Stem Cell Sources for Vascular Tissue Engineering and Regeneration

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This review focuses on the stem cell sources with the potential to be used in vascular tissue engineering and to promote vascular regeneration. The first clinical studies using tissue-engineered vascular grafts are already under way, supporting the potential of this technology in the treatment of cardiovascular and other diseases. Despite progress in engineering biomaterials with the appropriate mechanical properties and biological cues as well as bioreactors for generating the correct tissue microenvironment, the source of cells that make up the vascular tissues remains a major challenge for tissue engineers and physicians. Mature cells from the tissue of origin may be difficult to obtain and suffer from limited proliferative capacity, which may further decline as a function of donor age. On the other hand, multipotent and pluripotent stem cells have great potential to provide large numbers of autologous cells with a great differentiation capacity. Here, we discuss the adult multipotent as well as embryonic and induced pluripotent stem cells, their differentiation potential toward vascular lineages, and their use in engineering functional and implantable vascular tissues. We also discuss the associated challenges that need to be addressed in order to facilitate the transition of this technology from the bench to the bedside.

Vascular Tissue Engineering: Unmet Clinical Need

CARDIOVASCULAR DISEASE, and coronary artery disease (CAD) in particular, is the leading cause of mortality in the United States, necessitating ~500,000 coronary artery bypass graft (CABG) surgeries annually.¹ Surgically harvested autologous grafts, such as the left internal mammary and radial arteries or the greater saphenous vein, from patients are considered the gold standard for CABG procedures.²⁻⁵ Other autologous arterial/venous grafts, cryopreserved cadaveric grafts, umbilical vein grafts, and arterial allografts have also been tried but with limited success because of associated complications.⁶⁻¹¹ Although autologous vessels from patients remain the grafts of choice, in many cases, previous harvest, morbidity at the donor site, or disease progression limit the availability of native grafts.^{12,13} Clinical studies suggest that only a limited number of patients undergoing CABG surgeries have suitable arterial grafts and up to 30% of patients requiring venous grafts for peripheral vascular diseases lack transplantable veins.^{14,15}

While synthetic vascular prostheses such as expanded polytetrafluoroethylene (ePTFE) and Dacron are available alternatives for high-flow, low-resistance, large peripheral vessel pathologies, their clinical outcome for small-diameter (<6 mm) vessel replacement has been grim.¹⁶⁻²⁰ Prosthetic graft failure has been attributed to intimal hyperplasia,

thrombogenicity, compliance mismatch, and diameter mismatch between the graft and native artery.²¹⁻²⁴ Despite decades of effort, the successful fabrication of an ideal vascular graft still remains a challenge. Ideally, a vascular graft should be strong, biocompatible, nontoxic, nonimmunogenic, antithrombotic, compliant, vasoactive, and amenable to post-implantation remodeling by the host tissue. To this end, tissue-engineered vessels (TEVs) that can withstand the challenging arterial hemodynamic microenvironment and are amenable to physiological remodeling represent an attractive alternative.

Vascular Tissue Engineering Approaches

Three major approaches have been proposed for the tissue engineering of vascular grafts: (1) decellularized matrices; (2) cell-sheet engineering; and (3) biodegradable scaffolds from natural or synthetic polymers.

Decellularized blood vessels as well as small intestinal submucosa (SIS) have been used to fabricate vascular grafts. The main advantage of using decellularized tissue is that the native three-dimensional (3D) architecture of matrix molecules—mainly type 1 collagen and elastin—is preserved²⁵ and might be helpful in guiding tissue repair and remodeling postimplantation. Decellularized blood vessels

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provide an intact tubular acellular scaffold that can be implanted either directly or after the addition of endothelial and smooth muscle cells with the aim of improving patency.^{26–30} Similar to decellularized vessels, native decellularized tissues, that is, SIS, demonstrated sufficient mechanical strength as a vascular graft *in vitro*³¹ and *in vivo*.^{32–35} SIS was recently shown to support the attachment and proliferation of smooth muscle cells (SMCs) as well as endothelial cells (ECs) and has been successfully used for the development of mechanically robust TEVs after only 2 weeks in culture.³⁶

Cell-sheet engineering was pioneered by L'Heureux *et al.*³⁷ and requires no scaffold. Instead, it relies on the ability of the cells to form highly interconnected sheets that can be cultured around a mandrel for several weeks, yielding multilayered cylindrical tissues which can sustain burst pressure up to 2000 mmHg.^{37,38} Notably, cell-sheet engineered TEVs showed promising clinical results as arteriovenous shunt grafts in a challenging population of dialysis patients with end-stage renal disease.^{39,40} This impressive milestone represents a significant step forward in the clinical application of tissue-engineered vascular grafts.

Synthetic and natural polymers have been used as scaffolds that support cell growth and provide mechanical support necessary for implantation. In a pioneering study, Niklason *et al.* first demonstrated the feasibility of engineering a mechanically robust and implantable tissue-engineered blood vessel.⁴¹ Using a polyglycolic acid (PGA)-based scaffold that was molded into a cylindrical shape and seeded with ovine or porcine SMCs on the outer layer and autologous ECs in the lumen, they obtained vascular constructs with a high burst pressure (2150 mmHg) after 8 weeks in culture. The robust mechanical properties of these tissues enabled implantation into the right saphenous artery of Yucatan miniature pigs, where they remained patent for 4 weeks. Since then, many groups employed synthetic polymeric materials, including co-polymers of PGA with poly-L-lactic acid, polycaprolactone, poly-4-hydroxybutyrate, and polyurethane with various degrees of success.^{42–45} Recently, Dahl *et al.* also exploited the extracellular matrix (ECM) secretion potential of SMCs in order to fabricate off-the-shelf TEVs.⁴⁶ They seeded human cadaveric allogeneic SMCs into rapidly degradable polyglycolic acid scaffolds under the cyclic radial strain to fabricate TEVs that were subsequently decellularized and rendered nonimmunogenic using detergent. Decellularized TEVs could be stored for approximately 1 year at 4°C without compromising their mechanical properties and remained patent either for 1 year after implantation as CABG (with autologous ECs) in dogs or for 6 months as arteriovenous conduits (without ECs) in baboons.⁴⁶ These acellular TEVs underwent significant remodeling *in vivo*, as host α smooth muscle actin (α -SMA)-positive SMCs or fibroblasts populated the grafts and secreted elastin, predominantly near the anastomotic sites. However, very few α -SMA-positive SMCs or fibroblasts were seen, and no elastin was present within the mid sections of the grafts, suggesting limited cell infiltration and remodeling capacity, especially in the middle of longer grafts.⁴⁶ This approach has the potential to generate non-immunogenic TEVs from the allogeneic sources, but their clinical utility has not yet been established.

