Published in final edited form as:

Biomaterials. 2008 April ; 29(10): 1454–1463.

Small-diameter biodegradable scaffolds for functional vascular tissue engineering in the mouse model

Jason D. Roh^{a*}, Gregory N. Nelson^a, Matthew P. Brennan^a, Tamar L. Mirensky^a, Tai Yi^a, Ty Hazlett^b, George Tellides^a, Albert J. Sinusas^a, Jordan S. Pober^a, W. Mark Saltzman^a, Themis R. Kyriakides^a, and Christopher K. Breuer^a

^a*Yale University School of Medicine, Interdepartmental Program in Vascular Biology and Therapeutics, New Haven, CT 06510*

^b*Concordia Fibers, Coventry, RI, 02816*

Abstract

The development of neotissue in tissue engineered vascular grafts remains poorly understood. Advances in mouse genetic models have been highly informative in the study of vascular biology, but have been inaccessible to vascular tissue engineers due to technical limitations on the use of mouse recipients. To this end, we have developed a method for constructing sub-1mm internal diameter (I.D.) biodegradable scaffolds utilizing a dual cylinder chamber molding system and a hybrid polyester sealant scaled for use in a mouse model. Scaffolds constructed from either polyglycolic acid or poly-L-lactic acid nonwoven felts demonstrated sufficient porosity, biomechanical profile, and biocompatibility to function as vascular grafts. The scaffolds implanted as either inferior vena cava or aortic interposition grafts in SCID/bg mice demonstrated excellent patency without evidence of thromboembolic complications or aneurysm formation. A foreign body immune response was observed with marked macrophage infiltration and giant cell formation by post-operative week 3. Organized vascular neotissue, consisting of endothelialization, medial generation, and collagen deposition, was evident within the internal lumen of the scaffolds by post-operative week 6. These results present the ability to create sub-1mm I.D. biodegradable tubular scaffolds that are functional as vascular grafts, and provide an experimental approach for the study of vascular tissue engineering using mouse models.

1. Introduction

Tissue engineering and regenerative medicine have made significant progress toward the creation of autologous vascular grafts for the repair of damaged or malformed vessels [1–5]. To date, the vast majority of research focused on the development of these grafts has utilized large animal models, including ovine, porcine, and canine recipients [1–4]. Each animal model possesses unique characteristics that make it more or less suited for evaluation of various vascular biological phenomena important for the clinical translation of this technology. For example, lambs undergo accelerated ectopic calcification, making the ovine model uniquely suited for investigations focused on the development of vascular conduits for pediatric cardiothoracic applications, where ectopic calcification is a leading cause of graft failure [2, 6–8]. This use of large animal models has provided important preclinical data necessary for

*Corresponding Author, Jason D. Roh, fax: 203-785-3820, email: jason.roh@yale.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

the first attempts at clinical translation of vascular tissue engineering [9–11]. However, an understanding of the molecular and cellular mechanisms underlying the remodeling of these grafts and the formation of vascular neotissue has eluded investigators, in part due to a limited molecular characterization of vascular cell phenotypes in these animal models. A better understanding of the process of neotissue formation is critical to the continued development and rational design of improved, second-generation, tissue engineered vascular grafts.

The extensive molecular characterization and rapid advancements in mouse genetics have made this species a powerful tool for the study of developmental and pathological processes in cardiovascular biology [12–14]. The mouse is a recipient species in which genetic impairment of the immune system can allow implantation of devices containing human vascular cells without invoking potent xenograft responses, and in which a human immune system can be co-engrafted to study human allograft responses [15,16]. The ability to study human vascular constructs in the immunodeficient mouse, however, necessitates functional grafts of sub-1mm internal diameters (I.D.), which has yet to be accomplished in scaffold development. To this end, we have developed a method for constructing sub-1mm I.D. tubular scaffolds from a variety of commonly used biodegradable polymers used in vascular tissue engineering applications. These scaffolds can be used to create tissue engineered vascular grafts that can be surgically implanted as arterial or venous grafts in a mouse model using microsurgical technique with excellent patency and graft function.

2. Materials and methods

This study was in accordance with the institutional guidelines for the use and care of animals, and the institutional review board approved the experimental procedures described below.

