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Fabrication of tissue-engineered vascular grafts with stem cells and stem cell-derived vascular cells

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Abstract

Introduction—Cardiovascular disease is the leading cause of mortality worldwide. Current surgical treatments for cardiovascular disease include vascular bypass grafting and replacement with autologous blood vessels or synthetic vascular grafts. However, there is a call for better alternative biological grafts.

Areas covered—Tissue-engineered vascular grafts (TEVGs) are promising novel alternatives to replace diseased vessels. However, obtaining enough functional and clinically usable vascular cells for fabrication of TEVGs remains a major challenge. New findings in adult stem cells and recent advances in pluripotent stem cells have opened a new avenue for stem cell-based vascular engineering. In this review, recent advances on stem cell sourcing for TEVGs including the use of adult stem cells and pluripotent stem cells, and advantages, disadvantages, and possible future implementations of different types of stem cells will be discussed. In addition, current strategies used during the fabrication of TEVGs will be highlighted.

Expert opinion—The application of patient-specific TEVGs constructed with vascular cells derived from immune-compatible stem cells possesses huge clinical potential. Advances in lineage-specific differentiation approaches and innovative vascular engineering strategies will promote the vascular regeneration field from bench to bedside.

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Keywords

Adult stem cell; Pluripotent stem cell; Tissue-engineered vascular graft; Smooth muscle cell; Endothelial cell; Fibroblast

1. INTRODUCTION

1.1 Clinical background of cardiovascular disease and limitations of current treatments

Cardiovascular disease remains the leading cause of death in the world, accounting for 17.3 million deaths per year worldwide and 31.3% of all deaths, or about one in every three deaths, in the United States.[1] Vascular bypass graft and vascular replacement with autologous blood vessels or synthetic vascular grafts are the main treatments for coronary heart disease, aortic aneurysm, dissection, and peripheral vascular disease. For example, in 2010 in the US alone, there are about 1.4 million arterial bypass operations and a total of 397,000 coronary artery bypass procedures performed annually. However, patients with cardiovascular disease often lack optimal autologous vessels for grafting due to preexisting diseases. Although synthetic vascular grafts made of polymeric materials, including polyethylene terephthalate and expanded polytetrafluoroehylene, have been successfully applied to replace some diseased blood vessels, they can lead to many complications including acute thrombosis caused by the lack of functional endothelium coverage, restenosis resulting from chronic inflammatory responses, calcium deposition, and increased susceptibility to infection, especially in the case of small diameter grafts with an inner luminal diameter less than 6 mm.[2] Coating the intimal surface with heparin and other anticoagulant materials has been attempted, but these approaches need improvement[3] Moreover, in the pediatric population, children require multiple operations as they grow due to the lack of growth potential in current materials. Therefore, the current vascular grafts and conduits available for the treatment of cardiovascular disease are far from ideal.

1.2 Milestone works in the development of tissue-engineered vascular grafts (TEVGs)

The shortage of autologous vessel sources and the disadvantages of current synthetic vascular grafts has driven the development of TEVGs, which mimic the structure and function of native blood vessels. Milestone developments of TEVGs are summarized in Figure 1. In 1986, Weinberg and Bell first tried to construct a completely biological blood vessel by seeding cultured vascular cells such as smooth muscle cells (SMCs), endothelial cells (ECs) and fibroblasts into three-dimensional collagen gel. However, the lack of proper mechanical properties forced them to use Dacron mesh in the media layer as structural reinforcement. Many efforts were made thereafter to improve the functionality of biological blood vessels,[4–7] but the lack of proper mechanical properties, inadequate suture strength, limited ability to manipulate cell seeding, and susceptibility to rapid degradation by immune response undermined the possible advantages of completely biological scaffolds.[8–10] In 1998, Shinoka *et al* first reported *in situ* work to construct living pulmonary artery conduits by seeding an isolated and expanded vascular cell mixture into synthetic biodegradable polyglactin/poly(glycolic acid) (PGA) tubular scaffolds and facilitating vascular remodeling in an ovine pulmonary artery replacement model. In 1999, Niklason *et al* developed an *in*

vitro construction technique to produce small diameter arteries through the creation of a pulsatile perfusion bioreactor for TEVGS culture and modeling under simulated physiological mechanical stimuli. In 2001, Shinoka *et al* reported the first clinical application of TEVGs, after their early work in large animals, by *in situ* reconstructing a pulmonary artery with the patient's vascular cells and a pre-designed biodegradable scaffold for a four-year old girl with congenital cardiac defect. Short-term observation showed no evidence of graft occlusion or aneurysmal changes. Encouraged by the first success, the Shinoka group reported the successful reconstruction of low-pressure pulmonary outflow tracts with autologous bone marrow cell-seeded biodegradable scaffolds in twenty-three pediatric patients diagnosed with cyanotic congenital defects in 2005. In 2007, the L'Heureux group reported the preliminary use of cell-sheet engineered TEVGs in the adult arterial system as arterial-venous shunts for hemodialysis access for six patients. In 2012 and 2014, clinical trials of TEVGs constructed from allogenic fibroblasts for extra-hepatic portal vein obstruction replacement and hemodialysis access showed that engineered grafts can be used off-the-shelf.[9] In this hemodialysis access application, the TEVGs were implanted and patent for up to eleven months with no evidence of immune response. Although challenges still exist, there is no doubt that the milestone works mentioned above represent significant advances in the clinical application of TEVGs to promote the translation of basic research from the bench to the bedside.