Natural biomaterials such as collagen and fibrin have also been employed, because they can polymerize in the presence

of cells and contain inherent biological signals that influence cellular activity. Collagen gels have been utilized, as collagen is both a major component of the ECM of blood vessels and a natural cell substrate.⁴⁷ Although the application of cyclic strains improved cell alignment, matrix remodeling, and mechanical strength of the collagen-based constructs,^{48–50} the mechanical strength remained low for implantation. In contrast to collagen, fibrin stimulated the synthesis of collagen and elastin and yielded TEVs with improved mechanical properties,^{51–53} suggesting that fibrin may be a more appropriate scaffold for cardiovascular tissue engineering. Indeed, long-term culture of fibrin-based TEVs for 7–9 weeks yielded grafts with a burst pressure between 1400 and 1600 mmHg and a compliance comparable to that of native arteries, suggesting that fibrin-based vascular constructs might be sufficiently strong for arterial implantation.⁵⁴ Using fibrin hydrogels, our group demonstrated that fibrin-based small-diameter TEVs could be implanted as interpositional grafts in the jugular veins of lambs, where they remained patent for 15 weeks and displayed significant matrix remodeling, especially when prepared using bone marrow (BM)-derived stem cells.^{55,56}

Regardless of the scaffold that is being employed, engineering the correct mechanical microenvironment that mimics the cyclic stresses experiencing a vessel *in vivo* is necessary to enhance the function of vascular grafts and improve implantability.^{41,54} Ultimately, any strategy for engineering vascular substitutes aims at producing TEVs with adequate mechanical properties as well as blood compatibility and immunity characteristics, which are essential for long-term patency. Table 1 summarizes the advances in engineering functional and implantable TEVs.

Despite significant progress made in the development of biomaterials and the correct microenvironment for engineering 3D vascular constructs, cell sourcing remains a major problem. In particular, by increasing donor age, the cell's proliferative capacity and ECM synthesis decrease, leading to decreased mechanical properties such as elasticity and burst pressure.^{67,73} This is particularly important, as the majority of patients in need of a vascular implant are elderly. To overcome the replicative barrier of adult cells, the human telomerase reverse transcriptase subunit (hTERT) gene was expressed in ECs and SMCs from aged individuals, yielding an enhanced proliferation potential of these cells in culture but failed to reverse aging-associated cellular senescence and decreased life span.⁶⁷ Given the limited proliferative capacity and cellular senescence-associated changes in the cell's phenotype, it is necessary to seek alternative autologous cell sources that may be better candidates for TEVs. In this direction, multipotent adult stem cells as well as pluripotent stem cells are being currently explored as alternative cell sources for engineering cardiovascular tissues. In the next sections, we discuss various types of stem cells, their differentiation potential toward vascular lineages, and the challenges remaining before clinical applications can be realized.

Stem Cell Sources for Vascular Tissue Engineering

Adult multipotent stem cells

In this section, we discuss adult stem cells that have been used as sources of vascular cells, SMCs and ECs, for promoting vascularization and for engineering vascular grafts.

TABLE 1. TISSUE-ENGINEERED VASCULAR GRAFTS DEVELOPMENT

Year	Vascular graft/work	Use	Author
1986	Totally TEVs using bovine ECs, SMCs, fibroblast, and collagen, supported by Dacron mesh externally	Experimental	Weinberg and Bell ⁴⁷
1993	Human SMCs, ECs, fibroblasts, and collagen were used to generate three-layered tubular constructs	Experimental	L'Heureux <i>et al.</i> ⁵⁷
1994	TEVs made of canine ECs, SMCs, supported by Dacron mesh externally	Experimental	Hirai <i>et al.</i> ⁵⁸
1998	TEVs derived from self-assembly of human umbilical fibroblast, ECs, and SMCs around PTFE mandrel and implanted as femoral artery interposition graft in dogs	Experimental	L'Heureux <i>et al.</i> ³⁷
1998	TEVs fabricated from ovine autologous SMCs, ECs, and polyglactin woven mesh and implanted as ovine pulmonary artery interposition graft	Experimental	Shin'oka <i>et al.</i> ⁵⁹
1999	TEVs fabricated from bovine ECs, SMCs, and PGA conditioned with cyclic strain and implanted in pigs	Experimental	Niklason <i>et al.</i> ⁴¹
2000	Seeding of human ECs and myofibroblasts on acellularized xenogenic porcine aorta	Experimental	Bader <i>et al.</i> ²⁶
2001–2005	TEVs made of l-lactide and caprolactone scaffold and BMCs, transplanted into cardiac vessels into pediatric patients, no failure up to 32 months	Clinical	Hibino <i>et al.</i> ⁶⁰ Shin'oka <i>et al.</i> ^{61,62}
2003	Autologous bone marrow cells seeded on scaffold and implanted in dogs, first evidence that BMCs contribute to histogenesis	Experimental	Matsumura <i>et al.</i> ⁶³
2003	Biologically hybrid model for collagen-based tissue-engineered vascular constructs	Experimental	Berglund <i>et al.</i> ⁶⁴
2003	SMCs-seeded collagen gels were remodeled <i>in vitro</i> using biochemical and mechanical forces	Experimental	Stegemann and Nerem ⁶⁵
2003	Fibrin-based vascular media equivalents were constructed	Experimental	Grassl <i>et al.</i> ⁶⁶
2005	Fibrin-based TEVs with vascular ECs and SMCs was successfully implanted into an ovine model	Experimental	Swartz <i>et al.</i> ⁵⁶
2005	hTERT overexpressed in saphenous vein-derived ECs and SMCs seeded in PGA scaffold	Experimental	Poh <i>et al.</i> ⁶⁷
2005	BM-MNCs were induced into ECs and SMCs <i>in vitro</i> , seeded onto canine decellularized carotid arteries, and implanted into a canine model	Experimental	Cho <i>et al.</i> ⁶⁸
2006	Decellularized ovine pulmonary arteries seeded with autologous ECs and implanted as pulmonary conduits	Experimental	Leyh <i>et al.</i> ²⁸
2006	Endothelialization and flow conditioning of vascular media equivalents demonstrated <i>in vitro</i>	Experimental	Isenberg <i>et al.</i> ⁶⁹
2007	Ovine bone marrow progenitor cell-derived ECs and SMCs were used for TEVs fabrication and implantation into an ovine animal model. The bone marrow-derived TEVs demonstrated superior elastogenic potential	Experimental	Liu <i>et al.</i> ⁵⁵
2007	Poly(L-lactic acid) nanofibrous scaffolds were seeded with BM-MSCs and implanted into rats	Experimental	Hashi <i>et al.</i> ⁷⁰
2007	TEVs generated from autologous dermal fibroblasts and venous ECs were implanted in 10 patients as an arterio-venous shunt for dialysis	Clinical	L'Heureux <i>et al.</i> ³⁹
2008	PGA scaffold coated with l-lactide and ϵ -caprolactone seeded with BM-MNCs and implanted into the inferior vena cava of juvenile lambs	Experimental	Brennan <i>et al.</i> ⁴⁴
2010	TEVs constructed with PEUU scaffolds seeded with MD-SCs and implanted in the rat model as an abdominal aorta interpositional graft	Experimental	Nieponice <i>et al.</i> ⁷¹
2010	Fibrin-poly lactide-based TEVs seeded with explanted ECs and SMCs from carotid artery and implanted into as interpositional carotid artery grafts	Experimental	Koch <i>et al.</i> ⁷²
2010	Autologous bone marrow cells seeded on decellularized arterial scaffold and implanted as interpositional carotid grafts in an ovine model	Experimental	Zhao <i>et al.</i> ²⁹
2011	TEVs derived from allogeneic and cadaveric sources and implanted into primate and canine animal models. TEVs could be stored at up to 4°C	Experimental	Dahl <i>et al.</i> ⁴⁶

TEVs, tissue engineered vessels; SMCs, smooth muscle cells; EC, endothelial cells; PTFE, polytetrafluoroethylene; PGA, polyglycolic acid; BMCs, bone marrow cells; hTERT, human telomerase reverse transcriptase subunit; BM-MSCs, bone marrow derived mesenchymal stem cells; BM-MNCs, bone marrow mononuclear cells; PEUU, poly(ester urethane) urea; MD-SCs, muscle derived stem cells.