2.1. Small caliber scaffold fabrication

A dual cylinder chamber molding system was constructed from a 6.5mm diameter polypropylene rod. A 1.4mm diameter inner cylinder was cored through the center of the rod for a length of 30mm and gradually tapered out to 6.0mm at the inlet (Fig. 1). Either polyglycolic acid (PGA) or poly-L-lactic acid (PLLA) nonwoven felts [ConcordiaFibers, Coventry, RI] were used for the framework of the scaffold. Felts were 300 μ m thick with approximately 90% (PGA) or 83% (PLLA) total porosity. The gradual taper of the inner cylinder enabled flat felt sections (6.0mm $\times$ 4.0mm) to be easily shaped into tubes during their insertion through the inlet of the dual cylinder chamber system. Stainless steel needles (21g for PGA, 22g for PLLA) were then introduced into the opposing end to maintain the inner lumen and further compress the felt. A 50:50 copolymer sealant solution of ϵ -caprolactone and L-lactide (P(CL/LA)) [263,800Da, Absorbable Polymers International, Birmingham, AL] was created by dissolving the copolymer at 5% (w/v) in 1,4-dioxane or glacial acetic acid for the PGA and PLLA felts, respectively. The P(CL/LA) sealant was injected into the inlet of the chamber system and allowed to penetrate the felt and fuse the open seam. The hybrid polyester scaffolds were snap-frozen at -20°C for 30 minutes to enable rapid transformation of the P(CL/LA) sealant from liquid to solid phase, creating a sealed tube with a newly defined porous structure of P(CL/LA) polymer interconnecting the PGA or PLLA fibers. The scaffolds were lyophilized for 24 hours to eliminate the solvents before removing them from the dual cylinder chamber system.

2.2. Structural characterization of scaffold

Scaffolds were cut into 0.5mm thick cross-sections and imaged on a FEI XL-30 scanning electron microscope (SEM) [Hillsboro, OR]. Lumen diameters and wall thickness were measured from high magnification SEM images.

Porosimetry profiles of the scaffolds were acquired by micro-computed tomography (microCT) imaging [Scanco Medical microCT 40, Southeastern, PA]. Briefly, a 2048×2048 image matrix size over a 12.288mm field of view with $6\mu\text{m}$ slice increments was used to create an isotropic voxel size of $6\mu\text{m}$. A total of 200–250 slices were acquired for each scaffold section, and images were then binarized by applying a small gauss noise reduction filter using fixed threshold. Three-dimensional morphometric parameters as well as thickness and separation distribution maps were generated using previously published methods [17].

2.3. Biomechanical characterization of scaffold

Tensile strength, elastic modulus, and suture retention strength of the scaffolds were determined by tensiometry [Instron Model 5848 MicroTester, Norwood, MA]. For ultimate tensile strength, flat scaffold sections were incubated in phosphate buffered saline (PBS) in a 37°C shaker. Sections were tested at 0, 1, 2, 4, 8, 12, 16, 20, and 24 wk ($n=5$). Samples were inserted into the tensiometer using screw-action grips, and measurements were done with a crosshead speed of 1mm/min and analyzed using Bluehill software [Norwood, MA]. The elastic modulus of the scaffolds was calculated from the slope of the initial 10% elongation of the stress-strain curves of week 0 samples. Suture retention strength was measured on week 0 samples ($n=5$) by placing a 5-0 prolene suture approximately 1mm from the end of the scaffold. The suture was clamped into the upper grip with the scaffold immobilized in the lower grip. The force needed to displace the suture from the scaffold was measured using a crosshead speed of 1mm/min.

Burst pressure was measured using a closed polyethylene tube system. PGA-P(CL/LA) and PLL-A-P(CL/LA) scaffolds ($n=5$) were passed over the polyethylene tubing [Becton Dickinson, Sparks, MD]. A 20cc syringe and a 60psi pressure gauge [Ashcroft, Stratford, CT] were then attached to either end of the tubing using luer-lock needle adapters. Continuously increasing pneumatic pressure was introduced by the 20cc syringe until scaffold rupture. Scaffolds were observed under 2.5X magnification to visualize when and where rupture occurred while pressure gauge measurements were recorded by video camera.

