15773. [PubMed: 19720998]
78. Lammert E, Cleaver O, Melton D. Role of endothelial cells in early pancreas and liver development. Mech. Dev. 2003; 120:59–64. [PubMed: 12490296]
79. Teo AK, Wagers AJ, Kulkarni RN. New opportunities: Harnessing induced pluripotency for discovery in diabetes and metabolism. Cell Metab. 2013; 18:775–791. [PubMed: 24035588]
80. Zhang J, Lian Q, Zhu G, Zhou F, Sui L, Tan C, Mutalif RA, Navasankari R, Zhang Y, Tse H-F, Stewart CL, Colman A. A human iPSC model of Hutchinson Gilford progeria reveals vascular smooth muscle and mesenchymal stem cell defects. Cell Stem Cell. 2011; 8:31–45. [PubMed: 21185252]
81. Dash BC, Jiang Z, Suh C, Qyang Y. Induced pluripotent stem cell-derived vascular smooth muscle cells: Methods and application. Biochem. J. 2015; 465:185–194. [PubMed: 25559088]
82. Ge X, Ren Y, Bartulos O, Lee MY, Yue Z, Kim K-Y, Li W, Amos PJ, Bozkulak EC, Iyer A, Zheng W, Zhao H, Martin KA, Kotton DN, Tellides G, Park I-H, Yue L, Qyang Y. Modeling supravalvular aortic stenosis syndrome with human induced pluripotent stem cells. Circulation. 2012; 126:1695–1704. [PubMed: 22914687]
83. Carvajal-Vergara X, Sevilla A, D'Souza SL, Ang Y-S, Schaniel C, Lee D-F, Yang L, Kaplan AD, Adler ED, Rozov R, Ge Y, Cohen N, Edelmann LJ, Chang B, Waghray A, Su J, Pardo S, Lichtenbelt KD, Tartaglia M, Gelb BD, Lemischka IR. Patient-specific induced pluripotent stem-cell-derived models of LEOPARD syndrome. Nature. 2010; 465:808–812. [PubMed: 20535210]
84. Hitomi T, Habu T, Kobayashi H, Okuda H, Harada KH, Osafune K, Taura D, Sone M, Asaka I, Ameku T, Watanabe A, Kasahara T, Sudo T, Shiota F, Hashikata H, Takagi Y, Morito D, Miyamoto S, Nakao K, Koizumi A. Downregulation of Securin by the variant RNF213 R4810K (rs112735431, G>A) reduces angiogenic activity of induced pluripotent stem cell-derived vascular endothelial cells from moyamoya patients. Biochem. Biophys. Res. Commun. 2013; 438:13–19. [PubMed: 23850618]
85. Lippmann ES, Azarin SM, Kay JE, Nessler RA, Wilson HK, Al-Ahmad A, Palecek SP, Shusta EV. Derivation of blood-brain barrier endothelial cells from human pluripotent stem cells. Nat. Biotechnol. 2012; 30:783–791. [PubMed: 22729031]
86. Kanemura H, Go MJ, Shikamura M, Nishishita N, Sakai N, Kamao H, Mandai M, Morinaga C, Takahashi M, Kawamata S. Tumorigenicity studies of induced pluripotent stem cell (iPSC)-derived retinal pigment epithelium (RPE) for the treatment of age-related macular degeneration. PLOS One. 2014; 9:e85336. [PubMed: 24454843]
87. Dlouhy, BJ.; Awe, O.; Rao, RC.; Kirby, PA.; Hitchon, PW. J. Neurosurg. Vol. 21. Spine; 2014. Autograft-derived spinal cord mass following olfactory mucosal cell transplantation in a spinal cord injury patient: Case report.; p. 618-622.

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One-sentence summary

The ability to build functional blood vessels nears clinical translation, but important hurdles remain.

