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Review of Vascular Graft Studies in Large Animal Models

Rui Han Liu, BS,[1] Chin Siang Ong, MBBS,[1] Takuma Fukunishi, MD,[1]
Kingsfield Ong, MBBS,[2] and Narutoshi Hibino, MD, PhD[1]

As the incidence of cardiovascular disease continues to climb worldwide, there is a corresponding increase in demand for surgical interventions involving vascular grafts. The current gold standard for vascular grafts is autologous vessels, an option often excluded due to disease circumstances. As a result, many patients must resort to prosthetic options. While widely available, prosthetic grafts have been demonstrated to have inferior patency rates compared with autologous grafts due to inflammation and thrombosis. In an attempt to overcome these limitations, many different materials for constructing vascular grafts, from modified synthetic nondegradable polymers to biodegradable polymers, have been explored, many of which have entered the translational stage of research. This article reviews these materials in the context of large animal models, providing an outlook on the preclinical potential of novel biomaterials as well as the future direction of vascular graft research.

Keywords: vascular grafts, tissue-engineered vascular grafts, large animal models, biomaterials

Introduction

THE GLOBAL BURDEN of cardiovascular disease is on the rise, currently affecting one in three American adults and projected to affect one in two by 2030.[1] Many of these diseases require surgical intervention with grafts to correct perfusion insufficiencies and anatomic defects, including coronary and peripheral artery disease, end-stage renal disease (ESRD), and congenital heart defects. Approximately 400,000 bypasses, 446,000 AV fistulas, and over 6000 repairs for congenital heart defects occur annually, highlighting the exceeding demand for limited autologous graft options.[1]

Existing prosthetic options are limited to expanded polytetrafluoroethylene (ePTFE), polyethylene terephthalate (Dacron®), and polyurethane.[2] While prosthetic grafts are satisfactory in large-diameter applications, they fail to demonstrate long-term patency comparable to that of autologous grafts in small-diameter (<6 mm) settings due to their susceptibility to inflammation, thrombosis, intimal hyperplasia, and consequent compliance mismatch with the host vessels.[3–9]

The ultimate solution to combat the inflammation and thrombosis in existing prosthetic grafts is to create vascular prostheses that closely mimic the physiological responses and mechanical properties of autologous vessels without eliciting an immunogenic response. Structural components of blood vessels such as collagen and elastin promote compliance matching to host vessels, while the endothelial lining provides resistance

against inflammation and thrombosis.[10] Accordingly, the focus in vascular engineering has been on the facilitation of graft remodeling and reconstitution of native vessel wall anatomy.[11] Other important criteria to consider in the search for biomaterials for vascular grafts are ease of surgical anastomoses, resistance to infection, and off-the-shelf availability.[10]

Biomaterials, currently being investigated for use in vascular grafts, span a broad range of chemical and mechanical properties, but can be broadly categorized as nondegradable and biodegradable. Nondegradable biomaterials are composed of synthetic polymers of ePTFE and Dacron while biodegradable materials are composed of natural and synthetic polymers as well as decellularized tissue scaffolds. An important conceptual subcategory of vascular grafts that transcends the boundaries of biodegradability is tissue-engineered vascular grafts (TEVGs). Fundamentally, TEVGs require cells and a tubular scaffold. However, the attractiveness of TEVGs for clinical application lies in its projected ability to grow, remodel, and repair *in vivo*, offering the ideal solution to the thrombogenic conundrum.[12]

Before human application, the clinical potential of vascular grafts must be assessed *in vivo* in animal models (Fig. 1). Consideration for the selection of animal models include availability, cost, immunogenicity, implantability, reproducibility, and most importantly, similarity to normal human physiology.[13]

While small animal models such as mice and rabbits are widely available and relatively inexpensive to maintain,

[1]Division of Cardiac Surgery, The Johns Hopkins Hospital, Baltimore, Maryland.
[2]Department of Cardiac, Thoracic and Vascular Surgery, National University Health System, Singapore, Singapore.