2.4. In vitro biocompatibility assessment

In vitro biocompatibility of the scaffolds was assessed by implanting these constructs with human vascular cells. The ability of cells to attach and grow on these scaffolds was examined by statically seeding 10ul of a 10^8 cells/ml solution of human aortic smooth muscle cells (hAoSMC) onto 3mm long PGA-P(CL/LA) or PLLA-P(CL/LA) scaffolds ($n=4$). Seeded scaffolds were cultured in high glucose Dulbecco's modified eagles medium (DMEM) + 10% fetal bovine serum (FBS). After 72 hours, scaffolds were fixed in 10% neutral buffered formalin (NBF) and either embedded in glycolmethacrylate for hematoxylin and eosin (H&E) staining or dehydrated in serial ethanol washes and hexamethyldisilazane for SEM imaging.

Cell viability of seeded hAoSMCs on PGA-P(CL/LA) and PLLA-P(CL/LA) scaffolds was assessed with the CellQuanti-MTT assay [BioAssay Systems, Hayward, CA]. Scaffolds were cut into 0.5mm thick rings and seeded with 5×10^4 hAoSMCs. Seeded and unseeded scaffold rings ($n=5$) were cultured in 80ul of DMEM + 10% FBS. After 24 hours, MTT reagent was added, and samples were incubated for 4 hours at 37°C . Formazan produced by viable cells was then solubilized and absorbance was measured at 570nm. Human AoSMCs cultured directly on polystyrene tissue culture wells ($n=5$) were used as a positive control.

2.5. In vivo functional efficacy

Unseeded PGA-P(CL/LA) and PLLA-P(CL/LA) scaffolds ($n=7$) were implanted as inferior vena cava (IVC) and aortic interposition grafts, respectively, in female C.B-17 severe combined immunodeficient-beige (SCID/bg) mice, aged 3–4 mo [Taconic, Rensselaer, NY].

Anesthetized mice were placed in the supine position and opened with an abdominal midline incision. Infrarenal IVC or aorta were exposed under 5X magnification, cross-clamped, and excised. Three millimeter length scaffolds were then inserted as interposition grafts using a running 10-0 nylon suture for the end-to-end proximal and distal anastomoses. The animals were recovered from surgery and maintained without the use of any anticoagulation or antiplatelet therapy.

At post-operative weeks 3 or 6, grafts were examined by microCT analysis to evaluate for graft patency and morphology. Anesthetized mice were anticoagulated with 100IU heparin prior to sacrifice. A PE-10 [polyethylene] catheter was inserted into either the IVC or aorta and 300 μ l of Omnipaque (300mg/ml) was injected into the respective venous or arterial circulatory system. Mice were then imaged in an X-O* microCT [Gamma-Medica, Northridge, CA]. Three-dimensional reconstruction was done using COBRA software [Exxim Computing Corporation, Pleasanton, CA]. Images were combined using Total Commander [Ghisler & Co., Switzerland] and volume rendered with AMIDE imaging software [Loening, UCLA].

Immediately following microCT analysis, grafts were harvested and fixed in 10% NBF and paraffin embedded. Sections were stained with H&E, Gomori trichrome (collagen), and Verhoeff van Gieson (elastin) stains. Identification of endothelial cells, smooth muscle cells/myofibroblasts, and macrophages was done by immunohistochemical staining of unstained sections with rabbit-anti-human von Willebrand Factor [vWF, 1:2000, Dako, Carpinteria, CA, which cross reacts with mouse vWF], mouse-anti-human alpha smooth muscle actin [α SMA, 1:1000, Dako, which also cross-reacts with mouse α SMA], and rat-anti-mouse Mac-3 [1:50, BD Bioscience, Franklin Lakes, NJ]. Antibody binding for α SMA and Mac-3 was detected using biotinylated goat-anti-mouse IgG [1:500, Vector] or biotinylated goat-anti-rat IgG [1:500, Vector], followed by binding of streptavidin-horse radish peroxidase (HRP) and color development with 3,3-diaminobenzidine (DAB). Slides were then counterstained with hematoxylin. vWF expression was detected using a goat-anti-rabbit IgG-Alexa Fluor 568 [1:500, Molecular Probes, Eugene, OR] and a Dapi mounting medium counterstain [Vector].

