Advanced Review

Vascular tissue engineering: from *in vitro* to *in situ*

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Blood vessels transport blood to deliver oxygen and nutrients. Vascular diseases such as atherosclerosis may result in obstruction of blood vessels and tissue ischemia. These conditions require blood vessel replacement to restore blood flow at the macrocirculatory level, and angiogenesis is critical for tissue regeneration and remodeling at the microcirculatory level. Vascular tissue engineering has focused on addressing these two major challenges. We provide a systematic review on various approaches for vascular graft tissue engineering. To create blood vessel substitutes, bioengineers and clinicians have explored technologies in cell engineering, materials science, stem cell biology, and medicine. The scaffolds for vascular grafts can be made from native matrix, synthetic polymers, or other biological materials. Besides endothelial cells, smooth muscle cells, and fibroblasts, expandable cells types such as adult stem cells, pluripotent stem cells, and reprogrammed cells have also been used for vascular tissue engineering. Cell-seeded functional tissue-engineered vascular grafts can be constructed in bioreactors *in vitro*. Alternatively, an autologous vascular graft can be generated *in vivo* by harvesting the capsule layer formed around a rod implanted in soft tissues. To overcome the scalability issue and make the grafts available off-the-shelf, nonthrombogenic vascular grafts have been engineered that rely on the host cells to regenerate blood vessels *in situ*. The rapid progress in the field of vascular tissue engineering has led to exciting preclinical and clinical trials. The advancement of micro-/nanotechnology and stem cell engineering, together with in-depth understanding of vascular regeneration mechanisms, will enable the development of new strategies for innovative therapies.

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INTRODUCTION

Tissue engineering combines the principles and technologies of bioengineering, medicine, and biology to repair or regenerate tissues and organs by using the building blocks of native tissue, including cells and scaffolds.[1–4] In the past two decades, the field of vascular tissue engineering has developed in response to the need for the replacement of obstructed blood vessels and for the promotion of angiogenesis for tissue regeneration and wound healing. Given the prevalence of blood vessel diseases and the critical role of angiogenesis in tissue regeneration, vascular tissue engineering has emerged as an important field in tissue engineering and has undergone rapid development. Vascular tissue engineering involves multidisciplinary approaches, combining knowledge, and technologies in the fields of bioengineering, tissue engineering, vascular biology, biomaterials, cell engineering, and stem cell biology. Novel technologies derived from biomaterials research and regenerative cell therapies have produced promising results in animal models and clinical studies. Here, we provide a review on the approaches for vascular graft tissue engineering.

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ARTERIAL DISEASES AND NEED FOR VASCULAR GRAFTS

Cardiovascular diseases constitute one of the leading causes of death or impaired quality of life for millions of individuals. For example, in the United States alone, 600,000 individuals die from heart disease each year.[5] Atherosclerosis is a common arterial disease with a building up of plaque and a hardening of the arterial wall, resulting in stenosis, or obstruction of the blood vessel. Lack of blood supply can cause tissue ischemia and thus myocardial infarction, stroke, or claudication. While vascular disease is generally thought to be a result of endothelial cell (EC) dysfunction and inflammatory responses and smooth muscle cell (SMC) dedifferentiation,[6–8] recent reports suggest that vascular stem cells are also a major contributor to vascular disease development.[9–11] Current treatments for arterial diseases include bypass surgery, stent placement, anticoagulants, and changes in lifestyle. This review focuses on vascular grafts for bypass surgery.

Bypass surgery is usually performed to treat occlusive or aneurysmal diseases. In the United States, there are over 500,000 vascular grafts being used for bypass surgery each year, most of which are autologous venous and arterial grafts. However, autologous grafts are limited by availability, need for additional surgeries, donor site morbidity, and ~30% 10-year failure rate. On the other hand, synthetic vascular grafts are limited to large-diameter blood vessels due to thrombus formation and the frequent failure in small-diameter grafts. Therefore, there is a critical clinical need for small diameter vascular grafts that have superior patency and ideally can be available off-the-shelf for the replacement of small diameter arteries.[12] Tissue engineering offers promising approaches to engineer scaffold materials and cells, thus improving the biocompatibility and performance of vascular grafts. In the past decade, significant progress has been made towards translating research findings to clinical trials.[13–17] With extensive research in this area, the concept of tissue-engineered vascular grafts (TEVGs) has gradually evolved to reduce or avoid long cultivation time, as reflected by the trend from *in vitro* tissue engineering approaches to *in situ* tissue engineering approaches. It is worth noting that the majority of clinical and preclinical studies discussed below used the grafts for arterial replacement, unless specified otherwise.

CLINICALLY USED VASCULAR GRAFTS

In general, synthetic grafts work well as the replacement of large arteries such as the thoracic aorta, the abdominal aorta, and the iliac artery. For small-diameter arteries (inner diameter <6 mm) such as the coronary artery and peripheral arteries, autologous grafts are commonly used.