2. STEM CELL SOURCES FOR REGENERATING TEVGS

The fabrication of functional and clinically translatable SMC-based TEVGs involves three key components: obtaining numerous functional SMCs, fabricating optimal scaffolds for cell seeding and vascular tissue development, and facilitating the integration and remodeling of cell-scaffold constructs under various biochemical and biomechanical factors *in vitro, in vivo* and *in situ*.[9] All three aspects are complementary and essential in the development of functional TEVGs with a hierarchy of organized structure. In addition, selection of an optimal animal model for the evaluation of TEVGs is crucial, which has been comprehensively reviewed elsewhere.

As this review is focused on SMC-based vascular grafts, it is worth noting that TEVGs have also been successfully constructed using extracellular matrix (ECM)-embedded fibroblasts in a bioreactor.[9] The construction of TEVGs with different cell types and ECM components has been reviewed elsewhere. Some groups have reported that properly-designed acellular biodegradable synthetic grafts can regenerate short blood vessels in small animals by recruiting host vascular cells and improving vascular remodeling.[10–13] Since this strategy bypasses the *in vitro* cell culture step, it is more cost-effective and easier to control the batch quality. However, the potential of acellular grafts in regenerating longer blood vessels in large animals has not been exclusively demonstrated, largely due to limited trans-anastomotic host cell migration over an extended distance. Clinical evidence accumulated from the practice of implanting non-degradable synthetic grafts in humans over the past sixty years shows that trans-anastomotic vascular cell ingrowth only occurs in the immediate peri-anastomotic region. No more than 1 to 2cm migration distance is achieved even after years of implantation.[9] Given that many human peripheral bypass grafts are longer than 40

cm, it remains a great challenge for acellular biodegradable grafts to be translated into wide clinical use.

While many efforts have been made in the development of biomaterials and optimization of the microenvironment for SMC-based vascular tissue formation,[9] a vascular cell source remains a bottleneck problem for cell-based vascular therapy. Efforts to construct TEVGs using mature vascular SMCs isolated from explanted donor vascular segments have been extensively reported, especially at the early stage of TEVGs' development.[10–12] Thus, it is not surprising that the first clinical trial of TEVGs was carried out with autologous vascular cells. Unfortunately, mature vascular cells isolated from donor vessels are inadequate in quantity and suffer from limited proliferation potentials, reduction of collagen matrix production, and rapid decline of cell functions during extensive *in vitro* expansion. With potent proliferative capacity and differentiation potential into various subtypes of vascular cells, stem cells are becoming a promising cell source in vascular tissue engineering and regenerative medicine. Along with other researchers in the vascular regeneration field, we have successfully differentiated pluripotent stem cells such as mouse embryonic stem cells (ESCs), mouse induced pluripotent stem cells (iPSCs), and human iPSCs, into SMCs, and demonstrated the feasibility of harnessing pluripotent stem cells to serve as an advanced, unlimited cell source for vascular engineering.[13–15] However, the application of pluripotent stem cell-derived SMCs to the construction of TEVGs for clinical use still encounters challenges. Heterogeneous cell populations derived from pluripotent stem cells may lead to undesired tissue formation. Here, recent advances on stem cell sourcing for TEVGs including the use of adult stem cells and pluripotent stem cells, and advantages, disadvantages, and possible future implementations of different types of stem cells are reviewed. In addition, current strategies used during the fabrication of TEVGs are highlighted.

2.1. Adult stem cells

Adult stem cells are undifferentiated cells found in many tissues in the human body and capable of differentiating into specialized cell types. In fact, the application of adult stem cells as clinical therapies has existed for decades. Recent advances indicate that adult stem cells, particularly mesenchymal stem cells (MSCs), have the capacity to differentiate into SMCs and ECs and, therefore, may be used in vascular regenerative medicine. In this section, we will discuss the use of bone marrow-derived stem cells and other adult stem cells derived from a variety of tissues with resident MSC-like cells.

2.1.1. Bone marrow-derived stem cells—Bone marrow is a physiological active tissue wherein hematopoietic stem cells and MSCs sustain hematopoiesis. Bone marrow stem cells have many advantages such as simple isolation from bone marrow aspirates, abundant stem cell quantity,[16] and lack of an immune response due to the absence of major histocompatibility complex class II. These characteristics of bone marrow-derived stem cells are advantageous for use in regenerative medicine. In particular, bone marrow mononuclear cells and bone marrow mesenchymal stem cells can be used to construct TEVGs.