TABLE 2. *IN VITRO* STUDIES OF VASCULAR LINEAGE DIFFERENTIATION OF MULTIPOTENT STEM CELLS

Stem cell source	Vascular lineage	Differentiation factors	Selection and characterization	References
BM-MNCs	SMCs	TGF- β 1 $\pm$ PDGF-BB	α -SMA, SM22, Calponin, MHC, Contractility assay	74
BM-MNCs	SMCs	FBS	α -SMA promoter activity, α -SMA, Calponin, Contractility assay	55
BM-MNCs	SMCs	Co-culture with UCB EPCs in EGM2 complete medium (no hydrocortisone, bFGF, VEGF, heparin) + FBS	MHC expression	75
BM-MNCs	SMCs	FBS, bFGF, VEGF, IGF-1	Actinin, a-sarcomeric actin, L-type Calcium channels, Spontaneous Ca^{2+} transients	76
BM-MNCs	SMCs	RPMI medium plus FBS	Fibroblastic morphology, α -SMA, MHC	68
BM-MSCs	SMCs	TGF- β 1	α -SMA, Calponin	77
BM-MNCs	ECs		Cobblestone morphology, CD31, CD144, VWF, Dil-Ac-LDL uptake	55
BM-MNCs	ECs	Plasma fibronectin, EGF, bFGF	VWF, Dil-Ac-LDL uptake	76
BM-MNCs	ECs	VEGF	Cobblestone morphology, VWF, CD31	68
AD-SCs	SMCs	EBM-2 medium plus VEGF, bFGF, EGF, IGF	α -SMA, Calponin, MHC, Contractility assay	78
AD-SCs	SMCs	TGF- β 1	α -SMA, calponin, caldesmon, MHC, Contractility assay	79
AD-SCs	SMCs	MCDB131 + FBS + heparin	α -SMA, calponin, caldesmon, MHC	80
AD-SCs	ECs	FBS	CD31, CD144, VWF, eNOS, Dil-Ac-LDL uptake, Matrigel tube assay	80–83
AD-SCs	ECs	EGM2 medium $\pm$ Shear	CD31, eNOS, Dil-Ac-LDL uptake, Matrigel tube assay	84
AD-SCs	ECs	ECGS plus shear	CD31, CD144, CD34, Dil-Ac-LDL uptake, Matrigel tube assay	85
AD-SCs	ECs	bFGF, VEGF, Matrigel	α -SMA promoter activity, α -SMA, Calponin, Actinin, Contractility assay	86,87
HF-SCs (dermal papilla/sheath cells)	SMCs	FBS	α -SMA, Contractility assay	88
HF-SCs (bulge cells)	SMCs	TGF- β 1	MHC expression	75
UC-SCs (cord blood)	SMCs	Co-culture with UCB EPCs in EGM2 complete medium (no hydrocortisone, bFGF, VEGF, heparin) + FBS	CD31, CD34, Dil-Ac-LDL uptake, <i>In vivo</i> angiogenesis	89
UC-SCs (MSC from cord tissue)	ECs	bFGF, VEGF	α -SMA, Caldesmon, Desmin, gel contraction	90
UC-SCs (MSC from cord tissue)	Myofibroblast	FBS, EGF, PDGF-BB, ITS, BSA, Linoleic acid, TGF- β 1	Desmin, α -SMA	91
MD-SCs	SMCs	Coculture with bladder SMC, VEGF		

BM-SCs, bone marrow-derived stem cells; MNCs, mononuclear cells; MSCs, mesenchymal stem cells; AD-SCs, adipose-derived stem cells; HF-SCs, hair follicle-derived stem cells; UC-SCs, umbilical cord-derived stem cells; MHC, myosin heavy chain; FBS, fetal bovine serum; FLK1, fetal liver kinase 1; VEGF, vascular endothelial growth factor; TGF- β 1, transforming growth factor beta 1; bFGF, fibroblast growth factor basic; ECGS, endothelial cell growth supplement; PDGF-BB, platelet derived growth factor BB; EGM2, endothelial growth medium 2; α -SMA, smooth muscle alpha actin; ITS, Insulin-Transferrin-Selenium; eNOS, endothelial nitric oxide synthase; Dil-Ac-LDL, Acetylated Low Density Lipoprotein, labeled with 1,1'-dioctadecyl -3,3',3',3'-tetramethyl-indocarbocyanine perchlorate; EPCs, endothelial progenitor cells; UCB, umbilical cord blood.

In particular, we discuss the vascular cells derived from adult stem cells from BM, adipose tissue, hair follicle (HF), umbilical cord (UC), and muscle. In a separate section, we also discuss the endothelial progenitor cells (EPCs) that may be useful for endothelializing the lumen of vascular grafts. These efforts are summarized in Table 2.

BM stem cells. Bone marrow mononuclear cells (BM-MNCs): BM is the home of hematopoietic stem cells (HSCs) that sustain hematopoiesis and multipotent mesenchymal stromal cells (also known as mesenchymal stem cells [MSCs]), which are thought to support and maintain the HSC niche.⁹² MSCs are of mesodermal origin and constitute

0.1% to 0.5%⁹³ of the BM-MNCs, which are obtained by discontinuous density gradient centrifugation of BM aspirates.^{92,94} It was estimated that each gram of BM aspirate can give rise to $\sim 3 \times 10^5$ plastic adherent cells⁹⁵ and that 4–5 mL/kg body weight marrow can be easily aspirated from patients,⁶² thereby providing a rich source of stem cells for cell therapies.

BM-MNCs lack a major histocompatibility complex II as well as other immune co-stimulatory molecules, including CD40, CD80, and CD86.^{96,97} BM-MNCs were also shown to inhibit humoral and cellular immunity, as they suppressed the proliferation of B and T lymphocytes and the maturation of antigen presenting (dendritic) cells,^{98–100} suggesting that it might be possible to employ allogeneic BM-MNCs for the preparation of vascular grafts. When injected into mice blastocysts, BM-MNCs contributed to the development of all three germ layers, suggesting that these cells may be pluripotent.¹⁰¹ Indeed, BM-MNCs have been shown to differentiate into neurons,¹⁰² hepatocytes,¹⁰³ and cardiomyocytes,¹⁰⁴ indicating a broad differentiation potential.

The first evidence that BM-MNCs contribute to *in vivo* angiogenesis by differentiating into ECs and SMCs is derived from the studies conducted by Matsumura *et al.*⁶³ After removing blood cells, the cells from BM aspirates were labeled with a green fluorescent tracing dye and seeded onto polymeric scaffolds that were implanted in the inferior vena cava of dogs.⁶³ An immunohistochemical analysis of the explanted TEVs confirmed that labeled BM-MNCs had differentiated into ECs and SMCs.⁶³ SMCs and ECs were also derived from canine BM-MNCs and used to populate decellularized canine carotid arteries.⁶⁸ These TEVs had a suture strength that was similar to native carotid arteries and when implanted into a canine model, remained patent for 8 weeks. In another approach, Ross *et al.* derived multipotent adult progenitor cells (MAPCs) from BM-MNCs after removal of the CD45+/Glycophorin A+ hematopoietic fraction. MAPCs were shown to be multipotent, and they were successfully coaxed to differentiate into contractile SMCs using a combination of transforming growth factor beta 1 (TGF- β 1) and platelet-derived growth factor BB (PDGF-BB).⁷⁴

In a pioneering study, Shin'oka *et al.* started a clinical trial using TEVs that were prepared by seeding BM-MNCs in the operating room to avoid long culture times.^{60,62} To this date, 25 children with single-ventricle physiology have been implanted with such TEVs with no evidence of graft-related mortality and limited stenosis, as observed after a mean follow-up time of 5.8 years. This is a very significant step forward, demonstrating the clinical application of TEVs in the low-pressure circulation of young patients. It remains to be seen whether the same approach can be used to repair the arterial vasculature of adult patients.