Autologous Grafts

An autologous graft is a graft harvested from another part of the patient's body. Usually, saphenous veins and internal mammary arteries are used. Autologous grafts have the advantage of being native and biocompatible to the patient, thus diminishing the risk of immune rejection. Other advantages of this technique include the immediate availability of autologous vessels and the ability to implant this type of graft without prior FDA approval. Additionally, the native vascular tissue possesses the biological and structural properties for optimal vascular tissue performance. Vein grafts have thinner walls than arteries, and are remodeled and become thicker with time after transplantation into arteries. However, one-third of patients with peripheral arterial disease do not have suitable autologous grafts,[18,19] thus prompting the need for synthetic grafts or TEVGs. Harvesting autologous grafts also needs additional surgery that may result in morbidity or wound healing related complications at the donor site.

Polymer-Based Synthetic Grafts

When autologous vascular grafts are not available, polymer-based synthetic vascular grafts are used clinically. Synthetic vascular grafts in the market have been commercially available since the 1970s, often made of expanded-polytetrafluoroethylene (ePTFE), Dacron or polyurethane.[20,21] These polymers are biologically inert, but have limited resistance to thrombus formation on the surface, which may lead to the obstruction of small-diameter grafts. Therefore, synthetic grafts are generally used to replace arteries with inner diameters larger than 6 mm. Synthetic grafts can also be susceptible to infection.[22,23] In the past decade, heparin-coated ePTFE grafts have been used for clinical therapies and show promising improvement of patency (70–80%).[24] Furthermore, the patency of ePTFE grafts has been enhanced to ~65% using a tissue engineering approach with EC seeding.[25]

VASCULAR GRAFT TISSUE ENGINEERING

The field of tissue engineering provides promising new approaches to the next generation of vascular grafts. There are three distinct tissue engineering

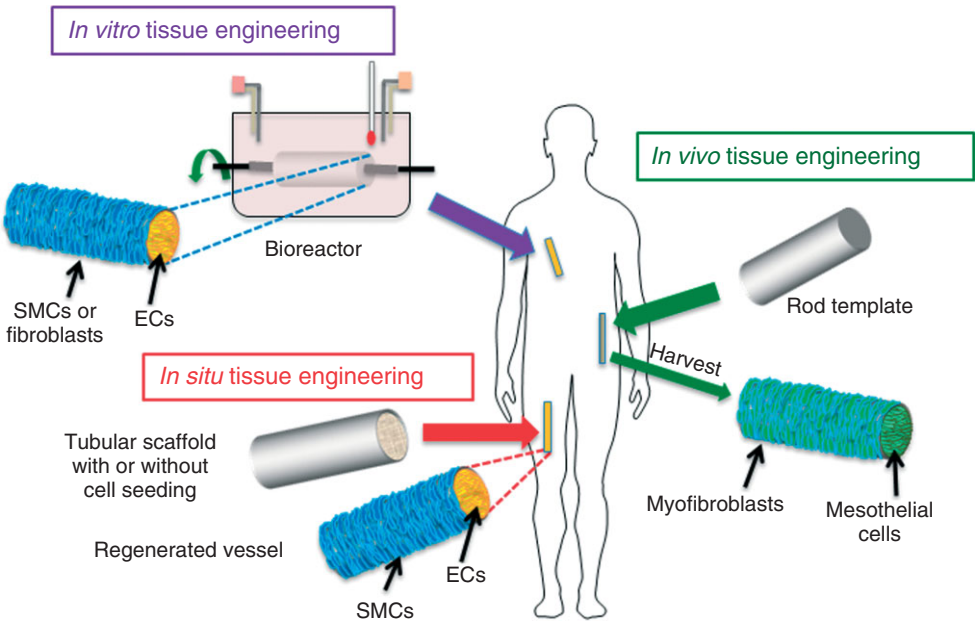

FIGURE 1 | Schematic illustration of *in vitro*, *in vivo*, and *in situ* tissue engineering of vascular grafts.

approaches to construct vascular grafts or regenerate blood vessels: *in vitro*, *in vivo*, and *in situ* (Figure 1). *In vitro* vascular tissue engineering is a traditional tissue engineering approach that constructs functional living vascular grafts outside the body by using cells, scaffolds and bioreactors.[26] For *in vivo* vascular tissue engineering, an autologous vascular graft is made *in vivo* by using the tissue environment of the body (e.g., in peritoneal cavity or under skin) as a bioreactor.[27] For *in situ* vascular tissue engineering, one avoids the extensive *in vitro* culture time period and fabricates a cellular or acellular vascular graft that has essential graft properties, taking advantage of the potential of the host cells to regenerate a blood vessel *in situ*. To reduce the *in vitro* cultivation time, scale-up production and make the grafts available off-the-shelf, there is a trend towards creating bioactive vascular grafts that can promote blood vessel regeneration *in situ*.

The most essential requirements for vascular grafts are biocompatibility, mechanical strength, and nonthrombogenicity. To be biocompatible, the grafts should be nontoxic and not induce inflammatory and immunogenic responses that lead to graft rejection. It is also desirable to make grafts that match the mechanical property of the native arteries to avoid detrimental remodeling at the anastomotic sites. Nonthrombogenicity is critical for patency of the grafts, and can be realized by either seeding ECs on the luminal surfaces or chemically modifying the surface with inhibitors of thrombus formation.[28] Since allogeneic ECs are highly immunogenic and can cause rejection, autologous ECs must be used for seeding. The success of the EC-seeding method relies on the capability of retaining ECs on the grafts under flow conditions *in vivo*. Upon implantation and in contact with blood, nonthrombogenic surface modification may be only effective for a certain period of time. To achieve long-term patency, endothelialization of the luminal surface is needed. *In vivo* endothelialization can be achieved in rodent and porcine models, but vascular grafts in humans usually have limited endothelialization.[29] Therefore, there is a need to develop bioactive grafts that can recruit ECs or their progenitors from circulating blood and surrounding tissues.