2.1.1.1. Bone marrow mononuclear cells (BM-MNCs): The first evidence that BM-MNCs contribute to the formation of vascular tissue through differentiation into SMCs and

ECs was shown in the *in situ* regeneration study by Matsumura *et al* in 2003. This study investigated how implanted adult stem cells interact in an *in vivo* microenvironment during vascular tissue formation. In this work, canine BM-MNCs were pre-labeled with green fluorescence and seeded into poly(L-lactide) (PLLA) mesh enforced poly(ε-caprolactone and L-lactide) (PCL/LA) copolymer tubular scaffolds that were 8 mm in diameter, 2 cm in length and 0.6 mm in thickness. The cell-seeded tubular scaffolds were implanted into inferior vena cavas of original respective BM-MNCs isolated dogs. Post-implantation immunohistochemistry observation showed that the blood vessels were composed of bone marrow-differentiated SMCs and ECs and remained patent for up to two years. This pioneering work demonstrated that BM-MNCs contribute to the construction of TEVGs. In the clinical trial and series work conducted by the Shinoka group,[,] autologous BM-MNCs were isolated and seeded into PLLA or PGA mesh enforced PCL/LA copolymer scaffolds. Cell-seeded-scaffold constructs were implanted as an extra cardiac cavopulmonary conduit in pediatric patients with single ventricle pathology. Middle and late term observations revealed that there was no graft-related mortality, no evidence of aneurysm formation, graft rupture, graft infection, or ectopic calcification.[] This clinical trial demonstrates that TEVGs can be successfully applied in a low-pressure pulmonary system in pediatric patients. It also shows that an *in situ* regeneration strategy has many advantages such as bypassing extensive *in vitro* cell culture and freedom from exposure to xenogeneic serum. However, the precise mechanisms underlying how seeded human bone marrow-derived cells proliferate, differentiate, and arrange themselves to contribute to a new tissue remain elusive, which requires advanced technologies to track the fate of donor cells, including genetic labeling and *in vivo* imaging.

Without an acellular control and cell lineage tracing observation, debates arise on if the seeded human BM-MNCs truly contribute to the formation of vascular tissue through *in situ* differentiation into SMCs and ECs. A recent study carried out in rodents found that seeded human BM-MNCs were replaced by mouse monocytes after one week of implantation, and then the scaffolds were repopulated by mouse originated SMCs and ECs. This study showed that human components are not maintained in developing vascular tissue. Instead, seeded human cells increased early host monocyte recruitment and improved vascular remodeling through an inflammation-mediated process. In corroboration with these findings, other studies have raised questions on the ability of BM-MNCs to differentiate into functional cells in ischemic disease therapies.[–] Cell fusion may account for the co-stain of fluorescent cell membrane labeling with differentiated vascular cell markers.[–] However, it remains unclear if studies on rodents can fully represent works on large animal models because host cell replacement and replenishment may occur much faster and more dramatically in small animals with shorter life span. Another concern is that BM-MNCs can differentiate into a variety of mature cells and are not lineage-specific for SMCs and ECs. To this end, it is important to distinguish the differentiated SMCs in synthetic or contractile phenotype from myofibroblasts or activated fibroblasts with similar marker expression patterns.

Another strategy is to seed BM-MNCs into a scaffold and differentiate the cells *in vitro* into mature SMCs and ECs before implantation. Cho *et al* first reported the utilization of BM-MNCs in generating small-diameter blood vessels with internal diameter of 3 mm. BM-MNCs fractions of SMC α-actin- and smooth muscle heavy chain-positive cells were

isolated and cultured in Medium 199 containing 10% fetal bovine serum, and BM-MNCs fractions of vWF/CD31-positive cells were cultured in medium supplemented with vascular endothelial growth factor, basic fibroblast growth factor, epidermal growth factor, insulin-like growth factor and ascorbic acid for 3 weeks prior to be seeded into decellularized arteries. Results showed that cell seeding and differentiation significantly improved the patency of vascular grafts in a canine carotid artery replacement model. The donor cells pre-labeled with a fluorescent dye were detectable in the retrieved vascular grafts. Furthermore, they found that the post-operative administration of granulocyte colony-stimulating factor enhanced *in vivo* endothelialization and reduced intimal hyperplasia. In addition, Brennan *et al* found that TEVGs constructed with BM-MNCs demonstrated growth potential in a juvenile animal model. It was found that all grafts explanted at 6 months were patent, and the grafts increased in volume as measured by difference in pixel summation in magnetic resonance angiography at 1 month and 6 months.

