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Evaluation of remodeling process in small-diameter cell-free tissue-engineered arterial graft

Shuhei Tara, MD, PhD,[a] **Hirotsugu Kurobe, MD, PhD,**[a] **Mark W. Maxfield, MD,**[b] **Kevin A. Rocco, MS,**[b] **Tai Yi, MD,**[a] **Yuji Naito, MD, PhD,**[b] **Christopher K. Breuer, MD,**[a] and **Toshiharu Shinoka, MD, PhD,**[a]
Columbus, Ohio; and New Haven, Conn

Objective: **Autologous grafts are used to repair atherosclerotic cardiovascular diseases; however, many patients lack suitable donor graft tissue. Recently, tissue engineering techniques have emerged to make biologically active blood vessels. We applied this technique to produce arterial grafts using established biodegradable materials without cell seeding. The grafts were evaluated in vivo for vessel remodeling during 12 months.**

Methods: **Poly(L-lactide-co-ε-caprolactone) scaffolds reinforced by poly(lactic acid) (PLA) fiber were prepared as arterial grafts. Twenty-eight cell-free grafts were implanted as infrarenal aortic interposition grafts in 8-week-old female SCID/Bg mice. Serial ultrasound and micro computed tomography angiography were used to monitor grafts after implantation. Five grafts were harvested for histologic assessments and reverse transcription-quantitative polymerase chain reaction analysis at time points ranging from 4 months to 1 year after implantation.**

Results: **Micro computed tomography indicated that most implanted mice displayed aneurysmal changes (three of five mice at 4 months, four of five mice at 8 months, and two of five mice at 12 months). Histologic assessments demonstrated extensive tissue remodeling leading to the development of well-circumscribed neovessels with an endothelial inner lining, a neointima containing smooth muscle cells and elastin, and a collagen-rich extracellular matrix. There were a few observed calcified deposits, located around residual PLA fibers at 12 months after implantation. Macrophage infiltration into the scaffold, as evaluated by F4/80 immunohistochemical staining, remained after 12 months and was focused mostly around residual PLA fibers. Reverse transcription-quantitative polymerase chain reaction analysis revealed that gene expression of Itgam, a marker for macrophages, and of matrix metalloproteinase 9 was higher than in native aorta during the course of 12 months, indicating prolonged inflammation (Itgam at 8 months: 11.75 ± 0.99 vs native aorta, $P < .01$; matrix metalloproteinase 9 at 4 months: 4.35 ± 3.05 vs native aorta, $P < .05$).**

Conclusions: **In this study, we demonstrated well-organized neotissue of cell-free biodegradable arterial grafts. Although most grafts experienced aneurysmal change, such findings provide insight into the process of tissue-engineered vascular graft remodeling and should allow informed rational design of the next generation of arterial grafts. (J Vasc Surg 2015;62:734-43.)**

Clinical Relevance: **Tissue-engineered vascular grafts (TEVGs) hold promise for correcting some types of congenital heart disease because they are biocompatible, are antithrombogenic, and possess the capacity for growth. Recently, some studies showed the feasibility of TEVG for arterial graft using animal models. The aim of this study was to evaluate and to characterize the tissue remodeling process of one such TEVG made from clinically approved biodegradable materials. In this study, we report a highly patent TEVG featuring organized neotissue but that had some instances of aneurysmal change. These findings provide insight into the TEVG remodeling process and enable better design of next-generation arterial grafts.**

Atherosclerotic cardiovascular disease (CVD) is a systemic narrowing and hardening of the arteries and includes conditions such as coronary heart disease, carotid artery stenosis, and peripheral arterial disease. CVD affects millions of patients and is the leading cause of morbidity and mortality in the United States.[1] Surgical intervention using autologous arterial and venous grafts is the most common corrective procedure for CVD; however, many patients lack suitable donor tissue because of previous surgery or as a result of their underlying vascular disease. Alternative synthetic grafts, such as expanded polytetrafluoroethylene (Gore-Tex) and polyethylene terephthalate (Dacron), have a history of long-term success when they are placed in large arteries whose flow is high and resistance low. However, current synthetic small-diameter (<6 mm) grafts are prone to occlusion by thrombogenesis and as a result are contraindicated. Synthetic materials have several other drawbacks, including risk of infection, persistent inflammation, calcification, and chronic need for anticoagulant therapy.

To address these challenges, tissue engineering techniques have emerged to make biologically active blood

From the Tissue Engineering Program and Surgical Research, Nationwide Children's Hospital, Columbus[a]; and the Department of Surgery, Yale University School of Medicine, New Haven.[b]

Author conflict of interest: C.K.B. and T.S. receive grant support from Gunze Ltd and the Pall Corp; this funding was not used to support the work described in this manuscript. H.K. (in 2011) and S.T. (in 2012) were recipients of the Banyu Fellowship from Banyu Life Science Foundation International (Tokyo, Japan). H.K. (in 2013) was recipient of a fellowship from Shinsenkai Imabari Daiichi Hospital (Ehime, Japan).

Correspondence: Toshiharu Shinoka, MD, PhD, 700 Children's Dr, Columbus, OH 43205 (e-mail: toshiharu.shinoka@nationwidechildrens.org).