Bone marrow-derived mesenchymal stem cells (BM-MSCs): When plated on plastic culture flasks, the mesenchymal stromal fraction of MNCs adhere to the plastic and give rise to fibroblastic colonies, while hematopoietic precursors do not adhere to the surface and are subsequently removed.^{92,94} As defined by the International Society for Cellular Therapy (ISCT), MSCs are adherent fibroblastic cells that are positive for CD105, CD90, and CD73 and negative for CD34, CD45, and CD14 or HLA-DR, CD19, CD11b, and CD79a.¹⁰⁵ In terms of differentiation potential, MSCs differentiate along the osteogenic, chondrogenic, and adipogenic

lineages.¹⁰⁵ Besides BM, MSCs have been isolated from different tissues, including blood, adipose tissue, HF, muscle, lung, spleen, and liver.^{94,101,106–111} Interestingly, a recent hypothesis postulated that the origin of MSCs may be in the perivascular space of all tissues throughout the body, where they promote tissue repair and homeostasis in a swift and highly localized manner.¹¹²

BM-MSCs were used with nanofibrous poly (L-lactic acid) (PLLA) to fabricate TEVs that were implanted as interpositional grafts into rat common carotid arteries. BM-MSCs-based grafts remained patent for 2 months and demonstrated extensive remodeling, with α -SMA+ cells in the medial layer and CD31+ cells in the lumen, suggesting that ECs from the host might have homed to the grafts.⁷⁰ Another study made use of GFP to label BM-MSCs before implantation and clearly demonstrated that the ECs covering the lumen of the TEV grafts originated from the host and not from the implanted BM-MSCs.¹¹³ In agreement, when co-implanted in immunodeficient mice, human BM-MSCs and blood-derived EPCs formed a patent microvasculature, while the implantation of BM-MSCs or EPCs alone failed to form vessels.⁷⁵ Taken together, these studies suggest that in contrast to BM-MNCs, which may contain endothelial progenitors, BM-MSCs have very limited—if at all—EC differentiation potential, but they serve in stabilizing the microvasculature generated by ECs. Finally, it remains to be seen whether the findings in rodent animal models can be translated into more physiologically relevant large animal models—a very challenging task.

Human BM-MSCs have been used as a source of SMCs as well as for TEVs fabrication. The Niklason group studied the role of ECM, growth factors, and mechanical forces in human BM-MSCs differentiation toward SMCs and highlighted the importance of combining mechanical and chemical factors to fabricate the vascular wall.⁷⁷ In our laboratory, we employed a tissue-specific promoter (α -SMA) and flow cytometry to purify SMCs from BM-MSCs and used them to engineer vascular grafts, which exhibited robust vascular contractility in response to receptor and non-receptor-mediated vasoconstrictors.⁵⁵ When implanted into the jugular vein of an ovine animal model, the grafts remained patent for 5 weeks and demonstrated extensive remodeling capacity. In contrast to vascular SMCs from the jugular veins of neonatal lambs, BM-MSCs-derived SMCs synthesized high amounts of collagen and, most notably, fibrillar elastin similar to native veins, indicating the great potential of these cells for regenerating the vascular wall.

Adipose-derived stem cells. Adipose-derived stem cells (AD-SCs) were first isolated from the stroma-vascular fraction (SVF) of adipose tissue harvested from liposuction.¹¹⁴ A few hundred milliliters to a few liters of adipose tissue is routinely liposuctioned from patients, and the lipoaspirate is subsequently digested with collagenase, washed, and filtered. AD-SCs are derived from the filtrate, also known as processed lipoaspirate, after culture for several days in the presence of fetal bovine serum.¹¹⁴ One group obtained 2×10^5 plastic adherent cells per gram of lipoaspirate⁹⁵ and $\sim 1.5 \times 10^{10}$ AD-SCs from 1 L of lipoaspirate after two passages,⁹⁵ and others showed that AD-SCs could undergo ~ 34 population doublings in culture, suggesting a high proliferation capacity.¹¹⁵ AD-SCs have a fibroblastic morphology,

MSCs-like immunophenotype (CD34+ /CD105+ /CD45- /CD31-,¹¹⁶ or CD29+ /CD44+ /CD71+ /CD90+ /CD105+ /STRO-1+ /CD49d+ /CD31- /CD34- /CD45-¹⁰⁹) and exhibit a clonal differentiation potential along all mesenchymal lineages.¹¹⁴ In addition, several studies demonstrated that the differentiation potential of AD-SCs extends beyond mesodermal boundaries and includes neurons,^{117–119} hepatocytes,^{120,121} and pancreatic islets.^{122,123} Finally, AD-SCs were found to suppress the mixed lymphocyte reaction and lymphocyte proliferation, supporting their potential for allogeneic transplantations.¹²⁴

AD-SCs are a rich source of vascular cells. Several studies demonstrated the successful differentiation of AD-SCs into contractile SMCs,^{78–80} which compacted hydrogels and proliferated on decellularized saphenous vein scaffolds, indicating their potential for reconstituting the vessel wall.⁷⁸ More recently, SMC were derived from AD-SCs by treatment with TGF- β 1 and bone morphogenetic protein 4 (BMP4) and were used on a PGA scaffold to generate the vascular wall.¹²⁵ Interestingly, the application of pulsatile force significantly improved both collagen synthesis and vessel wall mechanics¹²⁵; however, the implantability of these vascular grafts was not demonstrated.

Several groups reported that AD-SCs are a rich source of ECs.^{80,126} In addition to the total AD-SCs population, the CD34+ /CD31- cell fraction from the gluteal, abdominal, and visceral adipose tissue of human donors expressed endothelial markers and enhanced vascularization after implantation in a hind limb ischemia mouse model.¹²⁷ Another cell fraction, the FLK1+ /CD31- /CD34- /CD106- cells from SVF could also be coaxed to differentiate into ECs by culture on matrigel-coated dishes in the presence of vascular endothelial growth factor (VEGF) and fibroblast growth factor basic (bFGF).⁸⁵ Interestingly, the EC differentiation capacity of AD-SCs was not affected by either donor aging or vascular disease.^{83,128} Some studies suggested the presence of a common progenitor among AD-SCs that are capable of differentiating along both mesenchymal and endothelial lineages.^{126,129} However, others showed that in AD-SCs cultures, the CD31 and CD144 endothelial-specific promoters were methylated even after stimulation with EC-promoting growth factors, suggesting that the differentiation potential of AD-SCs toward endothelial fate might be epigenetically limited.¹³⁰ Given the heterogeneity of the SVF, it is not clear whether AD-SCs-derived ECs either differentiate directly from a common multipotent progenitor or represent a population of EC progenitors in SVF. Nevertheless, taken together, these studies support the notion that AD-SCs constitute an important stem cell source for vascular regeneration.