2.1.1.2. Bone marrow mesenchymal stem cells (BM-MSCs): In addition to the direct application of BM-MNCs, BM-MSCs are a special multipotent stromal fraction of bone marrow stem cells that can be cultured and expanded *in vitro* prior to scaffold seeding. The derived cells are capable of differentiating into a variety of mesodermal cell types including SMCs, osteoblasts, chondrocytes, and adipocytes.[–] BM-MSCs are isolated by plating the mononuclear cell fraction on a cell culture surface to remove the non-adherent cells by positive or negative surface marker sorting. Although the current research lacks specific markers for BM-MSCs, a few characteristics have been established. BM-MSCs are plastic-adherent. When maintained in standard culture conditions, the cells express surface markers including CD105, CD73 and CD90, and are negative for CD45, CD34, CD14, CD11b, CD79α, CD19 and HLA-DR. The cells can be differentiated into osteoblasts, adipocytes, and chondrocytes *in vitro* with standardized differentiation procedures. Though BM-MSCs have been widely studied, a disadvantage of the cells is clear as they account for only about 0.01% of the total mononuclear cell population, and both the percentage and differentiation capacity decrease with increased age.[,]

BM-MSCs have been used as SMC sources in fabricating TEVGs. In 2007, an *in situ* regeneration study by Hashi *et al* was successful in directly seeding BM-MSCs into electrospun PLLA/PCL scaffold to regenerate TEVGs. Then, BM-MSC seeded scaffolds were implanted in a rat common carotid artery replacement model. The results showed acellular control grafts had significant intimal thickening while BM-MSC seeded grafts resulted in well-organized layers of SMC and ECs and long-term patency. However, most of the seeded human BM-MSCs disappeared after seven days of implantation, and the majority of cells in the regenerated tissues were from the host. The results indicated that BM-MSCs mainly exerted their function through an antithrombogenic effect. In large animal models, Zhang *et al* seeded canine BM-MSCs into biodegradable three-layered tubular scaffolds with inner and outer layers made of poly(lactic glycolic acid) (PLGA) and an elastic polyurethane middle layer. After seven days of *in vitro* culture, the constructs were implanted into a canine abdominal aorta replacement model. The cell-seeded grafts remained fully patent without any signs of dilation or obstruction after three months post-implantation. In another study, the Andreadis group further purified smooth muscle progenitor cells from

differentiated bone marrow-derived cell mixture with smooth muscle α actin genetic labeling and fluorescence-activated cell sorting. TEVGs were successfully built from bone marrow-derived smooth muscle progenitor cells and bone marrow-derived endothelial cells and implanted in an ovine jugular vein replacement model. The TEVGs demonstrated extensive remodeling capability and a high amount of collagen and elastin synthesis from bone marrow-derived smooth muscle progenitor cells as compared with terminally-differentiated SMCs.

Similar to BM-MNCs, the application of BM-MSCs has been investigated with an *in vitro* regeneration strategy as well. By using nonwoven PGA scaffolds and a biomimetic perfusion system, Gong *et al* showed that human BM-MSCs can serve as a promising SMC source for small diameter vessel engineering through the optimization of various biochemical and biophysical factors including matrix proteins, growth factors, and mechanical forces that modulate SMC proliferation and differentiation. Zhao *et al* isolated ovine BM-MSCs and differentiated the cells into SMCs in medium supplemented with 5% fetal bovine serum, insulin and transforming growth factor-β1, and into ECs in medium supplemented with 5% fetal bovine serum, vascular endothelial growth factor, basic fibroblast growth factor and ascorbic acid before seeding the cells into decellularized blood vessel grafts. After one week of maturation *in vitro*, burst pressure and suture strength were measured and the results showed mechanical properties comparable to native arteries. Then the cell-seeded grafts were implanted in an ovine model and the grafts were demonstrated to be mechanically stable and patent over five months while the unseeded controls quickly occluded in two weeks.

2.1.2. Adult stem cells derived from other tissues with resident MSC-like cells—Although bone marrow was the original tissue and main location containing adult stem cells, many other tissue sources including adipose, hair follicles, and neonatal and infant thymus have been explored.[,] Several studies demonstrated the successful differentiation of adipose-derived stem cells into SMCs[–] and ECs.[–] In one study, adipose-derived stem cells treated with transforming growth factor-β1 and bone morphogenetic protein 4 were differentiated into SMCs. Then the derived SMCs were seeded into a nonwoven PGA scaffold to fabricate vascular wall tissue. In another study, adipose-derived stem cells were differentiated into contractile SMCs under treatment with sphingosylphosphorylcholine and transforming growth factor-β1. The derived cells can attach to decellularized vein grafts. Since adipose-derived stem cells are readily-available and easy to obtain, more attention is focused on this cell type.

Other studies have shown hair follicle-derived stem cells have similar proliferation and differentiation potential as compared to BM-MSCs and could be driven to differentiate into functional contractile SMCs.[,] Furthermore, Liu *et al* showed that miR-18b inhibited transforming growth factor-β1-induced differentiation of hair follicle stem cells into SMCs by targeting SMAD2, which revealed one molecular mechanism underlying SMC lineage differentiation.

