Validation of Biodegradable Synthetic Small Diameter Arterial Vascular Grafts Using a Large Animal Model

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ABSTRACT
Cardiovascular disease remains to be the number one killer worldwide and surgical intervention is often necessary for the patient survival. The autologous vascular and traditional prosthetic grafting remains to be the standard approach; however, this procedure has been plagued by several fatal pitfalls, including due to patient comorbidities and repeated bypass surgeries. Small-diameter (<6mm) prosthetic arterial grafts, made exclusively from biodegradable materials, is emerging as a highly promising alternative solution that eliminates the need for harvesting patient's native tissue, and dramatically reduces risks to calcifications and infections of non biodegradable synthetic grafts. Although human clinical trials are still distant, this new approach is being actively investigated by several groups around the world. In this review, we focus on several key large animal studies from the past two decades and highlight some of the critical challenges faced in the development of small-diameter biodegradable synthetic vascular grafts.

Abbreviations: PAD: Peripheral Artery Disease; TEAG: Tissue Engineered Arterial Grafts; EC: Endothelial Cell; SMC: Smooth Muscle Cell; ECM: Extracellular Matrix; PGA: Polyglycolide; PLGA: Poly (Lactic-Co-Glycolic Acid); PCL: Polycaprolactone; PLLA: Poly-L-lactic Acid; PU: Polyurethane; CS: Chitosan; PGS: Poly (Glycerol Sebacate); EPC: Endothelial Progenitor Cell

Mini Review
Peripheral artery disease (PAD) affects 8-12 million people in the United States, especially those over 50 [1]. Currently, the most important need for adult PAD patients is the lower limb bypass surgery. Although the autologous vessels and prosthetic grafts are gold standards for bypass surgeries [2] harvesting autologous vessels in an aging population often can be challenging. For instance, older PAD patients often have poor glyemic control due to diabetes, and the use of their already damaged vessels frequently result in repeated surgeries. Non-biodegradable graft is also associated with graft infection and calcification [3]. These and other challenges associated with antilogous vessels and non-biodegradable can be, in principle, overcome with the development of biodegradable arterial prosthetic grafts. Successful development of small diameter biodegradable Tissue Engineered Arterial Grafts (TEAG) that are capable of withstanding arterial pressure will dramatically increase the patient’s survival rate and quality of life. It effectively mitigates the need for multiple future operations, and by eliminating the time and effort spent in graft harvesting, it also significantly reduces the surgical duration and increases its success rate. Furthermore, the successful development and utilization of TEAG for peripheral arterial bypass surgery may eventually be adapted in the future for coronary artery bypass surgery and end stage renal disease hemodialysis as well.
Among the existing TEAGs under consideration, biodegradable synthetic scaffolds consisting solely of polymers are the most suitable for use in clinical practice due to its economic value, ease of production, and immediate off the shelf availability as compared to cellularized or decellularized biological scaffolds. In biodegradable grafts, the degrading foreign material is gradually replaced by the host endothelial (EC), smooth muscle cells (SMC), and the extracellular matrix (ECM). While the ideal biomaterial for vascular surgery should be non-thrombogenic, non-infectious, and highly durable during and after host regeneration [4], the existing materials and approaches can often lead to several critical problems. This review focuses on large animal trials aimed at addressing two most widespread complications, the loss of mechanical property and the thrombosis of the grafts. This work covers key studies from the last twenty years that were done on canine, ovine, and porcines.

**Mechanical Property Change and Tissue Regeneration**

The post implantation graft mechanical property sensitively depends on the competition between biodegradation (synthetic material) and regeneration (host tissue) rates. While it was not much of a concern during the initial human TEAG transplants in venous environments [5], the balance between these two rates become delicate in an arterial environment, where the mechanical stress on the graft is much higher. To produce a scaffold with the ability of surviving higher arterial pressure, without dilatation or burst, the electrospinning technology is commonly used on co-polymerizing biodegradable synthetic materials. Most research groups are currently exploring different combinations of Polyglycolide (PGA), Poly (lactic-co-glycolic acid) (PLGA), Polycaprolactone (PCL), and Poly-L-lactic Acid (PLLA) with or without natural materials such as Chitosan (CS) or Collagen (Table 1). The electrospinning method, originally developed for the textile industry [6], is now widely employed around the world to make biodegradable arterial grafts, and it offers the capability for a high degree of customization in diverse set of synthetic and biological materials. While it allows easier control for the fiber diameter, which is critical in producing scaffolds of optimal thickness and tensile strength suitable for the arterial environment, one notable challenge with the electrospinning approach has been the difficulty of increasing the pore size.