FIG. 1. Vascular graft testing in large animal models. Grafts are implanted into large animal models in either the arterial or venous circulation at the location indicated by the circle. Representative procedures at the time of implantation and follow-up at 6 months' postimplantation are shown. *White dotted lines* indicate the luminal and adventitial surfaces of the graft, *yellow arrows* indicate clip markings, and *asterisks* demarcate the suture line. IVC, inferior vena cava; RA, right atrium. Scale bar represents 1.0 cm.
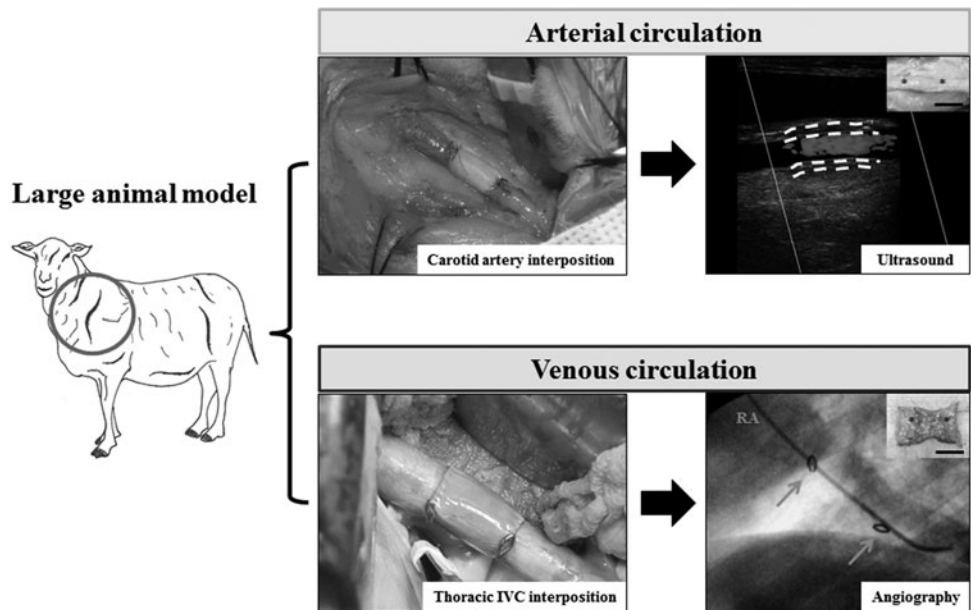
they lack similarity to human physiology with regard to vascular anatomy and hemodynamics and thus are only useful for dissecting the mechanical and molecular mechanisms of graft.[14] In contrast, large animal models, including dogs, pigs, and sheep, are limited in availability and costly to maintain, but are generally better able to mimic human physiology, although some variability is present between species.

For example, ovine models show the most similarity to humans in terms of the inflammatory response and thrombogenicity, while canine models are hyperthrombogenic, a property exploited for more rigorous vascular graft testing, but do not demonstrate spontaneous graft endothelialization.[14] On the contrary, porcine models exhibit endothelialization of the graft but consequentially are prone to intimal hyperplasia.[14,15]

The ideal animal model to simulate the normal human microenvironment would combine the thrombogenicity of the ovine model and the spontaneous endothelialization of the porcine model. In contrast, a combination of the hyperthrombogenicity of the canine model and the exaggerated endothelial healing response of the porcine model may create a more accurate depiction of diseased conditions. While it is apparent that there is no perfect animal model for human physiology and pathophysiology, testing grafts in large animals is essential for evaluation of long-term patency, graft remodeling, and ultimately, clinical translation.

Modified Synthetic Nondegradable Polymers: ePTFE and Dacron

Synthetic nondegradable polymers have been widely applied as vascular grafts due to the ease with which their mechanical properties can be modified. Two of the most common polymers used are ePTFE and Dacron (polyester). ePTFE is frequently used in femoropopliteal bypasses in the absence of available autologous vessels, whereas Dacron is more often used for large caliber aortic replacements. In canine carotid and femoral artery interposition models, Dacron grafts had increased early-platelet deposition compared to autologous vessel implants and exhibited 54% patency at 1 month.[16]

Unlike the other biomaterials discussed in this review, ePTFE and Dacron vascular grafts have already been in clinical application for over 50 years with extensive literature documenting clinical outcomes.[17]

As a replacement for large caliber (>8 mm) and medium caliber (6–8 mm) arteries, the thrombogenicity of prosthetic grafts can be largely offset by anticoagulation therapy, demonstrating 5-year patency rates rivaling that of autologous grafts at 90%.[8,9] However, even with rigorous anticoagulation and antiplatelet therapy, the 5-year primary patency rates for small-diameter prosthetic grafts in patients remain markedly inferior to that of autologous options in lower extremity bypasses: 44–62% for ePTFE and Dacron compared with 71–75% for autologous bypasses.[3] The poor patency rates of these polymers have prompted the development of new methods that primarily focus on promoting endothelialization. Two general methods of enhancing endothelialization have been tested in large animal models: cell seeding and surface modifications (Table 1).

TEVGs produced by electrostatic seeding of ePTFE and polyester grafts with host-derived endothelial cells (ECs) or EC precursors (CD34+ bone marrow cells) demonstrated reduced thrombosis, attributed to the production of prostacyclin by seeded EC, and increased surface endothelialization with increased microvessels in the neointima and graft wall in canine femoral artery and thoracoabdominal aorta interposition models.[18–20] The observed success of EC-seeded prosthetic grafts in canine models have also been corroborated by clinical trials, which showed that seeding of ePTFE femoropopliteal bypasses with EC increased the 1-year patency rate by 1.5 times and the 5-year patency rate by 3.5 times compared with unseeded controls.[21,22]

Surface modification of prosthetic grafts essentially applies the same principle as cell seeding, except the ECs that are autoseeded after implantation. In one study using a porcine arteriovenous graft model where ePTFE grafts were coated with human anti-CD34 monoclonal antibody, 85% of the graft surface was covered with ECs after 28 days compared with the 32% covered in uncoated grafts.[23] Similar

Table 1. Preclinical Large Animal Studies Using Synthetic Nonbiodegradable
Polymer-Derived Vascular Grafts