Recently, autologous amniotic fluid cells have been used as another cell source for cardiovascular tissue engineering applications. These cells were differentiated into several

mesodermal derivatives, which were then used for the *in vitro* fabrication of small- and large-diameter TEVGs and cardiovascular patches. The study exhibited feasible extraction, identification, and application of this cell source in cardiovascular tissue engineering.

2.1.3. Brief summary of adult stem cells—Adult stem cells are promising cell sources for vascular tissue engineering with advantages such as being readily attainable from various tissues, minimal or lack of *in vitro* cell culture time, ability to differentiate into SMCs and ECs, and most importantly, lack of risk of tumorigenesis.[9] However, cellular senescence and decreased differentiation capability associated with increasing donor age remain one of the largest barriers for their application in elderly patients.[10] For instance, a recent report Krawiec *et al* showed that adipose-derived MSCs from elderly patients may not be suitable for autologous TEVGs due to inadequate SMCs migration and differentiation. More research on basic physiological changes of adult stem cells during *in vitro* culturing and vascular remodeling is needed.

2.2. Pluripotent stem cells

Pluripotency refers to the ability to develop into all three germ layers: ectoderm, mesoderm, and endoderm. Embryonic stem cells (ESCs) were the first established pluripotent stem cells with unlimited growth and self-renewal abilities. Mouse ESCs were isolated from the inner cell mass of blastocyst and expanded *in vitro* by Martin *et al* in 1981. But not until almost twenty years later, the first human ESCs culture was established by the Thomson group. Similar to ESCs, induced pluripotent stem cells (iPSCs) reprogrammed from somatic cellular populations with four canonical reprogramming factors including OCT4, SOX2, KLF4, and cMyc have the ability to differentiate into almost all cell types.[10] Both ESCs and iPSCs have been differentiated into SMCs and used for generation of TEVGs (Table 1). In addition, human iPSCs derived from patients provide an excellent human cell-based tool for cardiovascular disease modeling and drug screening.[10]

2.2.1. Application of pluripotent stem cell-derived SMCs and ECs—More discoveries have fostered a deeper understanding of crucial extrinsic signals and intrinsic pathways involved in SMCs linage specification and improved approaches to differentiate and purify SMCs.[10] Based on these advancements, SMCs derived from pluripotent stem cells have been used in constructing TEVGs. We were the first to explore the potential use of SMCs differentiated from mouse iPSCs for vascular tissue engineering. Mouse iPSCs were treated with all-trans retinoid acid and the derived SMCs were seeded into three-dimensional macroporous nanofibrous PLLA scaffolds. Subcutaneous implantation results showed promising vascular tissue formation. Furthermore, we enriched and purified SMCs derived from mouse ESCs by developing a *LacZ* genetic label under the control of SM22α promoter as the positive sorting marker. We demonstrated the potential use of enriched SMCs to construct vascular tissues on three-dimensional scaffolds with better morphological and functional outcomes. In order to develop a highly efficient method to generate functional SMCs from human iPSCs and to construct patient-specific vascular tissues for translational study, we established human iPSCs from primary human aortic fibroblasts and differentiated human iPSCs into SMCs through an embryonic body-intermediated route. Contractile response to carbachol treatment and up-regulation of vascular collagen subtype genes and

matrix metalloproteinase subtype genes under pharmacological stimulation demonstrated that the differentiated SMCs were functional. Subcutaneous implantation of the SMC-scaffold constructs in nude mice showed vascular tissue formation with robust collagenous matrix deposition and maintenance of differentiated SMC phenotype. In corroboration with these findings, Breuer and his colleagues investigated the use of mouse iPSCs-derived cell mixture sheets in the construction of TEVGs. However, insufficient induction of the pluripotent cells led to significant teratoma formation in a mouse inferior vena cava interposition implantation model. These pioneering studies underline the importance of development of efficient methodologies for complete lineage specification in the construction of TEVGs. Moreover, as vascular SMCs have distinct embryonic origins, embryological origin-associated disease susceptibilities were observed in different aortic segments. By adopting a reprogramming strategy from peripheral blood mononuclear cells,[7] we recently established patient-specific, integration-free iPSCs and then differentiated the cells into SMCs with distinct embryonic origins. Recently published data revealed that SMCs differentiated from human iPSCs-derived cardiovascular progenitor cell intermediates could be a more promising cell source for TEVGs because the cells exhibit a functional phenotype comparable to primary cultured human aortic SMCs without the risk of tumorigenesis.

To reduce tumorigenesis and shorten the reprogramming time, the Xu group used a partially induced pluripotent stem cells (PiPSCs) strategy to reprogram human fibroblast cells into SMCs and ECs.[7] Through transient transfection with four reprogramming factors (OCT4, SOX2, KLF4 and c-MYC) for as short as four days, tumor formation was not observed *in vivo* and the transfected temporary cells displayed the potential to be further re-differentiated into SMCs on collagen IV-coated surface and ECs in differentiation factor-supplemented defined media. Human PiPSC-derived SMCs, together with human PiPSC-derived ECs, repopulated decellularized blood vessels and gave rise to functional TEVGs. The experimental TEVGS implantation group had a 60% survival rate at three weeks after mouse carotid artery interposition surgery as compared to a 20% survival rate of animals implanted with un-seeded controls.