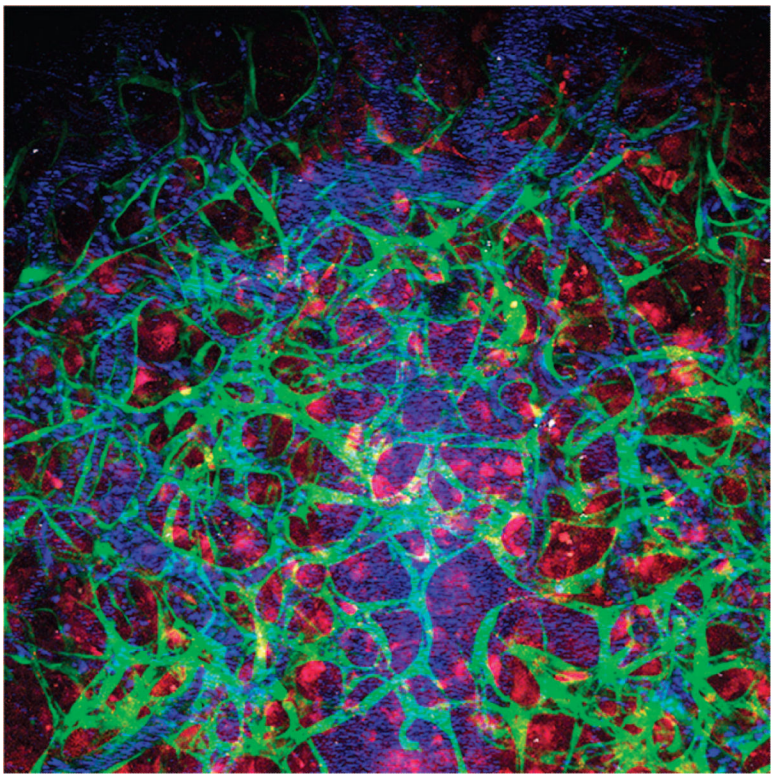

Fig. 1. Form and function

A multiphoton laser scanning microscopy image of iPSC-derived, engineered functional blood vessels observed through a cranial window in SCID mice is shown. hiPSC-derived ECs (green) co-implanted with perivascular 10T1/2 cells (red). RBCs labeled with DiD farred dye (blue). [CREDIT: REPRODUCED FROM (8) WITH PERMISSION]