Authors and year	Animal species	n	Graft type	Inner diameter of graft (mm)	Implantation model	Follow-up time (days)	Patency rate (%)
Clagett et al. (1984)[19]	Dog	16	EC-seeded Dacron	10	Thoracoabdominal aorta bypass	84	N/A
Bhattacharya et al. (2000)[18]	Dog	10	BMC-seeded ePTFE and PET composite	8	Descending thoracic aorta interposition	28	100
Fields et al. (2002)[20]	Dog	10	EC-seeded ePTFE	4	Femoral artery interposition	42	100
Li et al. (2005)[24]	Sheep	6	P15-treated ePTFE	8	Femoral artery/vein and carotid artery/jugular vein arteriovenous shunt	28	100
Rotmans et al. (2005)[23]	Pig	11	CD34-coated ePTFE	5	Carotid artery/internal jugular vein arteriovenous shunt	28	73

BMC, bone marrow cell; EC, endothelial cell; ePTFE, expanded polytetrafluoroethylene; PET, polyethylene terephthalate; N/A, not available.

results were also observed in a study using P15 (a Type-I collagen cell adhesion domain peptide)-treated ePTFE vascular grafts in ovine arteriovenous graft models.[24]

In terms of improving the patency rates of synthetic nondegradable prosthetic grafts such as ePTFE and Dacron, both EC-seeding and surface modification approaches have demonstrated success with regard to enhancing surface endothelialization and improving graft patency in large animal studies. However, these studies were much less effective in promoting the adhesion and proliferation of smooth muscle cells, an important characteristic of arterial walls, suggesting the possibility of targeting cell types in addition to ECs. Yet, because these prosthetic grafts are nondegradable, it may be less crucial to fully reconstitute the arterial anatomy than it is to simply extend the patency rate to a level comparable to that of autologous grafts.

According to the clinical trials published by Meinhart et al., the 5-year patency of EC-seeded ePTFE grafts was 73.8%, a figure that matched the reported 5-year patency of autologous saphenous vein grafts (72%).[3,21] While more translational studies moving from large animal models to humans are needed to establish the relative efficacy of cell seeding and surface modification of nondegradable prosthetic grafts, current methods have shown considerable promise in leveling out the playing field between autologous grafts and prosthetic grafts.

Biodegradable Scaffolds

Decellularized tissue scaffolds

The rationale behind using decellularized vessels are reduced antigenicity and preservation of native three-dimensional tissue architecture, which theoretically would facilitate host cell migration through the provision of physiological adhesion molecules.[25] Decellularized small intestinal submucosa (dSIS) has additionally been shown to have antibacterial properties.[26] Since the approach of using dSIS to construct vascular grafts emerged in the late 1980s, many preclinical implantation experiments have been conducted in large animal models (Table 2).

Badylak et al. was the first to evaluate dSIS in large animal models, specifically as large-diameter interposition grafts in canine aortas.[27] These first grafts had a higher elastic mod-

ulus and lower tensile strength compared to endogenous vessels, and while they did not exhibit endothelialization, the relatively high patency offered a potential solution to the plaguing problem of thrombosis. Development of small-diameter dSIS saw progressive success in recapitulating the properties of native vessels.

Initially, small-diameter dSIS in canine models exhibited no intimal formation and had low short-term graft patency due to graft occlusion, dilation, and rupture.[28] Subsequent canine studies implemented grafts with increased tensile strength, although compliance was still a fraction that of a native vessel; dilation and rupture were rare.[29] The grafts experienced complete endothelialization with smooth muscle cells in the neointima and patency was increased to 87.5–100% at 9–12 weeks postimplantation.[30,31] Mechanical remodeling of the dSIS grafts was further demonstrated by Roeder et al., who compared the mechanical properties of the grafts preimplantation and postexplantation and observed increased compliance over time, suggesting that the grafts were remodeling to approach the mechanical parameters of autologous vessels.[32]

Further breakthroughs in dSIS vascular grafts involved surface modifications in porcine models. Elastin is a natural polymer with low tensile strength, limiting its use as an arterial conduit. However, the tensile strength of elastin is boosted 10-fold upon strengthening with dSIS and the enhanced graft showed increased patency in carotid interposition models compared with ePTFE grafts.[33] Another surface modification that has been explored is heparin. Heparin-bound dSIS implanted into SVC of piglets was 100% patent at 90 days, although two out of nine grafts did show early signs of stricture and aneurysmal change.[34] Importantly, the implanted grafts grew with the animals, increasing in circumference and length with the native vessel without jeopardizing mechanical strength or interfering with the remodeling process.

The approach of treating dSIS with heparin was applied further by Koobatian et al., who coated dSIS with heparin-bound vascular endothelial growth factor to prevent thrombosis and promote endothelialization.[35] Within 3 months, complete endothelialization had occurred with the endothelium aligned in the direction of flow with smooth muscle cells organized circumferentially in the medial layer; the smooth muscle cells also exhibited vascular contractility in response to vasoconstriction agonists.