2.6. Statistical analysis

Statistical differences were measured using a Student's t-test. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Scaffold structures

Scaffold dimensions were easily manipulated by varying the gauge of the insert needle. To match internal diameters of mouse IVC and aorta, 21g and 22g needles were selected for PGA-P(CL/LA) and PLLA-P(CL/LA) scaffolds, respectively. Twenty-one gauge needles resulted in an internal diameter of approximately 0.9mm with a wall thickness of 150 μ m, while 22g needles yielded scaffolds with approximately 0.7mm internal diameters and 250 μ m wall thickness (Fig. 2(A) and (B)).

Using the P(CL/LA) sealant reduced the total porosity and narrowed the pore size distribution of both PGA and PLLA felts. Total porosity of PGA-P(CL/LA) and PLLA-P(CL/LA) scaffolds was 78.5% and 60.4%, respectively. Mean pore sizes were $45.4 \pm 17.6\mu$ m and $31.2 \pm 16.1\mu$ m for PGA-P(CL/LA) and PLLA-P(CL/LA) scaffolds, respectively (Fig. 2(C) and (D)).

3.2. Biomechanical properties of scaffolds

The initial burst pressure, suture retention strength, and elasticity were measured for both scaffolds to ensure that they possessed significant mechanical strength and properties to function as vascular grafts.

When exposed to increasing pneumatic pressure, scaffolds burst along the longitudinal seam created by the P(CL/LA) sealant. Resultant burst pressures of PGA-P(CL/LA) (2710 ± 282 mmHg) and PLLA-P(CL/LA) (2790 ± 180 mmHg) scaffolds were not significantly different from each other (Fig. 3(A), $P=0.6$), and were both above the range of physiologic venous burst pressures and within the range of arterial burst pressures [1,3].

Suture retention strengths of PGA-P(CL/LA) and PLLA-P(CL/LA) were also found to be greater than corresponding values for vein and artery [3]. Significant differences were observed between the scaffolds, with the PLLA-P(CL/LA) scaffolds (4.37 ± 0.67 N) having greater suture retention strength than the PGA-P(CL/LA) scaffolds (3.13 ± 0.72 N) (Fig. 3(B), $P=0.03$).

The intrinsic elasticity of the scaffolds was measured by calculating Young's modulus, and was not significantly different between PGA-P(CL/LA) (33.0 ± 7.0 MPa) and PLLA-P(CL/LA) (24.0 ± 5.9 MPa) scaffolds (Fig. 3(C), $P=0.06$). These values indicated that the scaffolds were stiffer than native venous and arterial tissue, but were more elastic than expanded polytetrafluoroethylene (e-PTFE), a commonly used vascular graft material [18,19].

The tensile strength of the scaffolds was followed over a 6 month time course to indirectly assess how the biodegradability of the scaffolds affected their strength. The tensile strength profiles of the scaffolds were found to be closely related to the biodegradation properties of the nonwoven felt materials. Initial tensile strength of the PGA-P(CL/LA) (5.3 ± 0.72 MPa) was greater than the PLLA-P(CL/LA) (3.7 ± 0.53 MPa), but rapidly decreased within 1 week (Fig. 3(D)). Tensile strength of the PLLA-P(CL/LA) scaffold did not decrease significantly from its original strength until after 4 weeks.

3.3. Scaffold biocompatibility

Seeded hAoSMCs were capable of penetrating and adhering to both the PGA-P(CL/LA) and PLLA-P(CL/LA) scaffolds (Fig. 4). No significant difference in cell viability was detected between hAoSMCs grown on PGA-P(CL/LA), PLLA-P(CL/LA), and tissue culture plastic (Fig. 4(G)).

A typical foreign body innate immune reaction was demonstrated in both PGA-P(CL/LA) and PLLA-P(CL/LA) scaffolds when implanted into the SCID/bg mouse venous and arterial vasculature. Increased porosity and faster biodegradation generated greater cellular infiltration into the PGA-P(CL/LA) scaffolds, compared to the less porous PLLA-P(CL/LA) scaffolds (Fig. 5(A) and (E)). By post-operative wk 3, the majority of the cellular infiltrate in both scaffolds was macrophages, as indicated by positive mac-3 expression and multi-nucleated giant cell formation (Fig. 5(B) and (F)). Collagen deposition was evident throughout the scaffold wall with increased density in the internal lumens (Fig. 5(C) and (G)). While no external collagen encapsulation was seen surrounding the PGA-P(CL/LA) scaffolds, there was the beginnings of encapsulation around the external periphery of the PLLA-P(CL/LA) scaffolds (Fig. 5(D) and (H)).