**Table 1:** TEAGs applied in Large animal studies.

| Author          | Animal Species | Study Duration | n  | Graft type                        | Graft Inner Diameter | Graft length | Implantation Position | Scaffold Technology          |
|-----------------|----------------|----------------|----|-----------------------------------|----------------------|--------------|-----------------------|-------------------------------|
| Niklason, et al. [25] | porcine        | 4 weeks        | 4  | PGA+EC, SMC                      | <6mm                 | 3.5 cm       | Right saphenous artery | chemically modified with sodium hydroxide |
| Shum Tim, et al. [26]  | ovine          | 5 months       | 11 | PGA/PHA+EC                       | 7mm                  | 3-4 cm       | Abdominal aorta        | -                             |
| Zhang, et al. [10]    | Canine         | 24 weeks       | 16 | Three-layers of porous PLGA-compact PUs-porous PLGA+BMCS | 6mm                  | 4cm          | Abdominal aorta        | dip-coating inner/spraying and salt leaching outer layer |
| Yokota, et al. [13]   | canine         | 12 months      | 16 | PGA/PLA + Collagen sponge        | 4mm                  | -            | Carotid artery         | Air jet spinning               |
| Huang, et al. [23]    | canine         | 3 months       | 8  | Heparin loaded P[LLA-CL]+EC      | 4mm                  | -            | Femoral artery         | coaxial electrospun            |
| Zhou, et al. [20]    | canine         | 3 months       | 6  | PCL/CS +EC                      | 3mm                  | 4-5 cm       | Carotid artery         | electrospun                   |
| Zhai, et al. [26]    | canine         | 1 month        | 8  | Heparin loaded P[LLA-CL]+EC      | 2.5-3.5 mm           | 5cm          | Femoral artery         | coaxial electrospun            |
| Wang, et al. [24]    | canine         | 24 weeks       | 20 | Heparin loaded P[LLA-CL]+polyurethane, collagen | 4mm                  | 5-6 cm       | Femoral artery         | coaxial electrospun            |
| Mrówczynski, et al. [17] | Porcine      | 1 month        | 11 | PCL                             | 4mm                  | 8cm          | Carotid artery         | electrospun                   |
| Fukunishi, et al. [27] | Ovine         | 6 months       | 6  | PCL/CS                          | 5mm                  | 1.5cm        | Carotid artery         | electrospun                   |
| Siang Ong, [28]      | Ovine          | 4 weeks        | 6  | PGA/PLCL+BMCS                  | 3mm                  | 5cm          | Carotid - external jugular vein AVF | electrospun                   |
| Min Ju, et al. [21]  | Ovine          | 6 months       | - | PCL+ EC, SMC, Collagen         | 4.75 mm              | 5cm          | Carotid artery         | electrospun                   |
Too small pores can halt cell infiltration - the initial step of vascularization and regeneration. Several methods have been explored to overcome this problem, such as salt leaching technique [7,8], laser radiation [9] and modifications to machine settings. For instance, when highly durable materials like PLGA and Polyurethane (PU) were constructed together and used salt leaching technique [10] to modify the pore sizes. Salt leaching technique increases scaffold pore sizes by adding salt particles and dissolving in the pre or leaching out salt particles in the post-polymer network creation stage [11]. Although, the rate of inflammation was found to be too high and the rate of regeneration too low. The compliance was shown to be lesser than native carotid arteries, which could be in part due to the slow degradation of PLGA and PU. However, a possible solution to this problem was reported by Wang et al [12], who modified the electrospinning condition with a thicker mat to produce durable PCL with larger diameter fibers and pores without a need of using other highly durable materials. The testing on rat abdominal aorta resulted in enhanced cell infiltration and vascularization similar to native aorta, as compared to thin diameter fibers with small pores. Although this is a highly promising result, extensive large animal model testing with synthetic materials with large pores and thick fibers are required to further verify the validity of this approach.

Vascular regeneration consists of EC, SMC, and ECM (collagen and elastin), where elastin and SMC play a key role in vascular compliance. In most large animal studies, the regeneration is dominated by collagen and SMC, while elastin lags behind [13,14]. For instance, when tested the PLLA/PGA copolymer with collagen micro sponge, the SMC and the collagen contents were similar to native carotid arteries; however, the elastin content tended to plateau only in 4 months of time [14]. It was speculated that the remaining slow degrading stiff layer of synthetic material might have prevented the tranmission of shear stress and pulsatile pressure to the scaffold wall [13]. Cross-linked elastin network is highly complex process and one of the biggest difficulties often faced in large animal studies [9]. A possible culprit for this problem has been hypothesized to be the fast degradation rate by the Wang et al [15], who have tested Poly (glycerol sebacate) (PGS), the fastest degrading material, with PCL sheath to prevent immediate bleeding. Result of post 3-month rat abdominal aorta implantation had shown compliant mechanical strength with synchronous pulsation due to higher expression of elastin. Currently the effect of fast degrading material has not been tested on large animals, where the regeneration rate is much slower than in a rat. Extremely fast degrading materials like PGS may not be suitable in a large animal setting [16]. In summary, these findings suggest to explore following two aspects in future large animal studies: (1) the effect of relatively faster degrading material for compliant vascular mechanical property with a careful consideration of large animal physiological slower regeneration rate; (2) the effect of fibers with larger pores and greater diameters for sufficient cell infiltration.