HF-derived stem cells. The HF is a dynamic mini organ containing cells of ectodermal and mesodermal origin. Anatomically, HF can be divided into four regions of ectodermal origin: (1) infundibulum; (2) isthmus; (3) suprabulbar region; and (4) bulb. Throughout life, HFs undergo many cycles of growth (anagen), regression (catagen) and quiescence (telogen), suggesting the presence of stem cells that support the process of continuous regeneration. However, the anatomic location where stem cells reside remained elusive until the early 1990s, when the first experiments were designed to follow the fate of label-retaining cells, showing

that these cells resided in the bulge region of the isthmus.¹³¹ This finding was later verified with sophisticated cell-fate mapping experiments^{132,133} that established the bulge as the stem cell niche of the HF. The multipotency of hair follicle-derived stem cells (HF-SCs) has also been demonstrated by coaxing them to differentiate into corneal epithelial cells,¹³⁴ melanocytes,^{88,135} neural stem cells,^{88,135} Schwann cells,¹³⁶ neurons,^{137,138} and myelinating glial cells.¹³⁷

The HF is surrounded by the dermal sheath (DS), which contains progenitor cells that maintain and regenerate the dermal papilla (DP), a mesodermal structure underneath the bulb region, which is very important for promoting hair regeneration.^{139,140} The DP and DS of rat HFs were shown to contain MSCs (termed HF-MSCs),^{141,142} which showed similar proliferation and differentiation potential as rat BM-MSCs.¹⁴³ Notably, HF-MSCs showed hematopoietic differentiation potential, as displayed by transplantation experiments in which cultured DP cells repopulated the BM of lethally irradiated mice.¹⁴⁴ In our group, we showed that the immunophenotypes of human HF-MSCs are similar to those of BM-MSCs (CD73+ /CD105+ /CD44+ /CD49b+ /CD90+ /CD309- /CD144- /CD34- /CD45-) and that they are clonally multipotent, as individual cells can be coaxed to differentiate into fat, bone, cartilage, and muscle cells.¹⁴⁵ We also noted that HF-MSCs were less susceptible to culture senescence than BM-MSCs, as they could undergo ~45 population doublings and maintained their myogenic differentiation potential as exhibited by the development of contractile properties even at late passages. Using a tissue-specific promoter (α -SMA) and flow cytometry sorting, we purified a proliferative population of ovine HF-MSCs that could be coaxed to differentiate into SMCs, as demonstrated by the expression of α -SMA, calponin, and myosin heavy chains as well as significant contractility in response to vascular agonists, suggesting that these cells were functional SMCs.^{86,87} When combined with the natural scaffold, SIS, HF-MSCs-derived SMCs generated vascular media with significant vascular contractility and mechanical properties approaching those of native arteries,¹⁴⁶ demonstrating the potential of HF-MSCs for use in vascular tissue engineering. Indeed, cylindrical TEVs generated with this approach in our laboratory are currently implanted as interpositional grafts in the arterial circulation of an ovine animal model (unpublished data).

We estimated that $\sim 10^{16}$ HF-MSCs can be obtained from single HF¹⁴⁵ and given the ease of accessibility and the high hair density on adult scalp (175–300 hairs/cm²;¹⁴⁷), HF-MSCs may be considered a robust autologous stem cell source for vascular progenitors. In an intriguing transgender experiment conducted on humans, where DS cells from two male donors were transplanted to a female recipient, it was observed that grafted DS/DP cells gave rise to intact HFs without immune rejection.¹⁴⁸ If proved to be true, this finding is very exciting, as it suggests that akin to BM-MSCs, HF-MSCs may also be immunoprivileged, thereby enhancing their potential as a readily available, allogeneic stem cell source for engineering cardiovascular tissues.

UC-derived stem cells. The UC connects the developing fetus with the placenta and is composed of three vessels—two umbilical arteries and one umbilical vein—surrounded by gelatinous mucopolysaccharides called Wharton's jelly. At term, newborn's UC is ~ 30 –50 cm long and weighs

~50 g.¹⁴⁹ Both umbilical cord blood (UCB) and umbilical cord tissue are repositories of multipotent stem cells. Approximately 75 mL of UCB can be obtained from each UC at birth that reportedly contains a heterogeneous cell population, including HSCs,^{150,151} MSCs,¹⁵² unrestricted somatic stem cells,¹⁵³ multilineage progenitors,¹⁵⁴ endothelial progenitors,¹⁵⁵ and very small embryonic-like stem cells.¹⁵⁶ Interestingly, UCB-derived HSCs have been in clinical use for the past 20 years for the treatment of malignant hematopoietic disorders.¹⁵⁰ MNCs, derived from UCB, have been shown to be MSCs that are based on plastic adherence, immunophenotypic profile, and multilineage differentiation potential toward adipogenic, chondrogenic, and osteogenic lineages.^{152,157} It was reported that 10^8 cord blood MNCs can give rise to 0.7 ± 0.2 MSC colonies,¹⁵⁸ and these cells can undergo approximately 20 population doublings in culture.¹⁵⁹ In addition, endothelial progenitors have been isolated from UCB^{160,162} using CD34+/¹⁶⁰ CD133+/^{163,164} CD34+ /CD133+¹⁶² and AC133- /CD14+¹⁶¹ surface markers, and their angiogenic potential has been demonstrated *in vivo*.^{155,163,164} Pesce and colleagues reported that 83% CD34+ cells were present among the MNCs of UCB,¹⁶² suggesting that UCB is a rich source of EPCs. UCB is amenable to cryopreservation and can serve as an autologous source of UCB-derived MSCs and EPCs.¹⁶⁵

The UC tissue and Wharton's jelly have been shown to possess MSCs.^{149,157} Specifically, MSCs have been isolated from umbilical artery,¹⁶⁶ umbilical vein, perivascular site,¹⁶⁷ Wharton's jelly,¹⁶⁸ and whole UC¹⁶⁹ and are collectively denoted here as UC-MSCs. Approximately 1.75×10^5 MSCs can be obtained per gram of UC tissue (total 5–6 g of UC tissue is obtained from each UC) that can reportedly undergo ~55 population doublings.¹⁴⁹ Approximately 10^{10} UC-MSCs can be obtained from $2\text{--}5 \times 10^6$ cells after 30 days of culture, suggesting that UC-MSCs represent a highly proliferative stem-cell source.¹⁴⁹

Besides mesenchymal lineages,^{149,157} UC-MSCs were shown to differentiate into cardiomyocytes,¹⁷⁰ hepatocytes,¹⁷¹ and neurons.¹⁷² In addition, UC-MSCs were shown to differentiate toward endothelial and myogenic cells *in vitro*. When cultured on matrigel-coated surfaces in the presence of bFGF and VEGF, UC-MSCs differentiated into ECs, which formed neo-vessels on implantation into the ischemic limbs of nude mice.⁸⁹ Farias *et al.* demonstrated that CD10+ /CD29+ /CD44+ /CD73+ /CD90+ /CD105+ /CD34- /CD45- UC-MSCs were ultra-structurally and functionally very similar to myofibroblasts, as they contracted collagen gels on cytokine treatment.⁹⁰ The myogenic differentiation potential of Wharton's jelly-derived MSCs (CD105+ /CD31- /KDR-) was demonstrated by their ability to promote healing of the rat tibialis anterior muscle that was damaged by bupivacaine treatment.¹⁷³ Similar to other MSCs, UC-MSCs have also been shown to possess immunomodulatory properties, suggesting that they may be suitable for allogeneic transplantations.^{174,175} However, their potential to generate contractile and implantable TEVs is yet to be demonstrated.