3.4. Functional vascular grafts

PGA-P(CL/LA) and PLLA-P(CL/LA) scaffolds could be successfully used as vascular interposition grafts in SCID/bg mouse abdominal IVC and aorta, respectively. All PGA-P(CL/LA)

LA) IVC grafts and PLLA-P(CL/LA) aortic grafts were patent without evidence of thrombosis or aneurysm formation throughout the six-week time course (Fig. 6).

Both PGA-P(CL/LA) venous grafts and PLLA-P(CL/LA) arterial grafts recovered at 3 and 6 wks post-implantation were completely infiltrated with cells and extracellular matrix (Fig. 5 and Fig. 7). The primary early cellular component within both scaffold walls was mac-3 positive macrophages (Fig. 5(B) and (F)). However, by post-operative week 6, organized vascular neotissue was evident in the internal lumens of both scaffolds. A medial layer, generated from infiltrating α SMA expressing cells (smooth muscle cells/myofibroblasts), was evident in both venous and arterial scaffolds (Fig 7(B) and (H)). The formation of the α SMA medial layer, however, was much more robust in the PLLA-P(CL/LA) arterial grafts compared to the PGA-P(CL/LA) vein grafts, correlating to the respective vessels they were implanted into (Fig. 7(E) and (K)). Partial endothelialization was also evident by post-operative week 6, with a monolayer of vWF expressing endothelial cells lining the internal lumen (Fig. 7(A) and (G)). Some collagenous neotissue formation was evident within the wall of both grafts, particularly in the inner lumen. However, no elastin production was detected by histological staining (Fig. 7(C) and (I)).

4. Discussion

The present study reports the development of sub-1mm biodegradable porous scaffolds for vascular tissue engineering in the mouse model. Non-woven PLLA and PGA felts were selected for this study as they are commonly used polymers in tissue engineering scaffolds. However, we anticipate that this technique can be translated to nearly any polyester sheet, including those generated from electrospinning technology. Additionally, scaffold dimensions (wall thickness, internal diameters, length) can be easily manipulated by customizing the polypropylene cylinder and insert needle gauges, making this technique useful for a wide range of animal models and enabling the rapid prototype testing of novel polymeric sheets for vascular tissue engineering scaffolds.

A biodegradable P(CL/LA) copolymer solution was developed to function as a sealant for the tubular constructs. PCL and PLA were chosen based on their intrinsic elasticity and tensile strength, which are critical for vascular tissue engineering scaffolds [20–22]. Utilizing dioxane and acetic acid solvents, with freezing temperatures at 11.8°C and 16.7°C, respectively, enabled us to quickly freeze the sealant into place and control porosity. Addition of the sealant did reduce total porosity by approximately 16% and narrowed the pore size distribution range (Fig. 2(C) and (D)). However, the resultant mean pore sizes and total porosity were sufficient for cellular penetration into the graft wall under in vitro and in vivo conditions (Fig. 4, Fig. 5, and Fig. 7). Furthermore, we observed no decrease in cell viability associated with this sealant (Fig. 4(G)).

Preliminary functional studies were conducted in the SCID/bg mouse because of its potential to accept xenotransplants, including tissue engineered grafts created with human cells [23–25]. Early use of human cells in preclinical development has multiple advantages, including early identification of biological characteristics of human cells that could potentially limit their clinical utility. Additionally, with the creation of a human-mouse chimeric system, detailed cell tracking experiments based on species-specific markers can be easily performed, providing further insights into the cellular mechanisms involved in vascular neotissue development.