Besides these essential requirements, biodegradability can also offer an advantage for tissue remodeling and regeneration. The principle is to have the graft materials degrade gradually in tune with the synthesis of extracellular matrix (ECM) by the cells, finally resulting in a completely regenerated artery. In addition, to scale up the feasibility of clinical therapies, vascular grafts should ideally be made available off-the-shelf, which is also important for the commercialization of vascular grafts.

In the past, vascular grafts have been fabricated out of many types of materials and cell types, as exemplified in Figure 2. The scaffold materials include reconstituted or decellularized ECM, synthetic polymer, and other biological materials. The cell types include ECs, SMCs, fibroblasts, stem cells, and reprogrammed cells. The grafts can be made out of a combination of a scaffold and cells, cells only, or

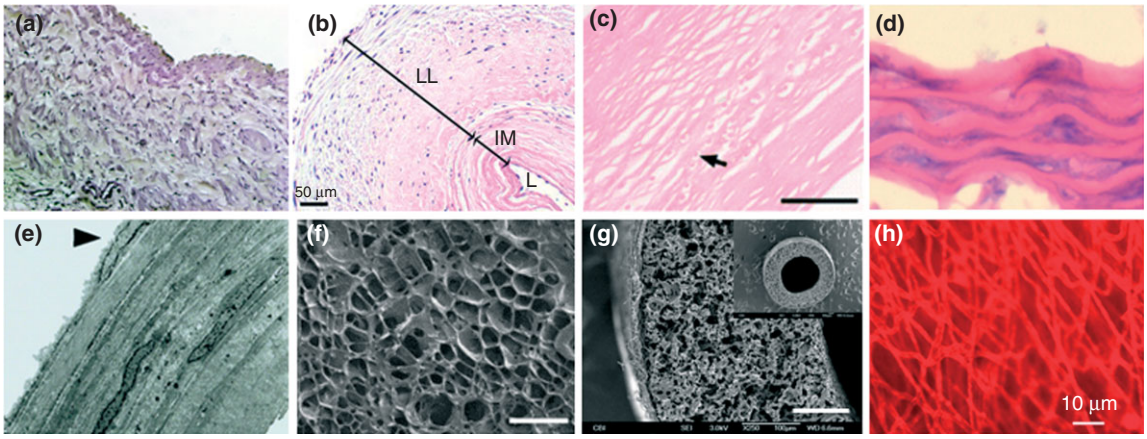

FIGURE 2 | Examples of vascular grafts and scaffolds made by using *in vitro*, *in vivo*, and *in situ* tissue engineering approaches. (a) Decellularized human iliac vein was recellularized with human autologous endothelial cells (ECs) and smooth muscle cells (SMCs) (courtesy of Suchitra Sumitran-Holgersson).[40] (b) Hematoxylin and eosin (H&E) staining reveals a decellularized internal membrane (IM), the fibroblast-seeded living layer (LL) and the EC-seeded lumen (L) of a tissue-engineered vascular graft (TEVG) made by cell-only approach.[44] (c) H&E-stained sections (arrow points to residual PGA) of a decellularized TEVG made *in vitro* by seeding cells in a PGA scaffold.[108] Scale bar = 100 μm. (d) H&E staining of decellularized aortic graft seeded with ECs and SMCs reprogrammed from fibroblasts.[98] (e) Electron micrograph of 2-week granulation tissue formed in the rat peritoneal cavity by *in vivo* tissue engineering. Arrowhead indicates a mesothelial cell lining several layers of myofibroblasts. Magnification 3500×.[101] (f) Scanning electron microscopy (SEM) of polycaprolactone–polyglycolic acid (PCL-PLA) copolymer for clinical studies.[116] Scale bar = 10 μm. (g) SEM images of composite grafts with a fast degrading inner layer. Scale bar = 100 μm.[162] (h) Electrospun PLA graft immobilized with heparin and stromal cell-derived factor-1α (SDF-1α; stained in red).[168]

scaffold only. The remodeling of vascular grafts can be realized *in vitro*, *in vivo*, or *in situ*. Here we will review *in vitro*, *in vivo*, and *in situ* vascular tissue engineering approaches and discuss the advantages and disadvantages of each.

In Vitro Vascular Tissue Engineering

To make functional vascular grafts outside the body, one needs to select the appropriate cell type, scaffolds, and biochemical/biophysical stimuli in bioreactors (Figure 1). Since the 1980s, many studies have used vascular cells (ECs and SMCs) to construct vascular grafts. However, since adult ECs and SMCs have limited expansion potential, recent studies have explored the use of adult stem cells and pluripotent stem cells to make the grafts. In general, these cells should be autologous to avoid immunogenicity. The scaffold materials can be native ECM, biodegradable polymers, or other biological materials such as silk, with their structure and porosity engineered by various fabrication methods. Alternatively, a cell-only approach can be used to generate native ECM in the grafts. Both biochemical and mechanical stimuli in bioreactors have been used to induce the remodeling of the vascular grafts for ECM synthesis and EC preconditioning.