Muscle-derived stem cells. Muscle-derived stem cells (MD-SCs) are another accessible cell source for regenerative medicine. These are the quiescent cells residing beneath the basal lamina of muscle fibers,¹⁷⁶ which are activated on injury and participate in the repair process.¹⁷⁷ MD-SCs were isolated from muscle biopsies and although the majority

demonstrated robust myogenic differentiation potential, an Sca-1 expressing fraction of late passage cells also exhibited tri-lineage differentiation potential in fat, bone, and cartilage.^{91,178,179}

Vascular grafts that were engineered using rat MD-SCs and polymeric poly(ester-urethane) urea scaffolds showed impressive mechanical properties with a burst pressure reaching ~2000 mmHg.¹⁸⁰ These TEVs remained patent and sustained arterial blood flow up to 8 weeks postimplantation as interpositional grafts in rats,⁷¹ suggesting that MD-SCs may be potentially useful as a source of autologous SMCs for vascular tissue engineering.

Endothelial progenitor cells. EPCs were initially isolated from peripheral blood as CD34+ or Flk1+ cells that differentiated into functional ECs *in vitro*.¹⁸¹ EPCs are produced in BM^{182,183} and are mobilized into the circulation by VEGF,¹⁸⁴ vascular trauma,¹⁸⁵ and several other cytokines.¹⁸⁶ EPCs have also been isolated from UCB^{160–164} and were shown to have enhanced proliferation potential as compared with those originating from peripheral blood.¹⁸⁷ Three strategies have been employed in the past to isolate EPCs: (1) adhesion to fibronectin-coated surfaces in conjunction with the ability to uptake the lectin Ulex Europaeus agglutinin-1 and fluorescence-labeled acetylated low-density lipoprotein (acLDL)^{181,188}; (2) CD34+ /FLK1+ /CD133+ immunophenotype^{181,189}; and (3) the capacity to form cobblestone colonies,¹⁹⁰ which were shown to correlate inversely with the risk of CAD.^{190,191} Accumulating evidence suggests that EPCs derived from BM or peripheral blood may represent hematopoietic lineage cells at various stages of maturation and with varying angiogenic potential,¹⁹² providing a potential explanation for the lack of standardized criteria and cell surface markers to define and isolate them.

Regardless of the method of isolation, EPCs have been shown to promote neo-angiogenesis.¹⁹³ The delivery of EPCs—either by a direct injection or after the seeding on scaffolds—enhanced the angiogenesis in animal models of ischemia or infarction.^{194–197} Shi *et al.* demonstrated that CD34+ EPCs from BM-colonized vascular prostheses *in vivo*,¹⁸² suggesting their potential application in vascular regeneration. In another study, Kaushal *et al.* isolated EPCs from ovine peripheral blood and seeded them onto decellularized porcine iliac vessels that were implanted into an ovine animal model.²⁷ The implanted vascular grafts remained patent for 130 days, suggesting that the EPCs were successfully substituted for mature ECs in the TEVs lumen.²⁷

EPCs are rare cells that are present in the blood at a frequency of 1 in 10,000 cells¹⁹⁸ or according to other reports as low as one cobblestone EPCs colony per 10^8 MNCs.¹⁹² In addition, EPCs can only undergo 20–30 population doublings *in vitro*,¹⁸⁷ and their proliferation capacity was shown to further decline with age^{198,199} and cardiac disease, for example, CAD,^{190,191} further limiting their use in vascular regeneration. However, a recent report showed that the peroxisome proliferator-activated receptor-gamma agonist, rosiglitazone prevented the decline of EPC function against the accumulation of advanced glycation end products.²⁰⁰ More studies are needed in this direction to better understand the molecular underpinning of aging and to develop strategies that attenuate the effects of senescence and realize the clinical potential of EPCs for vascular regeneration.

FIG. 1. Schematic diagram of pluripotent stem-cell derivation. iPS cells, induced pluripotent stem cells. iPS cells, induced pluripotent cells. Color images available online at www.liebertpub.com/teb

Challenges and limitations of multipotent stem cells. Maybe the most challenging obstacle hampering clinical applications of adult stem cells is cellular senescence, which is the decreased proliferation and differentiation potential by increasing donor age.²⁰¹ In addition to organismal aging, MSCs also suffer from culture senescence, limiting their culture time to about 8–10 passages and preventing their expandability to the large cell numbers required for cellular therapies. This is a major concern, as the patients who are mostly in need of cellular therapies, in general, and of vascular grafts, in particular, are elderly.

Age-related decline in cell number has been reported for BM-MSCs^{202,203} and AD-SCs.²⁰⁴ Our laboratory demonstrated that SMCs differentiated from adult BM-MSCs were not only less proliferative but also significantly less contractile than their neonatal counterparts.²⁰⁵ In another study, the Niklason group showed that terminally differentiated vascular cells isolated from aged patients exhibited reduced functionality and decreased ECM remodeling, resulting in failed grafts. Although ectopic expression of the hTERT subunit enhanced the proliferation of ECs and SMCs from aged patients, it failed to reverse the aging associated cellular senescence and decreased life span.⁶⁷ Interestingly, our recent studies showed that HF-MSCs were more resistant to donor aging compared with BM-MSCs, as evidenced by their ability to proliferate and maintain their vascular differentiation potential for longer times in culture (¹⁴⁵and unpublished data). In addition, the neutralization of insulin-like growth factor-1 reversed the aging defects in the HSC niche in the BM²⁰⁶ and pulsed the administration of rapamycin-rejuvenated HSCs in aged mice,²⁰⁷ but their effects on MSCs have not been evaluated as yet. More studies are required to understand the molecular underpinning of cellular senescence, which may lead to the design of rational treatments that delay or even reverse the loss of cellular function.

Increasing the use of adult stem cells in vascular tissue regeneration also calls for a better understanding of their interactions with host cells in balancing the inflammatory response and promoting tissue remodeling. Observations from vascular implants in rats suggested that MSCs remained in the grafts for a limited time period, as they were gradually replaced by host cells,⁷⁰ suggesting that the presence of cells in the vessel wall may not be necessary for graft patency. However, a recent study showed that the cell presence in the wall decreased macrophage infiltration and reduced the incidence of graft stenosis.²⁰⁸ The inhibition of macrophage infiltration also blocked the formation of vas-

cular neo-tissue, as evidenced by the absence of endothelial and smooth muscle cells, suggesting that macrophages play a critical role in vascular graft remodeling. This study also suggested that BM-MSCs might play an important role in promoting graft remodeling by balancing the immune response, an interpretation that is compatible with the notion that MSCs may be immuno privileged. Notably, the clearance of MSCs from the implants within a few weeks post-implantation strongly supports the use of allogeneic stem cells for engineering TEVs, as immunosuppression medication may need to be administered for a limited time post-surgery. The use of allogeneic cells will certainly facilitate the commercialization of TEVs and, therefore, the translation of this technology into the clinic.