**Possible Solutions to Thrombosis**

One of the major early stage challenges arising from the hydrophobic nature of the synthetic arterial grafts is thrombosis. This is well illustrated in a recent study [17] that performed a porcine carotid artery implantation of a small PCL scaffold 4-mm in diameter. Although an ideal late-term patency was found, PCL grafts were clearly shown to be susceptible to macrophage infiltration and thrombosis, due to graft hydrophobicity and the slow degradation rate. The results indicated that simple PCL graft materials should overcome its shortcomings by exploring cellular (EC and or SMC) or chemical surface modifications, and also by copolymering different materials. The steppingstone for thrombus formation is often the deposition of platelets to the scaffold wall, and early endothelization is found to be crucial to pre-layer the scaffold interior through autologous endothelial or bone marrow cells. We note that, while anticoagulant drugs are frequently used to avoid early thrombosis, there are number of studies [18-20] that found ideal patency rate without drug prevention, but with endothelization. There are two types of endothelial progenitor cells that can be seeded into scaffolds in vitro, endothelial progenitor cells and outgrowth endothelial cells. The outgrowth endothelial cells have a superior profile in terms of vascular regeneration and cellular marker expression, showing a high degree of similarity to well-differentiated endothelial cells. PCL/CS was seeded with outgrowth endothelial cells and transplanted into the canine carotid artery [20], and 83% was patent at 3 months, as compared to only 16% in unseeded PCL/CS of the control group.

Subsequently, Young Min Ju group has developed different pore size double-layered scaffolds seeded with autologous endothelial progenitor cell (EPCs) derived endothelial cells (ECs) and smooth muscle cells (SMCs), and then pretreated in a pulsatile bioreactor [21]. The lumen of the carotid artery at 6 months post-transplantation was covered by a mature EC, a proliferated middle layer of smooth muscle, and a collagenous outer layer, not only shown to be similar to the native vascular structure, but also the lumen of the graft was shown to be stable with no dilatation or narrowing throughout the study. These encouraging results warrant further explorations into outgrowth endothelial cells, double seeded scaffolds with SMC and their pretreatment in pulsatile bioreactor. Since the surface of most polymers in bio synthetic scaffold is hydrophobic, a weak interaction with fluid makes platelets deposit on the surface relatively quickly [22]. An emerging new approach to reduce the risk of thrombosis is the conjugation of heparin, a powerful anticoagulant, into the vascular lumen through coaxial electrospinning method. The release rate of the drug and its effect on the patency rate is currently being actively investigated. In a study that compared PLLA/CL scaffolds with loading heparin to its pre-endothelialized versions, has found that most of the release (68%) takes place by the first week with a graft patency rate of 100%. However, only 6% more was released in the second week, and the patency rate dropped to 50%.
A high release rate seems to not have a significant effect on the patency rate in the longer term, and in fact, better tensile properties were observed in the pre-endothelialized grafts in 3 months, as compared to heparin bonded ones [23]. However, a recent new study compared seeded heparin bonded PLLA/CL grafts with non-seeded group [24]. The inner layer of the graft was constructed with heparin bonded PLLA/CL nanofibers through coaxial electrospinning, and was embedded in an outer layer of woven PLLA/CL. For the first time, the heparin release was observed to be released sustainably throughout 12 weeks in pre-endothelialized heparin group, significantly longer than the early thrombotic period of about a month. Furthermore, the seeded heparin bonded graft patency was superior to non-seeded heparin group, with 88.9% patency rate in 24 weeks of observation as compared to 66.7%. These advances suggest that pre-endothelialization could be the key to sustaining the heparin release during the first month and to improving the long-term patency rate.

In conclusion, united application of heparin loading and double layered pre-endothelialization with outgrowth endothelial cells and SMC to be a highly promising path for the possible thrombosis prevention.

Conclusion

This review focuses on the two most common challenges faced in large animal studies of biodegradable synthetic TEAGs: loss of mechanical properties and graft thrombosis. In order to solve these problems, different groups have tried to change the strength, the elasticity and the surface properties of the scaffold and beginning to report promising results. Validating these preliminary findings in large-scale animal models will be a major step in pushing the development of bio synthetic TEAG a step closer to clinical use.

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Conflict of Interest

“The authors have read the journal’s policy and the authors of the manuscript have the following competing interests: TS have received grant support from Gunze Ltd. No authors have received salary support from Gunze Ltd. This does not alter our adherence to policies on sharing data and materials. There are no patents, products in development, or marketed products to declare.”

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