Constructing Grafts with Cells and ECM

A natural way of constructing vascular grafts is to use vascular cells and native ECM such as collagen, fibrin, and elastin. One of the early seminal studies in the field combined collagen gels with SMCs and ECs to make a blood vessel substitute[30]; however, the lack of sufficient mechanical strength of collagen matrix requires the use of a Dacron mesh as an additional support. Fibrin-based tubular tissue constructs can also be used to make the grafts, which are mechanically stronger than collagen matrix.[31,32] Cyclic distension was applied to collagen/fibrin-based constructs, and was found to dramatically increase the mechanical strength of the constructs.[33] Additionally, elastin can be incorporated into the graft to increase its mechanical strength or as a nonthrombogenic and anti-inflammatory coating.[34–39] While these studies have been promising, the reconstituted ECM scaffolds usually have low mechanical strength and need a long time period to remodel into usable grafts. A solution is to use decellularized arteries that have the desirable structure and mechanical strength and seed such grafts with cells. Recently this approach has been successfully used in a proof-of-concept clinical study to create an autologous bone marrow cell-derived EC/SMC-seeded graft for portal vein bypass[40] (Table 1; Figure 2(a)). To reduce the waiting time and make the graft available off-the-shelf, this approach has also been modified to seed only ECs or endothelial progenitor cells (EPCs) and rely on the *in situ* remodeling for blood vessel regeneration.[41,42]

TABLE 1 | Examples of TEVGs in Clinical Studies

Approach	Application	Scaffold	Cells	Preparation Time	Follow-up Time	Patency	Reference
In vitro, cells + polymer	Pulmonary artery	PCL-PLA copolymer reinforced with woven PGA	Autologous peripheral vein-derived cells	2.5 months	7 months	1/1	58
In vitro, cells only	Arteriovenous shunt	None	Autologous fibroblasts and vein cells	4 months	Up to 13 months	6/6	45
In vitro, cells + ECM	Portal vein	Decellularized vein	Autologous bone marrow stem cell-derived ECs and SMCs	1 month	1 year	1/1	40
In situ, cells + polymer	Extracardiac total cavopulmonary connection	PCL-PLA copolymer reinforced with woven PGA	Autologous bone marrow MNCs	Hours	1.3–31.6 months	23/23	117

ECM, extracellular matrix; MNCs, mononuclear cells; PCL, polycaprolactone; PGA, polyglycolic acid; PLA, polylactic acid; TEVGs, tissue-engineered vascular grafts.

Constructing Grafts with Cells Only

An alternative method to create vascular grafts involves the culture of organized cell sheets or constructs without the use of scaffolding material. This approach has been demonstrated in a series of elegant work from laboratory research to clinical trial in the past 10 years[43–46] (Table 1). Since cells secrete native ECM, long-term culture of cell sheets prior to implantation ensures that sufficient cell-secreted matrix of sufficient mechanical strength is produced. Autologous cells can be isolated from skin biopsies and superficial veins in patients. Matrix production is increased by using biochemical compounds such as sodium ascorbate. Vascular grafts are generated by rolling the cell sheets of fibroblasts or SMCs into tubular scaffolds, which are then seeded with ECs (Figure 2(b)). This approach has been successfully used for clinical trials that do not need immediate bypass treatment, e.g., as vascular grafts for hemodialysis access.[15] It is also possible to make the tubular scaffolds (before EC seeding) available off-the-shelf to reduce the time needed for graft fabrication.

Constructing Grafts with Cells and Synthetic Polymers and Other Biological Materials

While naturally occurring components of ECM can be used, polymer-based scaffolds confer certain distinct advantages. The use of synthetic materials results in reproducible scaffolds with controllable properties. A pioneering study demonstrated that functional vascular grafts could be created by using a combination of vascular cells and polymers—tubular polyglycolic acid (PGA) scaffolds that were surface-treated to increase hydrophilicity and seeded with SMCs.[47] The constructs were subjected to cyclic mechanical strain in bioreactors for eight weeks to increase collagen synthesis and mechanical strength, and ECs were subsequently seeded on the luminal surface and subjected to preconditioning flow for 3 days before implantation. These TEVGs were implanted in miniature pigs using autologous cells, and improved patency was demonstrated. In a later study, TEVGs were shown to be capable of remodeling and growing in lambs as they doubled their body weight within a 100-week period.[48] In addition, a variety of studies have been performed to develop bioreactors for vascular tissue engineering.[49] For example, a biomimetic waveform of pulsed flow was replicated in a bioreactor to increase the mechanical strength of the grafts.[50] Rotating bioreactor, perfusion bioreactor, and vacuum-enhanced seeding were used to achieve even, efficient, and faster seeding of ECs in the grafts.[51–53] Many type of polymers have also been used to construct vascular grafts *in vitro*, e.g., electrospun silk fibroin scaffold,[54] poly(carbonate-urea)urethane,[55] nonthrombogenic poly(ethylene glycol) (PEG)-based hydrogels,[56] poly-4-hydroxybutyrate[50] and elastic poly(trimethylene carbonate).[57] In 2001, the first clinical study using vascular cell-seeded biodegradable polymer grafts, polycaprolactone (PCL)–polylactic acid (PLA) copolymer reinforced with woven PGA, demonstrated the feasibility of cell-seeded polymer grafts for clinical application.[58] To reduce the waiting time for TEVGs, the mature constructs

seeded with allogeneic or xenogenic SMCs could be decellularized (Figure 2(c)), made available off-the-shelf, and implanted with or without EC seeding,[59] which is an *in situ* tissue engineering approach. Clinical studies using this approach are ongoing (Dahl and Niklason, unpublished communication).