Pluripotent stem cells

Embryonic stem cells and induced pluripotent stem cells. Pluripotency refers to the cell's ability to give rise to all three germ layers, that is, ectoderm, mesoderm, and endoderm.²⁰⁹ In 1981, researchers developed a technique to grow a pluripotent inner cell mass of the blastocyst mouse embryo *in vitro*, and these cells were termed mouse embryonic stem cells (mESCs).²¹⁰ Almost two decades later, the Thomson group established similar techniques for culturing human ESCs.²¹¹

In a breakthrough study conducted in 2006, Yamanaka and colleagues demonstrated that mouse embryonic fibroblasts and adult fibroblasts acquire properties similar to ESCs when transduced with retroviruses containing four transcription factors, OCT4, SOX2, KLF4, and cMyc^{212–215} (Fig. 1). hESCs-like colonies appeared at 3–4 weeks after viral transduction and were designated as induced pluripotent cells (iPSCs). These iPSCs exhibited molecular signatures similar to ESC and successfully produced chimeric mice, indicating germ-line transmission.²¹⁵ In addition, the Thomson group demonstrated that two of the transcription factors (KLF4 and cMyc) which were used for iPSCs generation could be replaced by NANOG and LIN28.²¹⁶ Since then, several groups generated iPSCs from many human cells, including blood cells,^{217–218} MSCs,²¹⁹ fetal²¹⁹ and neonatal fibroblasts,^{216,219} AD-SCs,²²⁰ adult testis,²²¹ HF primary keratinocytes,²²² β -pancreatic cells,²²³ and T lymphocytes²²⁴ using combinations of four or fewer transcription factors.^{225–229} In addition to applications in regenerative medicine, iPSCs derived from patients with genetic diseases provide an excellent model system that studies the genetics and pathophysiology of human disease.^{230–232}

Pluripotent stem-cell differentiation strategies. Three strategies have been employed for directed differentiation of pluripotent stem cells (Fig. 2): (1) embryoid bodies (EB); (2) co-culture with stromal cells; and (3) ECM-guided differentiation. The formation of EB, that is, 3D cell aggregates,²³³ in the presence of appropriate differentiating factors can direct the differentiation of pluripotent stem cells along all three germ layers, that is, endodermal,^{234–238} ectodermal,^{239,240} or mesodermal cells.²⁴¹ While the EB method provides the 3D environment that may be crucial for the recapitulation of certain developmental pathways *in vitro*, it yields highly heterogeneous cell populations with subsequent complications in separating the desired cell type and in dissecting the molecular pathways of differentiation. A co-culture with a stromal feeder layer (e.g., mouse OP9 cells) has also been employed fruitfully to differentiate pluripotent stem cells along the hematoendothelial lineages.²⁴² Although the co-culture system promotes efficient differentiation, the xeno-genic feeder layer complicates mechanistic studies as well as the application of differentiated cells in regenerative medicine applications. On the other hand, the monolayer culture on ECM-protein-coated surfaces is a straightforward differentiation strategy that obviates the need for other cell types.²⁴³ ECM-guided differentiation is better controlled and results in a more homogeneous population of differentiated cells. Irrespective of the particular differentiation strategy used, the differentiated cells should be selected based on the characteristic lineage/cell defining markers to obtain a pure cell population that can be either subsequently expanded in culture or used in cell therapies.

Pluripotent stem-cell differentiation toward vascular cells. Several studies involving vertebrate model systems, primarily mice and zebra fish, identified the key signaling pathways involved in vascular development.²⁴⁴ Others studied pluripotent stem cell differentiation toward vascular fate *in vitro* and determined the growth factors and timing schedule required for vascular specification *in vitro* (Fig. 3). These studies demonstrated that pluripotent stem cells differentiate *in vitro* into the mesendoderm and mesoderm fate within 2–4 days by cooperative interactions of the canonical Wnt,²⁴⁵ Activin/Nodal, and BMP4 signaling pathways.^{245–248} Activin and BMP4 mediated sustained β -catenin signaling for 3 days, coaxing about 80% of hESCs into FLK1+ / CXCR4+ mesodermal cells, while the inhibition of BMP4 signaling completely abolished mesoderm induction.²⁴⁶ In a recent study, Singh and colleagues demonstrated that Activin/Smad2,3 and canonical Wnt signaling up-regulated mesendodermal genes such as EOMES and MIXL1 within 2 days of differentiation.²⁴⁸ Further differentiation from mesendoderm into mesoderm also required BMP4 and sustained Wnt signaling.^{246,249} Another study reported that both Activin/Nodal and Wnt were necessary for induction into the primitive streak induction, while BMP4 promoted mesoderm formation.²⁵⁰ Interestingly, hESC gave rise to FLK1+ / CD117+ / CD34+ / CD31+ hemangioblasts with erythroid potential within 5–8 days of EB formation,²⁵¹ and this process was enhanced by several growth factors, including BMP4,^{251,252} bFGF,²⁵³ activin,²⁵³ canonical Wnt, and VEGF.^{250,254} Others dissected this pathway further, demonstrating that BMP4 was required for mesoderm differentiation into hemangioblasts,²⁵⁵

FIG. 2. Schematic diagram showing the strategies for *in vitro* differentiation of pluripotent stem cells. FACS, fluorescence-activated cell sorting. Color images available online at www.liebertpub.com/teb

FIG. 3. Schematic diagram depicting the pathways of human pluripotent stem cell differentiation toward vascular fate. Positive and negative markers and functional criteria for particular lineages are represented in italics. The time for each differentiation step is depicted in capital letters. Studies using the embryoid body, stromal cell co-culture, or monolayer culture methods are represented using thin lines, broken lines, and thick lines, respectively. FGF, fibroblast growth factor; BMP4, bone morphogenetic protein 4; TGF- β 1, transforming growth factor beta 1; PDGF-BB, platelet-derived growth factor BB; SCF, stem cell factor; VEGF, vascular endothelial growth factor.

but the expansion and differentiation of hemangioblasts into hematoendothelial fate depended on VEGF signaling.^{253,255} hESCs-derived hemangioblasts gave rise to a functional endothelium on further treatment with an endothelial cell growth medium (EGM2) for 3 days, as demonstrated by capillary tube and LDL uptake assays.²⁵⁶ In mice, Flk1+ mesodermal cells (putative hemangioblast precursors) differentiated directly into EC or mural cells when cultured in the presence of VEGF or PDGF-BB, respectively.²⁵⁷ *In vitro*, Flk1+ cells formed a vascular network consisting of α -SMA+ mural cells and CD31+ ECs surrounding CD45+/Ter119+ blood cells; and *in vivo*, the same cells contributed to angiogenesis on an injection into the chick embryo.²⁵⁷ This study clearly demonstrated that both types of vascular cells, that is, ECs and SMCs, could be successfully derived from Flk1+ hemangioblast precursors.

Human pluripotent stem cells have been successfully differentiated *in vitro* toward ECs^{258–263} and SMCs.^{258,260,264} Using the EB method, both vascular cell types (ECs and SMCs) could be derived from hESCs and could generate vasculature on implantation into mice.²⁶⁵ Using the same method, it was shown that the sequential treatment of EBs with BMP4, Activin A, FGF2, VEGFA, and SB431542 (a TGF β IIIR inhibitor), resulted in a robust EC fate.²⁶³ The co-culture of hESCs with OP9 cells for 8 days also yielded vascular progenitors (FLK1+ /TRA-1-60–), which on sorting and culture on collagen IV could differentiate to either SMCs (CD31– / α -SMA+) or ECs (CD31+), on treatment with serum or VEGF, respectively.²⁶⁰ In another approach, simply treating monolayer cultures of hESC with serum resulted in CD34+ cells, which could be coaxed to differentiate further into EC in the presence of VEGF-A and FGF2. The differentiated ECs were functional, as demonstrated by their capacity to form blood vessels when co-implanted with 10T1/2 cells in nude mice.²⁶⁶ A recent study took this approach a step further by demonstrating that a chemically defined

medium could induce monolayer cultures of pluripotent stem cells that give rise to the mesoderm and neuroectoderm, which on further treatment with PDGF-BB and TGF- β 1 yielded functional SMCs.²⁶⁷ Interestingly, hiPSCs-derived ECs were shown to have a limited proliferation potential as compared with hESCs-derived ECs, suggesting that reprogramming or the source of hiPSCs might affect the regeneration potential of differentiated vascular cells.²⁶⁸ More studies are needed to examine the potential of hiPSCs-derived vascular cells to generate functional and implantable small-diameter vascular grafts. Table 3 summarizes the studies in the direction of the vascular differentiation of pluripotent stem cells, including selection criteria and the necessary soluble factors.