2.2.2. Application of pluripotent stem cell-derived progenitor cells—Instead of directly seeding pluripotent stem cells-derived terminally-differentiated SMCs or ECs into scaffold, seeding pluripotent stem cell-derived progenitor cells may improve cellular engraftment. Recently, the Niklason group[9] showed that human ESCs- and iPSCs-derived mesenchymal progenitor intermediate cells were able to be seeded onto small-diameter vascular grafts *in vitro* in a bioreactor. However, markers representative of unwanted cartilage and bone tissues were detected in the engineered vessels, which raises concerns of lineage specification efficiency when incompletely differentiated progenitor cells are used. Moreover, the *in situ* vascular regeneration capability and the engraftment of pluripotent stem cells-derived progenitor cells remain to be explored.

2.2.3. Brief summary of pluripotent stem cells—The application of human ESCs is restricted by ethical concerns regarding their isolation from human embryos and the potential for immune rejection. In contrast, human iPSCs are not restricted by such

limitations and have additional advantages. Human iPSCs can be reprogrammed from a patient's own somatic cells to circumvent immune rejection and have the ability to develop into vascular cells similar to human ESCs. However, safety concerns arise from human iPSCs generation through the use of viruses, a genome-integrated method for reprogramming, and the accumulation of chromosomal abnormalities.[,] Recently, advanced integration-free reprogramming using non-integrated plasmid, mRNA, proteins, or small molecules[–] facilitates the generation of human iPSCs with reduced genotoxicity. Abnormal cells bearing chromosomal abnormalities resulted from either extensive *in vitro* culturing or the original mutant somatic cells[,] and should be excluded prior to further tissue regeneration manipulation. In addition, genetic aberrations like copy number variations during reprogramming have been reported.[,,] These genetic and epigenetic abnormality issues should be addressed before human iPSCs are translated into clinical use.

3. CONCLUSION

Though advances in the field of vascular regenerative medicine and the development of a variety of strategies to regenerate vascular tissues have occurred (Figure 2), the fabrication of clinically-translatable TEVGs is still at an early stage. Many challenges must be overcome to generate TEVGs with proper mechanical strength and functionality. In particular, vascular cell source, one of the key components in engineered vessels, still encounters many challenges and obstacles. Stem cells are a promising cell source, but current formulations have limitations. Adult stem cells are easy to obtain, but the viability is variable due to the donor age and health status. Human ESCs have unlimited self-renewal and differentiation capacities. However, the ethical concern regarding the sacrifice of embryos limit their therapeutic availability and utility. Human iPSCs have been proved to be an ideal cell source for TEVGs, but potential carcinogenic concerns and an unstable genome after reprogramming limit its prompt clinical application. Nevertheless, with continuous efforts to improve safety and more clinical trials studying the application of iPSCs in other fields, there is no doubt that human iPSCs will ultimately be widely used in regenerating blood vessels.

4. EXPERT OPINION

4.1. Current stage and challenges

With several decades of development and landmark studies throughout its infancy and exploratory stages, vascular regenerative medicine is advancing quickly. But it also faces challenges. Cell source is one of the crucial components in fabricating TEVGs and we summarized the advantages and disadvantages of different cell sources (Table 2). Some advantages and disadvantages to consider include accessibility to target cells, quantity of harvestable cells, exposure to xenogeneic serum in *in vitro* culture, functionality of derived cells, potential to become patient-specific, capability to correct genomic mutation in patients such as Loeys-Dietz Syndrome patients,[] and the total time needed to fabricate TEVGs. All cell sources have their limitations. Primary mature vascular cells were the first used cell sources and significantly contributed to the establishment of the vascular tissue engineering paradigm. However, the quantity and quality of isolated vascular cells are far from clinical

use, and most patients requiring vascular bypass and replacement surgery lack proper blood vessel segments for cell harvesting. Adult stem cells can be immediately used on patients after isolation. However, cell viability is correlated with patient age and health status. The use of embryonic stem cells is restricted by ethical concerns and is not currently applicable in the clinical setting. iPSCs are a newly established, widely investigated technology in a variety of regenerative medicine fields. With advantages including unlimited cell quantity, patient-specific cell sourcing, vascular lineage-specific differentiation, and ability to correct genetic mutations before scaffold seeding, iPSCs must be further researched for use in vascular tissue engineering.