Using Stem Cells as Cell Sources for Vascular Graft Construction

Since human ECs and SMCs have limited expansion potential, many recent studies have explored the use of stem cells or progenitor cells for vascular graft tissue engineering.[60–63] In adult tissues, bone marrow is an abundant source of stem cells for both ECs and SMCs. Bone marrow mesenchymal stem cells (MSCs) are an expandable multipotent population of bone marrow mononuclear cells (MNCs).[64–66] Generally, human bone marrow-derived MSCs express surface antigens that include STRO-1, CD29, CD44, CD105, and CD166. Bone marrow MSCs can resist platelet adhesion/activation as ECs[67] or differentiate into SMCs,[68] and thus can be used as a cell type for vascular graft coating or a cell source of SMCs. EPCs can be isolated from bone marrow, circulating blood, and umbilical cords. The EPCs that are positive for CD34, CD133, and VEGFR-2 can differentiate into ECs upon attachment to ECM,[69,70] although the exact characteristics of EPCs remain to be further defined.[71] In a recent clinical study, autologous bone marrow stem cells were differentiated into ECs and SMCs to repopulate a decellularized vein, which was successfully used as a bypass graft of a portal vein.[40] While late outgrowth EPCs may not always be readily available, particularly from older patients or patients suffering from heart disease,[72] the above clinical example demonstrates the potential of bone marrow stem cells for vascular graft tissue engineering. In addition to bone marrow stem cells, adult stem cells derived from other tissues such as adipose tissue and skeletal muscle can also be used for vascular tissue engineering.[62] For example, muscle-derived stem cells (MDSCs) were seeded into tubular poly(ester urethane) urea (PEUU) scaffolds to make TEVGs, in which MDSCs differentiated into SMCs and the grafts had sufficient mechanical strength.[73] Most of the studies using adult stem cells for vascular regeneration do not make functional TEVGs, and rely on an *in situ* regeneration mechanism to remodel the grafts, which will be discussed further in the section on *in situ* vascular tissue engineering.

Besides adult stem cells, pluripotent stem cells, including embryonic stem cells (ESCs)[74,75] and induced pluripotent stem cells (iPSCs),[76–79] can be differentiated into ECs and SMCs for vascular graft construction. The differentiation of ESCs into ECs has been optimized with defined medium components.[80–83] ESC-derived ECs are therefore capable of forming microvessels *in vivo* and restoring perfusion into ischemic hindlimb.[83,84] An example of vascular graft engineering showed that the use of mouse ESCs-derived ECs to construct TEVGs helped maintain the graft patency *in vivo*.[85] It is also established that ESCs can be driven to specifically differentiate into SMCs.[86–89] iPSCs can be generated from autologous fibroblasts, and possess the advantage of bypassing immunogenic and ethical issues. Similar to ESCs, iPSCs can be used to generate ECs[90] and SMCs.[91–93] An *in vitro* study showed that mouse iPSC-derived SMCs grew well in a three-dimensional (3D) environment of nanofibrous scaffold.[94] In an *in vivo* study, seeding of ECs and SMCs differentiated from iPSCs into polymer scaffolds were found to exert a paracrine effect to induce neotissue formation in the acute phase and to have a reduction in number by apoptosis at later time points.[95] The research on using ESC- and iPSC-derived vascular cells for vascular tissue engineering is still at an early phase.

A novel approach to generate autologous ECs and SMCs is the direct conversion of fibroblasts by cell reprogramming. Partial reprogramming technique with Yamanaka reprogramming factors has been developed to generate functional ECs (PiPS-ECs)[96,97] and SMCs (PiPS-SMCs).[98] ECs can also be reprogrammed from amniotic cells with defined factors.[99] PiPS-ECs improved neovascularization and blood flow recovery in a hindlimb ischemic model, and PiPS-ECs-seeded decellularized vessel scaffolds demonstrated good patency.[96] Furthermore, TEVGs seeded with PiPS-ECs and PiPS-SMCs (Figure 2(d)) showed excellent performance *in vivo*.[98] These findings indicate that reprogramming of fibroblasts into ECs and SMCs has a potential for clinical applications.