TABLE 2. PRECLINICAL LARGE ANIMAL STUDIES USING DECELLULARIZED TISSUE SCAFFOLD-DERIVED VASCULAR GRAFTS

Authors and year	Animal species	n	Graft type	Inner diameter of graft (mm)	Implantation model	Follow-up time (days)	Patency rate (%)
Badylak et al. (1989)[27]	Dog	12	Autogenous dSIS	10	Infrarenal aorta interposition	364	75
Lantz et al. (1990)[28]	Dog	18	Autogenous dSIS	4.3	Carotid and femoral artery interposition	336	75
Hiles et al. (1995)[29]	Dog	8	Xenogenic porcine dSIS	10	Infrarenal aorta interposition	60	100
Sandusky et al. (1995)[30]	Dog	8	Xenogenic porcine dSIS	3.5–5.0	Carotid artery interposition	180	87.5
Robotin-Johnson et al. (1998)[34]	Pig	11	Autogenous dSIS	36.8	SVC interposition	90	100
Nemcova et al. (2001)[31]	Dog	5	Xenogenic porcine dSIS	4	Femoral artery interposition	63	89
Roeder et al. (2001)[32]	Dog	7	dSIS	5–8	Carotid artery interposition	63	100
Kaushal et al. (2001)[41]	Sheep	11	EPC-seeded xenogenic porcine decellularized iliac vessels	4	Carotid artery interposition	130	100
Conklin et al. (2002)[36]	Dog	2	Heparin-treated xenogenic porcine decellularized carotid artery	<5	Carotid artery bypass	67	100
Tamura et al. (2003)[39]	Dog	1	Heparin-treated xenogenic porcine decellularized carotid artery	—	Abdominal aorta interposition	126	N/A
Cho et al. (2005)[77]	Dog	12	BMC-seeded canine decellularized carotid artery	3	Carotid artery interposition	56	100
Ketchedjian et al. (2005)[37]	Sheep	16	Decellularized sheep pulmonary trunk	—	Pulmonary artery or aortic artery patches	140	N/A
Cho et al. (2006)[40]	Dog	6	BMC-seeded canine decellularized abdominal aorta	7	Abdominal aorta interposition	56	100
Hinds et al. (2006)[33]	Pig	6	Elastin-strengthened xenogenic porcine dSIS	4.3	Carotid artery interposition	0.25	33
Tillman et al. (2012)[38]	Sheep	11	EC-seeded xenogenic porcine decellularized carotid artery	5	Common carotid artery/external jugular vein arteriovenous shunt	180	0
Syedain et al. (2014)[42]	Sheep	9	Decellularized graft derived from fibrin gel and ovine dermal fibroblasts	4	Carotid artery interposition	168	100
Koobatian et al. (2016)[35]	Sheep	12	Heparin and VEGF-treated xenogenic porcine dSIS	5	Carotid artery interposition	90	92

dSIS, decellularized small intestinal submucosa; EPC, endothelial progenitor cell; SVC, superior vena cava; VEGF, vascular endothelial growth factor.

136

Small-diameter decellularized vascular grafts have also been derived from the carotid artery, abdominal aorta, pulmonary trunk, and iliac vessels, although literature on preclinical evaluation in large animal models is much less robust than for dSIS.[10,14] In contrast to dSIS, decellularized carotid arteries exhibit compliance similar to that of native vessels.[33]

In terms of large animal studies, decellularized carotid artery grafts have mainly been investigated in canine models and relied on cell seeding (TEVGs) and surface modification to overcome the challenges of prosthetic grafts. TEVGs constructed from decellularized carotid arteries seeded with bone marrow cells increased patency to 8 weeks from the 2 weeks for unseeded grafts.[37] However, when EC-seeded TEVGs were implanted in sheep, all grafts developed venous anastomotic stenosis by 4 months as commonly seen in clinical arteriovenous graft failure.[38] Heparinization of decellularized carotid arteries seems to have found considerable success as testing in canine models revealed full patency at four and half months postimplantation.[39]

Taking a different approach from direct surface modification, Cho *et al.* administered granulocyte colony-stimulating factor subcutaneously postimplantation and saw enhanced endothelialization with reduced intimal hyperplasia.[40] Similar successes were observed for decellularized pulmonary trunks implanted as patches into the pulmonary aorta of sheep.[37] In addition to exhibiting full patency at 130 days postimplantation, endothelial progenitor cell-seeded decellularized iliac vessels were also shown to exhibit contractile activity and nitric oxide-mediated vasodilation akin to endogenous vessels.[41]

A remarkably different approach has been undertaken, in which decellularized grafts are synthesized *in vitro* using dermal fibroblasts and a fibrin gel mold.[42] Grafts were implanted into ovine femoral arteries and found to be 100% patent at 24 weeks with extensive recellularization and with compliance and mechanical properties comparable to that of native arteries. Importantly, this approach produced grafts that have off-the-shelf availability, as the grafts were synthesized 5 weeks before implantation and could be stored stably until implantation.

The use of decellularized biological scaffolds has been widely explored in vascular graft development due to its inherent physiologic properties, conferring the ability to adapt to and grow with the host, overcoming a major pitfall of synthetic nondegradable polymer grafts. In addition, vessels used for subsequent decellularization and implantation can be harvested autologously, reducing the reactive inflammatory response, effectively decreasing the risk of thrombosis and increasing patency.