4.2. Future of the field

With future advances in SMC differentiation methodology and knowledge on SMC physiology, lineage-specific SMCs differentiated from stem cells will be widely used and more research is needed to explore the underlying mechanisms of how scaffolds are remodeled and new vascular tissues are developed *in situ*. As for adult stem cell sources, recently Tang *et al* identified a new type of adult stem cell in the vessel wall called vascular multipotent stem cells and showed that the cells may contribute to regenerated vascular tissue after vessel injury. This type of vascular resident stem cell may be another source for TEVGs, although the identity of the cells is still under debate due to the limited methodology available to precisely trace the cell fate *in situ*. As for cell reprogramming, research is needed on the direct cell conversion of SMCs from other somatic cells. Recently, the Niklason group reported the application of decellularized TEVGs made from extracellular matrix secreted from allogeneic primary mature SMCs.[,] This study suggests that SMCs derived from other cell sources may be used in the same strategy and will open a new avenue to fabricate off-the-shelf biological grafts in the future.

In conclusion, with more researchers investigating how seeded cells coordinate with scaffolds and microenvironments, future advancements will facilitate the regeneration of vascular tissue *in situ*, achieve long term application of TEVGs in high-pressure adult arterial system, and elucidate the utility of TEVGS in disease modeling and drug screening.

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** of outstanding interest

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Article Highlights Box

1. Autologous mature vascular cells contribute to the foundation of vascular tissue engineering, but are greatly restricted by cell quantity and quality.
2. Adult stem cells demonstrate promising outcomes, but lack lineage specificity and are limited by donor age and health status.
3. Pluripotent stem cells, particularly human iPSCs cells, are a feasible option and possess advantages that other cell sources do not.
4. Advances in knowledge on SMC physiology and advances in differentiation approaches of vascular cells will promote the application of lineage-specific vascular cells.

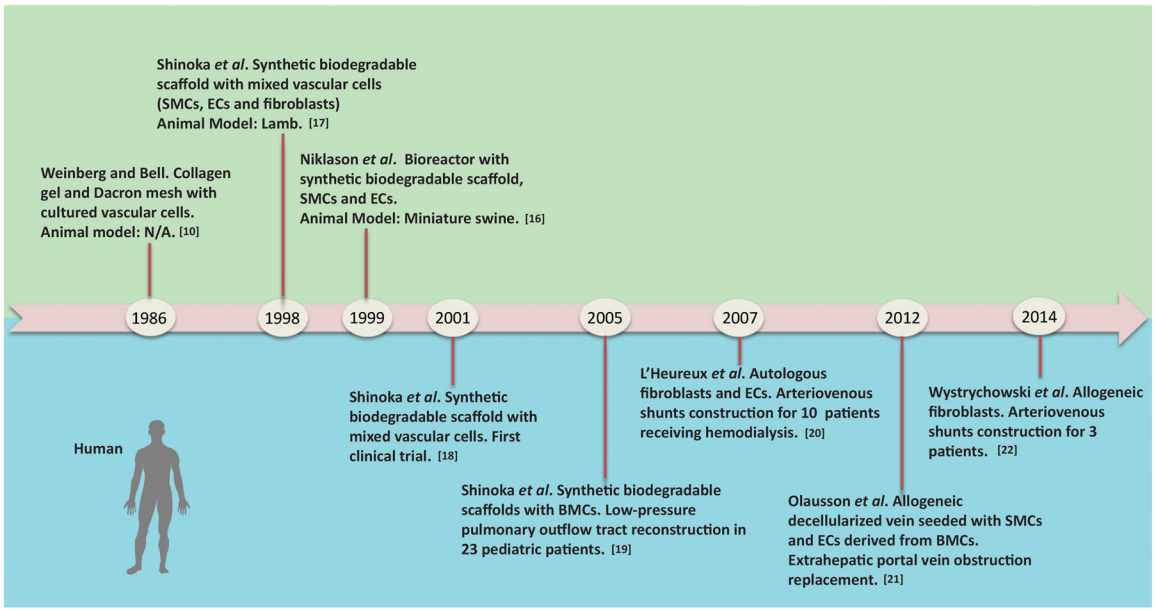

Figure 1. Historic development of tissue-engineered vascular grafts

Green area above the timeline refers to studies on animals, while blue area below the timeline refers to clinical trials on human. SMCs: smooth muscle cells; ECs: endothelial cells; BMCs: bone marrow cells.