In Vivo Vascular Tissue Engineering

In the past decade, an *in vivo* vascular tissue engineering approach has been developed to fabricate autologous grafts in the peritoneal cavity or subcutaneous pouch.[27,100] The principle is to use the microenvironment of the body as a bioreactor and allow the formation of a capsule layer around a tubular template, and the tube formed by living tissue can be harvested as a graft (Figure 1). In an early study, silastic tubing with variable length and diameter was inserted into the peritoneal cavity of rats or rabbits. By 2 weeks, the tubing was covered by myofibroblasts (several layers), collagen matrix, and a single layer of mesothelium.[101] The Silastic

tubing was removed from the harvested implants, and the tube of living tissue was everted such that it now resembled a blood vessel with an inner lining of nonthrombotic mesothelial cells and an outer mesenchymal layer (Figure 2(e)). Autologous transplantation demonstrated the long-term patency of these grafts. This approach has also been proved feasible in a large animal model.[102] To improve blood compatibility, a shear-resistant confluent monolayer of ECs could also be seeded to cover the luminal surface of the conduit.[103] Interestingly, an implantable device capable of imposing cyclic stretching was developed to increase the mechanical strength of the developing tissue in the peritoneal cavity.[104]

By using templates of appropriate shape, myofibroblast-rich tissue capsules can be used to engineer other tissues such as the bladder, uterus, and vas deferens.[105] *In vivo* implantation studies showed that myofibroblast tissue produced in the peritoneal cavity was able to differentiate into bladder, vas deferens, or uterine SMCs. An important question is what cell types contribute to the capsule formation in the peritoneal cavity. Bone marrow transplantation experiments showed that bone marrow-derived hematopoietic cells could trans-differentiate into myofibroblasts in the capsule layer.[106]

A more convenient location for *in vivo* tissue engineering is the subcutaneous pouch. The capsule layer forms in a way dependent on the type of polymer rods.[100] The wall thickness of capsule layer showed a trend of poly(ethylene) (PE) > poly(urethane) > poly(methyl methacrylate) (PMMA) > poly(vinyl chloride) (PVC) > poly (fluoroacetate) in a rabbit model. After one month of implantation, the wall thickness ranged from 50 to 200 microns, and could sustain 200 mmHg pressure. The degrees of stiffness of the capsule layers around the rods of PMMA, PE, and PVC were similar to those of the human coronary, femoral, and carotid arteries, respectively. Recently, the subcutaneous implantation approach has been used to generate autologous hemodialysis grafts.[107] However, the mechanism of capsule formation in subcutaneous pouch is not clear. It may or may not be the same as that in the peritoneal cavity, and further investigations are needed. Besides the manipulation of rod materials, bioactive tubing templates may be developed to engineer the formation of capsule layer in future studies. The advantage of the *in vivo* vascular tissue engineering approach is that it needs minimal *in vitro* manipulation and can generate autologous grafts. However, the waiting time of graft maturation (2–4 weeks) limits its application to nonemergency clinical treatment.

In Situ Vascular Tissue Engineering

The advantage of *in vitro* and *in vivo* approaches is the generation of living functional vascular grafts with properties similar to autologous grafts. However, it takes weeks to make the grafts, and an *in vitro* approach may require additional bioprocesses for storage, shipping, and handling. These approaches are valuable in addressing some clinical problems, but are limited by scalability. A recent trend in academic research and industry commercialization is to move towards making the vascular grafts available off-the-shelf and taking advantage of the regenerative capability of the body to regenerate blood vessels *in situ* (Figure 1). The grafts for *in situ* vascular tissue engineering can be either (1) cellular grafts that are seeded with cells followed by either implantation or a short culture period, or (2) acellular grafts that require the engineering of the structure, chemistry, degradability, and bioactivity of the grafts to promote *in situ* regeneration; these are discussed below.

Cellular Grafts for In Situ Vascular Tissue Engineering

Early studies have shown that EC seeding improves the patency of ePTFE grafts to ~65%.[25] However, ePTFE is nondegradable and does not allow extensive remodeling *in vivo*. In the past 10 years, native ECM and biodegradable polymers have been used as scaffolds for *in situ* tissue engineering. In most cases, ECs or EPCs were used to seed the grafts to improve hemocompatibility and thus patency.[41,59,108–110]

Bone marrow MSCs have been used to construct vascular grafts,[67,111,112] and an *in vivo* study demonstrated suppressions of thrombogenic response and neointima formation.[67] Similarly, MDSC and pericyte seeding allows endothelialization, improves patency and contributes to the remodeling of vascular grafts *in situ*.[113,114] However, in MSC-seeded grafts, it was noted that after weeks, the majority of the cells incorporated into the graft were from the host rather than the implanted human MSCs. A study using unsorted bone marrow MNCs also showed that the transplanted cells did not have long-term engraftment even in an immunocompromised host.[115] Instead, host cells repopulated the vascular graft. It was observed that monocyte recruitment to the region of engraftment was increased in the presence of MNCs, subsequently leading to remodeling of the vascular grafts and incorporation into the host. These results suggest that there is clear benefit to using cells. MNC-seeded biodegradable scaffolds (Figure 2(f)) have been successfully used for clinical studies in Japan,[116,117] and are currently in clinical trials to treat congenital heart disease in the United

States.[118] Importantly, autologous cells are used in order to ensure hemocompatibility. In this case, autologous MNC harvesting, MNC seeding, and the implantation of the grafts are performed within hours. The replacement of transplanted cells by the host cells suggests that it is also possible to harness the endogenous regeneration potential for vascular tissue engineering without cell seeding, which is discussed in the next section.