The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.
0741-5214

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http://dx.doi.org/10.1016/j.jvs.2014.03.011

vessels, called tissue-engineered vascular grafts (TEVGs). The traditional concept of tissue engineering consists of the following three components: (1) a tissue-inducing scaffold material, (2) the isolation and use of cells or cell substitutes, and (3) the integration of the cells and the scaffold by a seeding technique.[2] We have successfully applied this technique in a low-pressure environment (<30 mm Hg)[3] and have performed TEVG implantation in 25 pediatric patients in Japan.[4] Currently, we have begun a clinical trial in the United States with approval of the Food and Drug Administration (FDA) for implantation of TEVGs in patients undergoing extracardiac total cavopulmonary connection procedures. To achieve this, we employ highly porous, biodegradable grafts composed of poly(L-lactide-co-ε-caprolactone) (PLCL) reinforced by mesh of poly(glycolic acid) that are seeded with bone marrow-derived mononuclear cells. As the synthetic scaffolding degrades away, a new blood vessel is formed in its place by the infiltration of the host's own smooth muscle cells and endothelial cells from the adjacent native blood vessel.

For the TEVG strategy to be translated to arterial applications, the graft must withstand arterial pressures while maintaining sufficient porosity for cellular infiltration. Moving toward that reality, we confirmed the feasibility of TEVGs with and without cell seeding in a small-diameter arterial model.[5,6] Furthermore, several groups have also demonstrated different types of TEVGs without cell seeding for small-diameter arterial grafts.[7,8] The electrospinning technique, which enables the production of nanofiber-based scaffolds, has been proposed as a promising technique for fabrication of arterial TEVGs[9] and has shown good surgical and mechanical properties with a high patency rate in an arterial implantation model.[10] However, we believe that cellular migration into the scaffold was likely inhibited by the tightly knitted nanofiber, causing prolonged neotissue remodeling and foreign body reaction. Moving forward, we created a cell-free TEVG for arterial circulation constructed from PLCL and reinforced by poly(lactic acid) (PLA) fiber mesh (PLA-PLCL grafts) to enhance cell migration into the scaffold. Herein, we used the PLA-PLCL grafts to evaluate the process of vessel remodeling with implantation in a mouse abdominal aorta model during a 12-month period.

METHODS

Animals. All animals received humane care in compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. The Institutional Animal Care and Use Committee at Yale University approved the use of animals and all procedures described in this study. The 8-week old female SCID/Bg mice were purchased from Jackson Laboratories (Bar Harbor, Me).

Scaffolds. PLA-PLCL grafts were constructed with use of a dual-cylinder chamber molding system from a nonwoven 100% PLA fiber mesh (molecular weight, 120,000; Biomedical Structures, Warwick, RI) and a 50:50 PLCL (molecular weight, 360,000; Gunze Co, Ltd, Kyoto, Japan) sealant solution on the basis of conventional grafts previously described.[6] Pore size of the scaffold was about 30 μm, and wall thickness was about 250 μm. Wall thickness changed over time according to tissue remodeling. Each scaffold was 3 mm in length, and inner luminal diameters were between 500 and 600 μm (Fig 1, *A*). All scaffolds were sterilized by overnight ultraviolet radiation preceding implantation.

Graft implantation. Twenty-eight grafts were implanted as infrarenal aortic interposition conduits with a running 10-0 nylon suture for the end-to-end proximal and distal anastomoses by standard microsurgical technique (Fig 1, *B*). Details of the method for graft implantation were described in our previous report.[5] Neither antiplatelet nor anticoagulant agents were used during aortic cross-clamping or the perioperative period in this study. Because we observed an entirely equivalent model with the same graft for 6 weeks in our previous study,[6] we decided the time point for evaluation of late-term tissue remodeling to be 4, 8, and 12 months. Five mice were selected randomly and sacrificed at each time point, and harvested grafts were separated in half for different analysis.

Twelve sham operations were performed (opening and closing of the abdomen with exposure of the aorta) to evaluate the natural causes of aortic disease, such as aortic calcification and dilation, for 12 months.

Ultrasound. Serial ultrasonography (Vevo Visualsonics 770; Visualsonics, Toronto, Ontario, Canada) was used to serially monitor grafts after implantation. Before ultrasonography, mice were anesthetized with 1.5% inhaled isoflurane.

Contrast-enhanced micro computed tomography (CT) angiography. We selected five mice at each time point randomly, and in vivo micro CT angiography was performed under anesthesia with the GE eXplore Locus in vivo micro CT scanner (GE Healthcare, Milwaukee, Wisc). One minute before image acquisition, animals were given an intrajugular 0.3 mL bolus of Ultravist (370 mg I/mL; Bayer Healthcare, Wayne, NJ). Micro CT data were transferred to the Advanced Workstation (version 4.4; GE Healthcare) for further reconstruction and quantitative analysis. Measurements of graft length, inner luminal diameter, and graft volume were performed. Similar measurements were obtained in controls having undergone sham operation.

Histology and immunohistochemistry. Explanted grafts at 4, 8, and 12 months after implantation and native abdominal aortas were fixed in 4% paraformaldehyde and embedded in paraffin. Sections 5 μm thick were then stained by standardized techniques for hematoxylin and eosin, Masson trichrome, elastica-van Gieson, and von Kossa.

Identification of endothelial cells, smooth muscle cells, macrophages, and matrix metalloproteinase 2 (MMP-2) was done by immunohistochemical staining of paraffin-embedded explant sections with rabbit anti-CD31 (1:50; Abcam, Cambridge, Mass), mouse anti-smooth muscle actin (1:500; Dako, Carpinteria, Calif), rat anti-F4/80 (1:1000; AbD Serotec, Oxford, UK), and rabbit anti-MMP-2

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