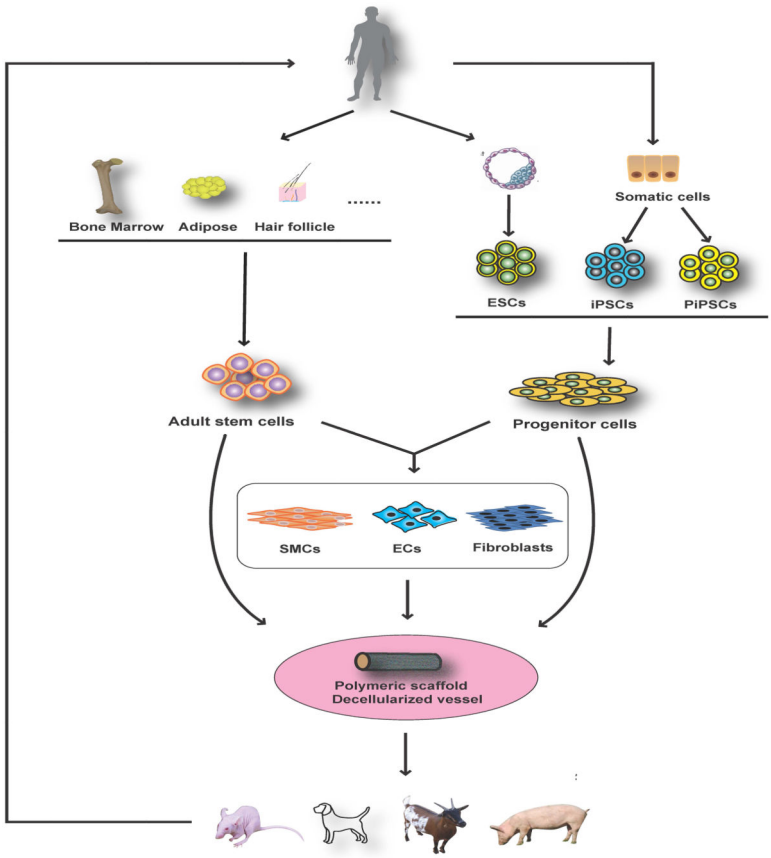

Figure 2. Current strategies applied in fabricating tissue-engineered vascular grafts

ESCs: embryonic stem cells; iPSCs: induced pluripotent stem cells; PiPSCs: partially induced pluripotent stem cells; SMCs: smooth muscle cells; ECs: endothelial cells.

Table 1

Application of pluripotent stem cells in constructing tissue-engineered vascular grafts

Cell Source	Implanted Cell Type	Scaffold	Animal Model	Implantation Site	Duration (weeks)	Reference
mESCs	SM22á-*LacZ* selected SMCs	Macroporous nanofibrous PLLA	Nude mouse	SQ	2	[35]
miPSCs	SMCs	Macroporous nanofibrous PLLA	Nude mouse	SQ	2	[34]
miPSCs	Differentiated mixture	Nonwoven PGA mesh with P(CL/LA) sealant	SCID mouse	Interior vena cava interposition	10	[102]
hESCs	MSCs	Nonwoven PGA mesh	*N/A	*N/A	8	[109]
hiPSCs	SMCs	Macroporous nanofibrous PLLA	Nude mouse	SQ	2	[36]
hiPSCs	MSCs	Nonwoven PGA mesh	*N/A	*N/A	8	[110]
hPiPSCs	SMCs, ECs	Decellularized vessel	SCID mouse	Carotid artery interposition	3	[107,108]

Abbreviations: ECs: endothelial cells; hESCs, human embryonic stem cells; hiPSCs, human induced pluripotent stem cells; hPiPSCs, human partially-induced pluripotent stem cells; mESCs, mouse embryonic stem cells; miPSCs, mouse induced pluripotent stem cells; MSCs, mesenchymal stem cells; PLLA, poly(L-lactide); PGA, poly(glycolic acid); P(CL/LA), poly(å-caprolactone and L-lactide); SCID, severe combined immunodeficiency; SMCs, smooth muscle cells; SQ, subcutaneous

** in vitro* bioreactor culture

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Table 2

Advantages and disadvantages of various cell sourcing in clinical utility

	Mature Vascular Cells		Adult Stem Cells		ESCs		iPSCs	
	Advantage	**Disadvantage**	**Advantage**	**Disadvantage**	**Advantage**	**Disadvantage**	**Advantage**	**Disadvantage**
Isolation methodology	Easy	-	Easy	-	-	Ethnical Concerns	-	Reprogramming Safety
Extracted Cells Numbers	-	Limited	-	Limited	Unlimited	-	Unlimited	-
Xenogeneic Serum Exposure	-	Yes	*in situ*: No	*in vitro:* Yes	No	-	No	-
SMC Contractile Function and ECM Secretion Ability	-	Limited	-	Limited	Unlimited	-	Unlimited	-
Patient-Specific	Yes	-	Yes	-	-	No	Yes	-
SMC Lineage Specific	Yes	-	-	No	Yes	-	Yes	-
Genomic Mutation Correction Capability	-	No	-	No	Yes	-	Yes	-
Key Steps for TEVGs Construction	**1** Vascular cells isolation **2** Expansion **3** Cell seeding		*in situ:* **1** Stem cell isolation **2** Cell seeding *in vitro:* **1** Stem cell isolation **2** SMC differentiation **3** Cell seeding		**1** SMC differentiation **2** Cell seeding		**1** iPSCs reprogramming **2** SMC differentiation **3** Cell seeding	

Abbreviations: ECM, extracellular matrix; ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; SMC, smooth muscle cells; TEVG, tissue-engineered vascular grafts

Definitions: Limited, limitations due to age and health status; Unlimited, no limitations since ESCs and iPSCs have unlimited self-renewal and full differentiation capabilities.