Acellular Grafts for In Situ Vascular Tissue Engineering

Acellular grafts are usually made of native ECM or biocompatible/biodegradable polymers with sufficient mechanical strength. In addition, anti-thrombogenic treatment of inner surface of the grafts is generally needed for small-diameter grafts. To maximize the *in situ* regeneration potential, the structure, chemistry, degradation rate, and/or bioactivity can be further engineered.

An early demonstration of the feasibility of acellular grafts for *in situ* regeneration was the use of heparin-treated decellularized submucosa of the intestine as a vascular graft.[119] This collagen-based graft was integrated into the host tissue and remodeled into a functional blood vessel *in situ*. Similarly, it is feasible to decellularize native vessels such as the saphenous vein, which exhibits satisfactory strength, reduced antigenicity compared to fresh allograft, and the capacity to support cellular repopulation.[120] In addition to decellularized blood vessels, recent studies have shown that it is possible to decellularize entire organs such as heart, lung, liver, and kidney and preserve the structure of the microvascular network.[121–125]

The synthetic polymer grafts can be fabricated by various methods such as casting, extrusion, weaving, and electrospinning. In the past decade, electrospinning technique has evolved as a major method to make synthetic polymer grafts[126–129] because this technique is scalable and the electrospun fibrous structure resembles the fibrous structure of native ECM in the vessel wall, thus allowing cell infiltration and cellularization of the grafts. The high surface-to-volume ratio of electrospun scaffolds also makes it feasible to incorporate various drug delivery strategies.[130–133] In addition, electrospinning is a consistent, versatile method that allows the production of nonwoven, three-dimensional fibrous structures with controllable fiber diameter.[134–136] The components of native ECM (e.g., collagen, elastin, hyaluronic acid), commonly used synthetic polymers (e.g., polyesters) and other biological materials (e.g., silk) can all be electrospun into graft scaffolds. For example, protein-based materials such as silk have been used to electrospin tubular grafts.[137] Many other protein-based and protein-engineered biomaterials[138] are being developed as materials that may be used for vascular tissue engineering. Synthetic materials that simulate ECM properties[139] (e.g., proteolytic degradation) or are tuneable can also be used to make graft scaffolds.

To promote cell infiltration and graft remodeling, several methods have been developed to increase pore size and overall porosity of electrospun scaffolds and scaffolds in general, including the incorporation of sacrificial fibers and porogens,[140–142] modification of fiber diameter,[143] post-processing by photopatterning or ultraviolet radiation treatment,[144,145] laser ablation,[146] and electrospinning on micropatterned collector.[147] Cell infiltration can also be enhanced by heparin coating, fiber alignment or the incorporation of hyaluronan into the electrospun fibers.[148,149] To enhance the cell compatibility of polymer scaffolds, composite grafts with native ECM (e.g., collagen) and polymer scaffolds (electrospun or woven) have also been developed.[150,151] Interestingly, bare electrospun poly(epsilon-caprolactone) (PCL) grafts showed faster endothelialization than ePTFE grafts in an abdominal aorta anastomosis model.[152] However, long-term studies for 12–18 months showed cell regression and graft calcification.[153] Whether this happens to other polymers with various surface modifications needs further investigation.

The surface modification of the grafts is critical for the prevention of thrombus formation.[28,154] For example, PEG and carbohydrates can form brush-like layers on the surface and resist platelet adhesion, and immobilized heparin and hirudin coatings can inhibit thrombin activity locally. In general, covalent linkage of these molecules to the surface is more stable and is preferred over passive adsorption onto the surface, especially under hemodynamic conditions. To increase the surface conjugation sites in polyesters, hydrolysis, and plasma treatment methods can be used.[155,156] Heparin has been conjugated to a variety of surfaces and has shown thromboresistant effect *in vitro* and *in vivo*, including the scaffold made of collagen, PLA, ePTFE, and polyurethane.[157–160] Similarly, hirudin-conjugated grafts also showed improved patency of PLA grafts.[161] In a preclinical study of a canine femoral arteriovenous access model, heparin-coated electrospun grafts demonstrated the advantage of early accessibility and self-healing,[160] with the time to hemostasis after cannulation ~10 times faster than ePTFE. Histological analysis demonstrated functional endothelialization (nitric oxide expression), positive wound healing (cellular infiltration into the wall of the graft), and hemocompatibility of the electrospun grafts.

The degradation rate of the grafts may impact the remodeling of the grafts. An interesting recent finding suggests that fast degradation of the grafts (Figure 2(g)) enables accelerated remodeling of the grafts *in situ*.[162] The fast degradation of the elastomeric poly(glycerol sebacate) scaffold resulted in the infiltration of the graft by host cells and fast regeneration of blood vessels that are similar to the native vessels. The recruitment of M2 type macrophages may participate in a constructive remodeling of the tissues.[163] The elastomeric construct was able to degrade away in 3 months, resulting in almost complete host-based artery development.[162] Further studies are needed to determine the optimal degradation rate and the regeneration mechanisms of the grafts.