While the use of autologous vessels to combat thrombosis is a sound conceptual idea, the practicality of this method falls short when considering that goal of vascular graft research is to find alternatives to overcome the supply limitation of autologous vessels in cases of systemic vascular disease. Therefore, the focus has shifted away from using autologous implantation toward xenogenic implantation, using surface modifications and cell-seeding (TEVGs). While preclinical experiments using decellularized scaffolds have demonstrated the ability to withstand arterial flow as well as the potential to reconstitute native vessel anatomy, they must be able to overcome the limited off-the-shelf availability of using biological tissues before becoming a possibility in clinical practice.

Natural polymers

Fibrin is a natural protein that has been explored as a biomaterial for vascular graft, particularly TEVG, synthesis because it promotes cell adhesion, has high biocompatibility, and can be easily isolated from host blood.[43] However, its lack of stability often limits its application.[44] Two general approaches have emerged in response to this limitation, enabling the application of fibrin in translational vascular graft research: fibrin gels and fibrin compaction.

ECs, smooth muscle cells, and bone marrow-derived smooth muscle progenitor cells embedded in fibrin gel tubes show physiological contractility in response to vasoreactive agonists and displayed complete endothelialization with circumferentially aligned smooth muscle cells in the media layer in as little as 5 weeks postimplantation into sheep.[45,46] The second approach of fibrin compaction, utilized in the sheep model, while demonstrating recapitulation of native artery wall anatomy, saw patency rates that fell noticeably short of autologous vessels.[43]

Hyaluronan-based scaffolds (HYAFF-11) were developed to target the insufficiency of elastin biosynthesis in small-diameter vascular grafts in many large animal studies. While graft patency was only 70% at 5 months due to intimal hyperplasia and thrombosis, the patent grafts showed complete degradation and replacement of the scaffold with a neoartery with laminar circumferential arrangement of smooth muscle and elastin fibers.[47]

Few studies have evaluated the preclinical potential of natural polymer-derived vascular grafts in large animals, with a large spread of synthetic method as well as patency results (Table 3). The deterrence in the use of natural

TABLE 3. PRECLINICAL LARGE ANIMAL STUDIES USING NATURAL POLYMER-DERIVED VASCULAR GRAFTS

Authors and year	Animal species	n	Graft type	Inner diameter of graft (mm)	Implantation model	Follow-up time (days)	Patency rate (%)
Swartz *et al.* (2005)[45]	Sheep	4	SMC-embedded fibrin gel	4	Jugular vein interposition	105	100
Liu *et al.* (2007)[46]	Sheep	3	BM-SMPC-embedded fibrin gel	4	Jugular vein interposition	35	100
Zavan *et al.* (2008)[47]	Pig	10	Hyaluronan-based scaffold (HYAFF-11)	4	Carotid artery interposition	150	70
Aper *et al.* (2016)[43]	Sheep	6	EC and SMC-seeded compacted fibrin	5.3–6.0	Carotid artery interposition	180	50

BM-SMPC, bone marrow-derived smooth muscle progenitor cell; SMC, smooth muscle cell.

polymer-derived vascular grafts is largely attributed to the difficulty in balancing graft stability and graft degradation. While the ease of degradation facilitates the replacement of the implanted graft with neoartery, the simultaneous graft instability jeopardizes the integrity of the graft during the initial remodeling process. However, natural polymers have shown substantial promise in smoothing out the transition between graft degradation and host cell recruitment, leading to a remodeled vessel bearing remarkable resemblance to native arteries.

Biodegradable synthetic polymers

Scaffolds made from biodegradable polymers have been widely explored as an approach to recapitulate autologous vessel anatomy and function in large animal trials (Table 4). Theoretically, as the scaffolds degrade, the seeded cells and the host's cells will endothelialize the graft and secrete extracellular matrix (ECM), allowing for the seamless transition from a synthetic to endogenous vessel. Widely used materials include polyglycolic acid (PGA), polylactic acid (PLA), and poly ε-caprolactone (PCL). Polymers or combinations of polymers are used to customize mechanical properties and degradation speed.[48]

The feasibility of using biopolymers was first demonstrated by Shinoka et al., who implanted a TEVG, consisting of a PGA tubular scaffold seeded with autologous ECs and myofibroblasts, as an interposition graft into the pulmonary artery of lambs.[49] The PGA scaffolds were absent upon histological examination by week 11 and the remodeled vascular grafts showed endothelialization as well as ECM production. Similar results were observed in PGA scaffolds implanted in the venous microenvironment, specifically as inferior vena cava (IVC) interposition grafts in lambs.[50] These PGA grafts remained patent for up to 24 days in porcine saphenous artery grafts and up to 1 year in canine coronary bypass models, showing short- and long-term patency.[51,52]

PCL nanofiber grafts have not been as extensively tested in large animal models as PGA grafts; one short-term study revealed that PCL grafts had improved patency and endothelialization compared with ePTFE controls and also showed significant macrophage infiltration and thrombosis formation.[53]