The SCID/bg mouse does lack a functional adaptive immune system, which could potentially influence results. However, prior reports using scaffolds constructed from similar polymers have demonstrated no evidence of an adaptive immune response to the biomaterials used in our scaffolds [26,27]. In these studies, PGA, PLLA, and PCL constructs elicited foreign body

innate immune responses in immunocompetent hosts. The innate immune system is intact in the SCID/bg mouse, enabling it to mount similar inflammatory responses to our scaffolds that would be observed in immunocompetent mice. A typical foreign body response to both PGA-P(CL/LA) and PLLA-P(CL/LA) scaffolds was replicated in this study in their functional setting as intravascular grafts, validating the use of this animal model for the study of tissue engineered vascular grafts (Fig. 5). Furthermore, recent studies have demonstrated that the foreign body innate immune response to biomaterials is more robust in intravascular hemodynamic settings, as opposed to traditionally used subcutaneous implantation assessments, suggesting that the ability to study these constructs as interposition vascular grafts presents a more accurate depiction of their actual in vivo biocompatibility [27]. Additionally, the reconstitution of a circulating human immune system in immunodeficient mouse strains potentially offers an even more relevant model for the preclinical study of tissue engineered human vessels [15,16].

The PGA-P(CL/LA) and PLLA-P(CL/LA) scaffolds demonstrated appropriate initial mechanical properties to be effective as venous and arterial interposition grafts. Initial burst pressure, suture retention strength, and ultimate tensile strength were within or above the physiologic ranges of venous and arterial tissue, and exhibited greater elasticity than current synthetic graft materials (i.e. ePTFE) (Fig. 3). As evidenced by the decreasing tensile strength of the scaffolds from hydrolytic degradation (Fig. 3(D)), these initial biomechanical properties, however, likely change when exposed to the hemodynamic and inflammatory environments of the circulatory system.

Despite their gradual biodegradation, PGA-P(CL/LA) and PLLA-P(CL/LA) scaffolds were functional as venous and arterial interposition grafts, respectively, exhibiting no thrombus or aneurysm formation over the course of 6wks (Fig. 6). The continued biomechanical strength and functionality of the scaffolds over prolonged in vivo times is likely secondary to the vascular remodeling of the scaffolds as they slowly degrade. Even without cell seeding prior to implantation, early stages of organized vascular neotissue formation, consisting of partial endothelialization, medial generation, and extracellular matrix deposition, were evident by post-operative wk 6 (Fig. 7). At the present time, the mechanism of how vascular neotissue forms in these engineered constructs is unknown. The differences in medial generation in the PLLA-P(CL/LA) arterial grafts and PGA-P(CL/LA) venous grafts suggests that differing hemodynamic forces between the venous and arterial circulation may potentially be a contributing factor to the recruitment and proliferation of α SMA expressing cells in tissue engineered vascular grafts. Additionally, a significant macrophage infiltration was evident within the walls of both scaffolds, implicating a possible role for inflammation in vascular neotissue formation. Current studies are underway to determine the role of seeded cells, immune responses, and hemodynamic forces in the development of tissue engineered vascular grafts using these small-diameter scaffolds in mouse models.

5. Conclusion

This study presents the development of sub-1mm I.D. biodegradable scaffolds. Composed of biodegradable polymers commonly used in tissue engineering, these tubular scaffolds demonstrate adequate porosity, biomechanical properties, and biocompatibility to function as interposition vascular grafts in the venous and arterial abdominal vasculature of SCID/bg mice. The ability to study tissue engineered vascular grafts in the mouse model will provide a powerful experimental tool for answering the mechanistic and developmental questions in vascular tissue engineering.

Acknowledgements

Processing and embedding of samples was done by the Yale Core Center for Musculoskeletal Disorders (NIH/NIAAMS AR46032). The authors would also like to thank Tony DeSimone for his help in constructing the dual cylinder chamber

system. This study was funded by the NIH through KO8-HL083980, PO1-HL070295, RO-1GM072194, T32-preddoctoral research fellowship, and through an AMA Foundation Seed Grant and ASA Grant.