The anti-thrombogenic coating on the grafts may only be effective *in vivo* for a limited period of time. To improve the patency of vascular grafts, rapid endothelialization is desirable. Since human endothelium barely regenerates, it is necessary to incorporate bioactivity into the grafts to recruit EPCs from the circulation and ECs/mesenchymal cells from the surrounding tissues. In a porcine arteriovenous graft model, capturing EPCs by coating anti-CD34 antibody on ePTFE grafts accelerated endothelialization but increased neointima formation at venous anastomosis.[164] Whether the recruited $CD34^+$ cells contribute to neointima formation by paracrine signaling or differentiation remains to be determined. Stromal cell-derived factor-1α (SDF-1α) is a chemokine that can recruit EPCs to promote angiogenesis.[165,166] Woven polyester grafts adsorbed with fibronectin and SDF-1α increased the recruitment of EPCs and enhanced endothelialization.[167] Using heparin as a binding adaptor to immobilize SDF-1α significantly increased the stability of SDF-1α on electrospun PLA graft surface (Figure 2(h)) under static and flow conditions.[168] In addition, heparin facilitated cell infiltration into the vascular wall, and immobilized SDF-1α also promoted the recruitment of SMC progenitors for the regeneration of the vascular wall.[168] The exact mechanism by which EPCs and SMC progenitors are recruited by SDF-1α is not clear; it is likely that this recruitment process is mediated by the chemotactic effect of SDF-1α. It is worth noting that heparin binds to both SDF-1α and its receptors,[169] and thus heparin administration in the circulating blood should be avoided when SDF-1α is used to recruit EPCs. Another approach is to increase the number of circulating EPCs by using granulocyte colony-stimulating factor (G-CSF). Subcutaneous injection of G-CSF increased circulating bone marrow progenitor cells and enhanced endothelialization of synthetic grafts.[170] Overall, bioactive grafts and stem cell recruitment approaches have shown promising results, but more in-depth studies on stem cell biology and scaffold engineering are needed.

CONCLUSIONS

The area of vascular graft tissue engineering is at an exciting juncture. In the past decade, significant progress has been made in the development of technologies in scaffold engineering, cell engineering, and fabrication technologies, and some of the products are already in clinical trials. The variety of scaffolds, cell types, biophysical factors, and biochemical factors used has led to a multitude of approaches to address the clinical needs. Preclinical/clinical studies using *in vitro*, *in vivo*, and *in situ* approaches (Box 1) showcase some of the techniques used for building the next generation of vascular grafts. The development of these studies and their translation into clinical settings will determine the direction of future vascular implants. Currently, only cell-seeded grafts are in clinical trials; however, there is a trend towards reducing the time of *in vitro* manipulation and harnessing the regeneration potential in the body. *In vitro* and *in vivo* tissue engineering approaches have the advantage of making living functional grafts with excellent biocompatibility

BOX 1

IN VITRO, IN VIVO, *AND* IN SITU *TISSUE ENGINEERING OF VASCULAR GRAFT*

In vitro tissue engineering of vascular grafts is a traditional tissue engineering approach that constructs functional living vascular grafts outside the body by using cells, scaffolds, and bioreactors. It may take weeks to grow the cells (e.g., autologous ECs) and fabricate the mature grafts.

In vivo tissue engineering of vascular graft uses the tissue environment of the body (e.g., in peritoneal cavity or subcutaneous pouch) as a bioreactor to grow autologous grafts by implanting a rod with desirable diameter and length for 2–4 weeks. This approach needs minimal *in vitro* manipulation.

In situ tissue engineering of vascular graft takes advantage of the regeneration potential of the host cells to regenerate a blood vessel *in situ* following anastomosis implantation. The graft preparation does not need extensive *in vitro* culture and manipulation, and the grafts can be acellular or seeded with cells.

and performance, and can address the clinical problems that do not need immediate bypass surgeries. To make vascular grafts available off-the-shelf and scale up the production for commercialization, *in situ* tissue engineering approaches using either cellular or acellular grafts have distinct advantages and potential. The successful use of autologous ECs and stem cells in preclinical and clinical studies suggests that cell-based vascular grafts may play an important role in future vascular therapies. It is encouraging that acellular vascular grafts show promise for the *in situ* regeneration of blood vessels, as this may facilitate clinical translation. Multiple approaches to vascular graft development will ensure the selection and customization of the approach best suited to the specific and effective treatment of vascular diseases.

With the recent advancement of knowledge and technologies in the tissue engineering of vascular grafts, there are still many scientific questions to be addressed and there is still enough space for creativity and innovation in research. For example, to realize effective *in situ* regeneration, the mechanisms of regeneration needs further investigation. The activation and recruitment of stem cells from circulating blood and surrounding tissues may play an important role in the tissue remodeling and regeneration process, which is still poorly understood. The role of inflammatory cells in the blood vessel regeneration is also unclear. The micro and nanostructure of the scaffolds can be engineered to minimize detrimental inflammatory responses, facilitate beneficial remodeling, enhance cell recruitment, and optimize cell function. Nonthrombogenic, tuneable, and smart materials can be developed to enhance the remodeling process and deliver drugs. Various degradation mechanisms could be incorporated into the grafts to tailor the degradation rate for optimal regeneration. Novel bioactive grafts can be designed based on the understanding of stem cell biology and regenerative medicine. In the next 10 years, the collective efforts of bioengineers, biologists, and clinicians will lead to important breakthroughs in both basic research and clinical translation in the area of vascular graft tissue engineering.

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