Challenges and limitations of pluripotent stem cells. Although pluripotent stem cells such as human (h)ESCs and hiPSCs are great potential cell sources; they also suffer from major disadvantages. hESCs are plagued by ethical concerns regarding their isolation from human embryos as well as potential immunogenicity. On the other hand, hiPSCs are more attractive, because they can be generated from the patient's own cells obviating immunogenic rejection and can differentiate into vascular cells using similar protocols as those developed for ESCs.^{274,276,278} However, several issues currently prevent the use of hiPSCs in regenerative medicine, including (1) low reprogramming efficiency; (2) use of viral vectors for reprogramming; (3) accumulation of chromosomal abnormalities^{281,282}; (4) oncogenic potential; and (5) variations in differentiation potential depending on the donor cell type.²⁸³

The safety concerns over iPSCs generation by the use of recombinant retrovirus led to the development of novel methods to avoid transgene integration, including standard transfection, viral vectors that can be excised from the genome after integration, mRNA, and protein delivery

TABLE 3. *IN VITRO* MESODERMAL AND VASCULAR LINEAGE DIFFERENTIATION OF PLURIPOTENT STEM CELLS

Differentiation strategy/pluripotent stem cell	Differentiated cell type/gcrm layer	Selection marker	Growth factor/supplement	Reference
EB/mESCs	Mcsoendoderm	GFP-Goosecoid, E-cadherin, PDGFR- α	Activin A/Nodal	269
Collagen IV dishes/mESCs	Paraxial and lateral mesoderm, ECs	PDGFR- α , FLK1	FBS	270
EB/mESCs	Mesodermal and hematopoietic cells	GFP-T ⁺ , FLK1	BMP4, Activin A, Wnt3A	250
EB/mESCs	Hematoendothelial	SCL, FLK1	VEGF, BMP4, Activin A, TGF- β 1	255
EB/mESCs	Hematoendothelial	GFP-T ⁺	BMP4, Activin A, bFGF, VEGF	253
EB/mESCs	Mesodermal, hematopoietic	GFP-T ⁺ , FLK1	BMP4, Activin A, Wnt3A	271
EB/mESCs	Hematoendothelial		VEGF, IGF1, bFGF, ECGS	272
Collagen IV/mESCs	Hematoendothelial	FLK1, CD144	VEGF, SCF, IL3, EPO, G-CSF	243
Collagen IV/mESCs	ECs and SMCs	FLK1	VEGF, PDGF-BB	257
Collagen IV/miPSCs	ECs and SMCs	FLK1	SMGM2 + PDGF-BB, EGM2 + VEGF	273
OP9 and Collagen IV/miPSCs	Hematoendothelial	FLK1	FBS, mSCF, hTPO, mIL3, hEPO	274
Gelatin/miPSCs and mESCs	SMCs	α -SMA, MHC	atRA	275
EB/hESCs	ECs	CD31	EGM2	259
Collagen IV/hESCs	ECs and SMCs	cell size and shape	VEGF 165, PDGF-BB	258
OP9/hiPSCs	Hemato ECs	CD31, CD43	FBS	276
OP9/hESCs	Hematoendothelial	CD34	FBS	277
OP9 and collagen IV/hESCs	ECs and SMCs	FLK1, CD144, TRA-1-60	VEGF, PDGF-BB	260
OP9 and Collagen IV/hiPSCs	ECs and SMCs	FLK1, CD144, TRA-1-60	VEGF, PDGF-BB	278
S17/M210B4 cells/hESCs	ECs and SMCs	CD34	EGM2, PDGF-BB, TGF- β	279
EB/hESCs	ECs	GFP-CD144	BMP4, Activin A, bFGF, SB431542	263
Collagen IV/hESCs	ECs	CD31	SCF, bFGF, VEGF, BMP4, Activin A	262
EB/hiPSCs	SMCs	α -SMA	SMCM	280
Gelatin/hESCs	SMCs	α -SMA, MHC	atRA	264

ESCs, embryonic stem cells; mESCs, mouse embryonic stem cells; miPSCs, mouse-induced pluripotent stem cells; hESCs, human embryonic stem cells; hiPSCs, human-induced pluripotent stem cells; EB, embryoid bodies; T, brachyury; SFM, serum-free media; BMP4, bone morphogenetic protein 4; Pax6, paired box gene 6; GFP, green fluorescent protein; PDGFR- α , platelet-derived growth factor receptor alpha; SCL, stem cell leukemia protein; SCF, stem cell factor; IL3, interleukin3; EPO, erythropoietin; G-CSF, granulocyte colony stimulating factor; SMGM2, smooth muscle growth medium 2; TRA-1-60, tumor rejection antigen 1-60; mSCF, mouse stem cell factor; mIL3, mouse interleukin3; hTPO, human thrombopoietin; hEPO, human erythropoietin; SB431542, TGF- β 1 receptor ALK5 inhibitor; SMCM, smooth muscle cell medium; atRA, all trans retinoic acid.

strategies.^{215,284–291} Small molecules are being increasingly used and can be substituted for some of the genetic factors, either by affecting histone deacetylases or through other mechanisms.^{292–299} These studies increased the safety as well as the efficiency of reprogramming and facilitated iPSCs generation from the somatic cells of patients, thereby increasing the potential of iPSCs for studying the molecular mechanisms of disease and for regenerative medicine.

On the other hand, recent studies documented chromosomal aberrations in pluripotent stem cells, resulting either from culture adaptation or the original parental cells.^{300,301} In addition, iPSCs were found to acquire genetic aberrations such as copy number variation and mutations as a result of the reprogramming process.^{281,282,302} Finally, iPSCs were recently found to contain on an average five-point mutations in protein coding regions, independently of the method of reprogramming.²⁸² Therefore, before clinical use, it is necessary to ensure that iPSCs are rendered free from any genetic or epigenetic abnormalities which can compromise the long-term function of implanted cells or tissues. In addition, the epigenome as well as the differentiation potential of iPSCs may depend on the donor cell type,²⁸³ calling for

studies to compare the vascular differentiation potential of iPSCs originating from different donor cells. Finally, the development of rigorous approaches for selecting large numbers of differentiated cells from undifferentiated ones is also required to eliminate the tumorigenic potential of the latter before delivery to the patient.

Conclusions

Despite progress in the development of biomaterials and engineering the correct microenvironment with the mechanical and biological cues supporting blood vessel function, the source of cells remains a major challenge in engineering a successful human vascular graft. Multipotent and pluripotent stem cells have tremendous potential for vascular tissue engineering. Autologous adult multipotent stem cells such as MSCs and EPCs have shown robust differentiation potential toward vascular lineages and beneficial attributes in balancing the inflammatory response and promoting neotissue formation. However, their limited lifespan due to organismal or culture senescence and slow rates of ECM synthesis pose challenges in engineering vascular

grafts from elderly patients. On the other hand, hiPSCs can be prepared from the patient's own cells and have a significantly more protracted lifespan, but concerns about chromosomal aberrations, oncogenic potential, incomplete differentiation, and cumbersome and lengthy reprogramming strategies limit clinical applications at the moment. Although currently ongoing clinical trials demonstrate the efficacy of TEVs using differentiated autologous vascular cells, the lengthy culture times and the custom-made preparation of the grafts increase the cost of treatment significantly. In this regard, the enhanced proliferation and differentiation potential along with the immune-privileged attributes of stem cells may increase the quality, decrease the time and cost of tissue development, and even facilitate the preparation of off-the-shelf grafts that can be employed on demand.

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