PGA has a short degradation period, which confers an advantage in terms of remodeling, but is also a pitfall as the scaffold may degrade before the remodeled graft has attained adequate mechanical strength. Therefore, PGA has been explored as copolymers with PLA and PCL, which have longer degradation periods. While PGA and PLA have similar tensile strength and elasticity, PCL is notably different in that it has much less tensile strength but increased elasticity.[48]

A few large animal studies have looked at the translational potential of PGA-PLA, or poly (lactic-co-glycolic acid) (PLGA), copolymer grafts, which consists of a PGA core surrounded by woven PLA for mechanical reinforcement. The PGA core was completely degraded by 2 months postimplantation while the PLA sheath remained unabsorbed for the duration of the study.[54,55]

Torikai et al. implanted relatively large-diameter grafts (10 mm) into the descending thoracic aorta of pigs and observed complete endothelialization with smooth muscle cells in the subendothelium, which had good collagen content but poor elastin deposition.[54] In contrast, a patch graft

in canine pulmonary arteries revealed elastin content comparable to native vessels at 6 months postimplant.[56] These remodeled vessels also demonstrated physiologic vasomotor responsiveness to norepinephrine, showing functional recapitulation on top of gross anatomic mimicry.

Small-diameter (4 mm) PGA-PLA grafts have been tested as well as carotid bypass grafts in canine models.[55] As the previous study, endothelialization occurred with subendothelial smooth ECs and upon mechanical evaluation, it was found that the tensile strength and elastic modulus were comparable to that of native carotids. Importantly, despite the small diameter and high-flow vascular environment, these grafts remained patent at 12 months postimplantation with no signs of thrombosis or aneurysmal transformation.

Another combination, PLA-PCL copolymer, commonly referred to as poly (L-lactide-co-ε-caprolactone) (PLCL), has been evaluated in a TEVG study utilizing a canine IVC interposition model.[57] This study demonstrated that bone marrow cells seeded onto the biodegradable scaffold expressed endothelial lineage markers at 8 weeks after implantation but did not explicitly evaluate the mechanical or histological properties of the scaffold itself.

All three common biopolymers (PGA, PLA, and PCL) have been integrated into vascular grafts and have undergone extensive preclinical testing in large animal models; PLCL can either be interwoven or layered with PGA. Compared with PGA-PLA grafts, PGA-PLGL grafts have a longer degradation period of 6 months.[58,59]

The success of large-diameter (≥6 mm) PGA-PLCL grafts have been very reproducible in both ovine and canine models, consistently showing endothelialization with ECM and smooth muscle deposition similar to that of native vessels and the absence of dilation, thrombosis, or calcification.[58,60–62] However, one canine study reported poor vascular smooth muscle layer development at 12 months, although hydroxyproline and elastin content of the remodeled graft was similar to that of a native vessel while another study reported nonuniform distribution of subendothelial smooth muscle cells, leading to lesions within the graft.[60,62]

Furthermore, in a canine IVC interposition study, TEVGs constructed from PGA/PLCL seeded with bone marrow-derived vascular cells have demonstrated biochemical mimicry of native vessels, specifically the IVC, by releasing nitric oxide in a dose-dependent manner in response to acetylcholine stimulation.[63] The same study also showed that the inflammatory response attenuates as the biopolymer scaffold is replaced by autologous components, with CD4^{+} T cell infiltration inversely correlated to percentage degradation of the scaffold, providing a proof-of-concept for biodegradable vascular grafts in general. Small-diameter PGA-PLCL grafts have also experienced considerable preclinical success in overcoming thrombogenesis, the main challenge of small-diameter prosthetic vessels.[64,65]

A novel approach that has been explored is binding heparin to scaffolds such that the scaffolds continuously release heparin after implantation to discourage thrombosis and subsequent graft stenosis. Using this approach, one group reported an extension of the patency period by 24 weeks in a canine femoral artery replacement model.[65]

One emerging copolymer is PGA and polyhydroxyalkanoate (PHA); PHA has higher tensile strength than PGA