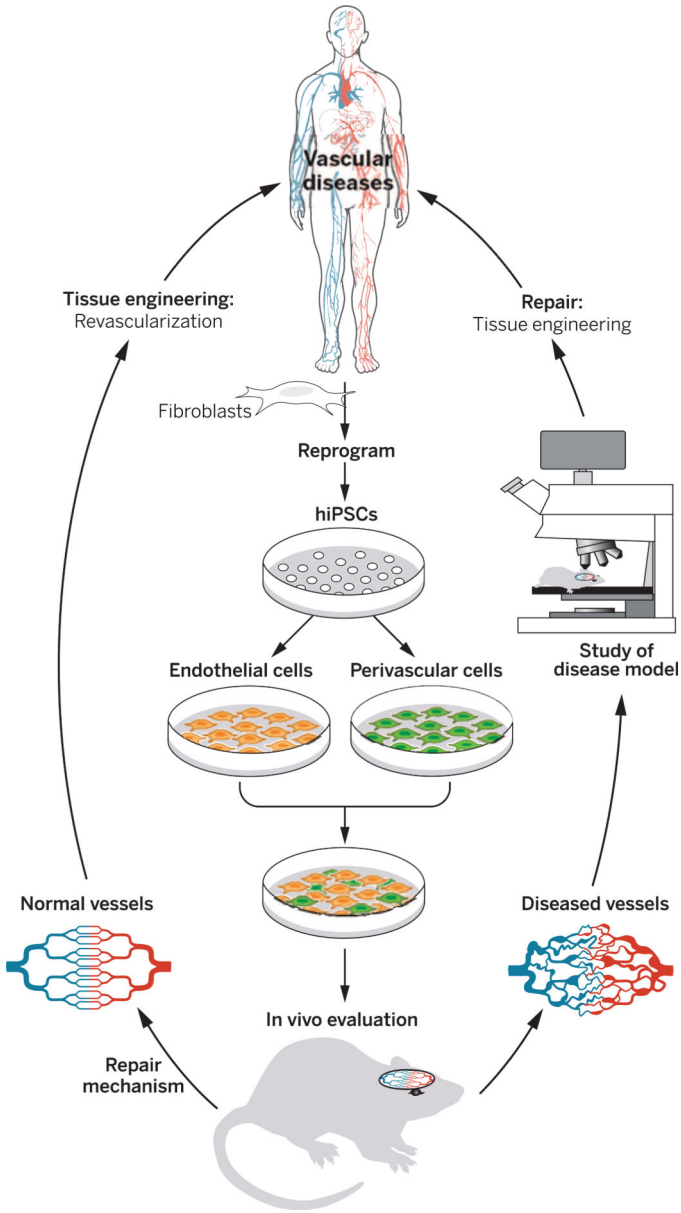

Fig. 2. Models and medicine

Potential applications of hiPSC-derived blood vessels: Disease models and regenerative medicine. Skin fibroblasts are collected from patients with various vascular complications (top). The patient-derived cells and a cocktail of reprogramming factors (OCT4, SOX2, KLF-4, and C-MYC, the "Yamanaka factors") are used to derive hiPSCs (top to center). Two different types of vascular cells, ECs and PVCs, are differentiated from these hiPSCs, and then tissue-engineered vessel constructs are created (center). Vessel constructs are implanted into various animal models and evaluated (bottom). If the hiPSC-derived, engineered vessels maintain the abnormalities observed in the patients' blood vessels, then the engineered vessels provide sophisticated in vitro and in vivo vascular disease models that would facilitate our understanding of patho-physiology and perhaps suggest treatment strategies for the various diseases (right). On the other hand, if these hiPSC-derived vessel

constructs generate normal vessels, then this engineered vessel approach can be used for revascularization of patients (left).

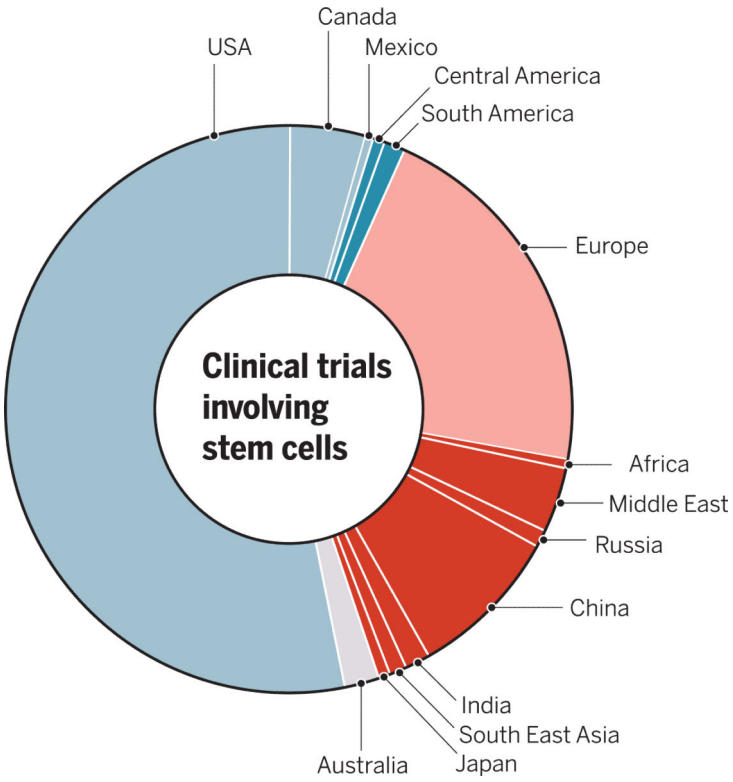

Fig. 3. Stem cell–related clinical trials around the world

This figure was generated with data available from clinicaltrials.gov.