TABLE 4. PRECLINICAL LARGE ANIMAL STUDIES USING BIODEGRADABLE SYNTHETIC POLYMER-DERIVED VASCULAR GRAFTS

Authors and year	Animal species	n	Graft type	Inner diameter of graft (mm)	Implantation model	Follow-up time (days)	Patency rate (%)
Shinoka et al. (1998)[49]	Sheep	7	PGA/PLGA	15	Pulmonary artery interposition	168	100
Niklason et al. (1999)[51]	Pig	4	PGA	<6	Right saphenous artery interposition	24	100
Shum-Tim et al. (1999)[66]	Sheep	11	PGA/PHA	7	Abdominal aorta interposition	150	100
Watanabe et al. (2001)[60]	Dog	4	Femoral vein cell-seeded PGA/PLCL	10	IVC interposition	180	100
Izhar et al. (2001)[75]	Dog	8	PELA	6	Carotid artery interposition	90	100
Matsumura et al. (2003)[57]	Dog	16	BMC-seeded PLCL	8	IVC interposition	56	100
Iwai et al. (2004)[56]	Dog	20	PLGA with collagen microsponge	15	Pulmonary artery patch	180	N/A
Hibino et al. (2005)[59]	Dog	13	Venous cell or bone marrow-seeded or unseeded PGA/PLCL	8	IVC interposition	28	100 for seeded 40% for unseeded
Hoerstrup et al. (2006)[67]	Sheep	14	PGA/PHA	18	Pulmonary artery interposition	700	100
Matsumura et al. (2006)[63]	Dog	34	BMC-seeded PGA/PLCL	8	IVC interposition	180	N/A
Roh et al. (2007)[61]	Sheep	8	BMVC-seeded PGA/PLCL	13	IVC interposition	30	100
Brennan et al. (2008)[50]	Sheep	8	PGA/PLCL	13	IVC interposition	180	100
Torikai et al. (2008)[54]	Pig	15	PGA/PLA	10	Thoracic aorta interposition	360	100
Yokota et al. (2008)[55]	Dog	16	PGA/PLA	4	Carotid artery and coronary artery bypass	360	100
Zhang et al. (2008)[64]	Dog	16	BMSC-seeded PGA/PLCL	6	Abdominal aorta interpostion	90	100
Dahl et al. (2011)[52]	Dog	8	PGA	3–4	Carotid artery and coronary artery bypass	360	83
Cummings et al. (2012)[69]	Sheep	14	Myofibroblast and EC-seeded PGA/PHA	—	Pulmonary artery interposition	560	100
Kelm et al. (2012)[68]	Sheep	14	PGA/PHA	18	Pulmonary artery interposition	1680	N/A
Matsumura et al. (2013)[62]	Dog	7	PGA/PLCL	8	Pulmonary artery interposition	360	100
Wang et al. (2013)[65]	Dog	20	Heparin-treated PLA/PLCL and polyurethane-collagen type 1 composite	<6	Femoral artery interposition	168	88.9
Lu et al. (2013)[76]	Dog	—	Heparin-treated PCL	2.5			
Mrowczynski et al. (2014)[53]	Pig	11	PCL	4	Carotid artery interposition	30	78
Zhou et al. (2014)[73]	Dog	6	EC-seeded PCL/CS	3	Carotid artery interposition	90	83
Fukunishi et al. (2016)[58]	Sheep	6	PCL/CS	5	Carotid artery interposition	180	67
Fukunishi et al. (2016)[58]	Sheep	6	PGA/PLCL	12	IVC interposition	180	100

BMSC, bone marrow stromal cell; BMVC, bone marrow-derived vascular cells; CS, chitosan; IVC, inferior vena cava; PCL, poly ε-caprolactone; PELA, poly(ethylene glycol); PGA, polyglycolic acid; PHA, polyhydroxyalkanoate; PLA, polylactic acid; PLCL, poly (L-lactide-co-ε-caprolactone); PLGA, poly(lactic-co-glycolic acid).

and provides mechanical strength to the graft as the PGA layer is being remodeled.[66]

PGA-PHA grafts have only been tested in ovine models thus far, but has shown clinical potential as successes have been reproducible. In aortic, IVC, and pulmonary artery interposition graft systems, extensive remodeling of PGA-PHA grafts occurred, with proteoglycan percentage, DNA content, and endothelial surface proteins reminiscent of native vessels with no evidence of thrombosis, calcification, stenosis, or aneurysm.[66–68] However, two groups did note that these grafts, while mechanically stronger due to higher collagen content, had less elastic properties than native vessels.[67,68] An upregulation of matrix metalloproteinases, markers of ECM remodeling, have been correlated with collagen deposition in these grafts, providing evidence that the environment provided by PGA-PHA grafts are conducive to vascular remodeling.[69]

Recently, a novel hybrid polymer of synthetic PCL and chitosan (CS) has been investigated in several large animal models. CS is a natural protein with high biocompatibility, low toxicity, antibacterial properties, and rapid degradation.[70,71] Unfortunately, its cationic nature has the potential to facilitate aggregation of anionic platelets.[72]

Very small-diameter grafts (3 mm) have been constructed and evaluated in canine and ovine models, both of which have demonstrated adequate endothelialization with ECM composition close to that of autologous vessels.[73,74] Perhaps due to the combination of the small diameter and the thrombogenic tendency of CS, unseeded scaffolds had patency rates below 100% at 3 and 6 months after implantation. However, seeding of the graft with outgrowth endothelial cells produced TEVGs that could increase patency significantly, from 17% to 83% at 3 months postimplantation.[73]

Another possibility for small-diameter vascular grafts is Lycra (polyester-polyurethane copolymer) fibers bound by poly(ethylene glycol) (PELA)-PGA copolymer, which seals off initially the porous scaffold. In the canine common carotid interposition model, this graft exhibited limited neointima formation with a lack of organized ECM but interestingly showed increased compliance over time, eventually to a degree close to that of the canine aorta.[75] In addition, the porosity and hydrophilicity of the Lycra fibers and PELA-PGA copolymer, respectively, were found to be positively correlated to endothelialization and vascularization of the graft. Small-diameter heparin-conjugated PCL/polyurethane-collagen type I composite grafts have also been briefly evaluated in the canine model, revealing patent grafts at 8 weeks after implantation without aneurismal dilatation or stenosis.[76]

Vascular grafts constructed from biodegradable synthetic polymers demonstrate the most versatility and potential of all the biomaterials discussed thus far. Unlike vascular grafts made from decellularized vessels and natural polymers that have a narrow window of adaptation with regard to mechanical and degradative properties, the distinct properties of individual biopolymers have enabled the emergence of customized hybrid and composite grafts that continues to close the functional gap between synthetic grafts and autologous vessels.

Currently, the methodology of vascular graft research using biodegradable synthetic polymers entails a substantial amount of trial-and-error with biomaterial combinations and subsequent fine-tuning of the proportions of each biomaterial. Furthermore, the emergence of novel bio-materials, synthetic protocols, cell-surface modifications, and cell-seeding technologies provides additional parameters that not only expands the scope and depth of research but also increases the chances of stumbling upon the perfect alternative to autologous grafts.

Outlook

Global burden of cardiovascular disease and ESRD continues to climb while treatment options remain limited to small diameter autologous vessels and suboptimal large-diameter prosthetic grafts. Novel biomaterials have emerged as a promising solution to meet this increasing demand but these grafts must first overcome many challenges in both design and evaluation before becoming a clinical possibility. These grafts must be able to withstand systemic arterial pressure, resist inflammation, and have the capacity to remodel, ideally acquiring native vessel anatomy. Importantly, the thrombogenic tendencies of current prosthetic grafts must be overcome, allowing for long-term patency of small-diameter vascular grafts.

To gauge the clinical potential of vascular graft candidates, they must be evaluated in large animal models, which simulates, although imperfectly, the human vascular environment. As mentioned in the introduction, there are many barriers to the routine use of large animal models for preclinical evaluation of vascular grafts; chief among these is the exorbitant cost of maintaining the animals and facilities. There are two major ways that this obstacle can be surmounted: high-throughput in vitro screening and increased graft implantations per animal.

Rigorous in vitro experimentation with graft candidates would increase selectivity for the grafts that make it to the preclinical testing stage, ensuring that each large animal subject is used prudently. Double-graft implantation in which the bilateral symmetry of the vascular system is exploited to implant both the candidate graft and control graft into the same animal would not only produce more methodically sound experiments but also maximize the utility of each animal.

It is important to note that the cost of preclinical testing in large animals may be minimized but will never become zero because large animal models bridge the chasm between basic science research and clinical trials. Ultimately, the cost of preclinical experimentation becomes trivial in comparison to the cost of running clinical trials with human subjects.

While many biomaterials and synthetic technologies are currently in the translational stage of development undergoing testing in large animals, there are no standard protocols for preclinical testing specific to the intended purpose of each vascular graft. Thus, clinical trials must rely on preclinical results from studies that are not directly comparable and often lack valid controls. Standardized preclinical protocols, ideally producing models that recapitulate severe pathophysiology, and grafts that are deliberately ''over-engineered'' to withstand chronic inflammatory and diseased microenvironments would improve predictability when translating to human clinical trials.

As this review has outlined, there are many materials, natural as well as synthetic and biodegradable as well as nonbiodegradable, that are currently available and being explored in the development of vascular grafts with long-term patency rates rivaling that of autologous grafts. Based on existing studies in large animal models, vascular grafts

constructed from biodegradable synthetic polymers show the most clinical potential because of the unique mechanical and degradative properties of the polymers themselves. Superimposed onto the versatile combinations of individual polymers are the varying synthetic techniques that offer additional layers of fine-tuning. Furthermore, surface modifications and preimplantation cell seeding will be imperative to graft remodeling and maximizing long-term patency.

Compared to decellularized tissue scaffolds and natural polymers, the development of a clinically applicable vascular graft using biodegradable synthetic polymers may be a lengthier and more tedious process, simply due to the large number of manipulatable parameters. As evidenced by Tables 1–4, surface-modified vascular grafts and TEVGs composed from decellularized vessels and from biodegradable synthetic polymers exhibit high patency rate most consistently and thus may be the first ones to proceed to clinical trial. However, given the inherent compliance mismatch of the decellularized tissue with the host vessels, it is likely that most of the vascular grafts moving to clinical trials will be combinations of biodegradable synthetic polymers.

There have been promising results seen in large animal models as the challenges are addressed using a combination of cell sourcing, graft surface modifications, and the utilization of biodegradable materials that collectively facilitate graft remodeling. Large animal studies have provided evidence of graft remodeling and in many instances have been able to largely recapitulate native vessel anatomy via histological analysis. There is direct evidence that this new generation of grafts has improved patency compared with existing prosthetic options, as many studies have exploited ePTFE grafts as a control. This may be an important step in facilitating the movement from large animal studies to human clinical trials.

Nonetheless, with continual elucidation of biochemical pathways in vessel endothelialization and the discovery of novel polymers and synthetic techniques, the development of a small-diameter vascular graft with long-term patency, and good off-the-shelf availability may not be far off in the horizon.

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Address correspondence to:
Narutoshi Hibino, MD, PhD
Division of Cardiac Surgery
The Johns Hopkins Hospital
Zayed 7107, 1800 Orleans Street
Baltimore, MD 21287

E-mail: nhibino1@jhmi.edu