References

1. Niklason LE, Gao J, Abbott WM, Hirschi KK, Houser S, Marini R, Langer R. Functional arteries grown in vivo. *Science* 1999;284:489–493. [PubMed: 10205057]
2. Kaushal S, Amiel GE, Guleserian KJ, Shapira OM, Perry T, Sutherland FW, Rabkin E, Moran AM, Schoen FJ, Atala A, Soker S, Bischoff J, Mayer JE. Functional small-diameter neovessels created using endothelial progenitor cells expanded ex vivo. *Nat Med* 2001;7:1035–1040. [PubMed: 11533707]
3. L'Heureux N, Dusserre N, Konig G, Victor B, Keire P, Wight TN, Chronos NA, Kyles AE, Gregory CR, Hoyt G, Robbins RC, McAllister TN. Human tissue-engineered blood vessels for adult arterial revascularization. *Nat Med* 2006;12:361–365. [PubMed: 16491087]
4. Watanabe M, Shin'oka T, Tohyama S, Hibino N, Konuma T, Matsumura G, Kosaka Y, Ishida T, Imai Y, Yamakawa M, Ikada Y, Morita S. Tissue engineered vascular autograft: inferior vena cava replacement in a dog model. *Tissue Eng* 2001;7:429–439. [PubMed: 11506732]
5. Campbell JH, Efendy JL, Campbell GR. Novel vascular graft grown within recipient's own peritoneal cavity. *Circ Res* 1999;85:1173–1178. [PubMed: 10590244]
6. Rashid ST, Salacinski HJ, Hamilton G, Seifalian AM. The use of animal models in developing the discipline of cardiovascular tissue engineering: a review. *Biomaterials* 2004;25:1627–1637. [PubMed: 14697864]
7. Roh JD, Brennan MP, Lopez-Soler RI, Fong PM, Goyal A, Dardik A, Breuer CK. Construction of an autologous tissue-engineered venous conduit from bone marrow-derived vascular cells: optimization of cell harvest and seeding techniques. *J Pediatr Surg* 2007;42:190–202.
8. Shinoka T, Shum-Tim D, Ma PX, Tanel RE, Isogai N, Langer R, Vacanti JP, Mayer JE Jr. Creation of viable pulmonary artery autografts through tissue engineering. *J Thorac Cardiovasc Surg* 1998;115:536–545. [PubMed: 9535439]
9. Shin'oka T, Imai Y, Ikada Y. Transplantation of a tissue-engineered pulmonary artery. *N Engl J Med* 2001;344:532–533. [PubMed: 11221621]
10. Matsumura G, Hibino N, Ikada Y, Kurosawa H, Shin'oka T. Successful application of tissue engineered vascular autografts: clinical experience. *Biomaterials* 2003;24:2303–2308. [PubMed: 12699667]
11. Shin'oka T, Matsumura G, Hibino N, Naito Y, Watanabe M, Konuma T, Sakamoto T, Nagatsu M, Kurosawa H. Midterm clinical results of tissue-engineered vascular autografts seeded with autologous bone marrow cells. *J Thorac Cardiovasc Surg* 2005;129:1330–1338. [PubMed: 15942574]
12. Yutzey KE, Robbins J. Principles of genetic murine models for cardiac disease. *Circulation* 2007;115:792–799. [PubMed: 17296868]
13. Plump A. Atherosclerosis and the mouse: a decade of experience. *Ann Med* 1997;29:193–198. [PubMed: 9240624]
14. Daugherty A. Mouse models of atherosclerosis. *Am J Med Sci* 2002;323:3–10. [PubMed: 11814139]
15. Pober JS, Bothwell AL, Lorber MI, McNiff JM, Schechner JS, Tellides G. Immunopathology of human T cell responses to skin, artery, and endothelial cell grafts in the human peripheral blood lymphocyte/severe combined immunodeficient mouse. *Spring Semin Immunopathol* 2003;25:167–180.
16. Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. *Nat Rev Immunol* 2007;7:118–130. [PubMed: 17259968]
17. Hildebrand T, Rueggsegger P. A new method for the model-independent assessment of thickness in three-dimensional images. *J Microscopy* 1997;185:67–75.
18. Catanese J III, Cooke D, Maas C, Pruitt L. Mechanical properties of medical grade expanded polytetrafluoroethylene: the effects of internodal distance, density, and displacement rate. *J Biomed Mater Res* 1999;48:187–192. [PubMed: 10331912]

19. Silver FH, Snowhill PB, Foran DJ. Mechanical behavior of vessel wall: a comparative study of aorta, vena cava, and carotid artery. *Annals Biomed Eng* 2003;31:793–803.
20. Lillie MA, Gosline JM. Limits to the durability of arterial elastic tissue. *Biomaterials* 2007;28:2021–2031. [PubMed: 17240445]
21. Sarkar S, Salacinski HJ, Hamilton G, Seifalian AM. The mechanical properties of infrainguinal vascular bypass grafts: their role in influencing patency. *Eur J Vasc Endovasc Surg* 2006;31:627–636. [PubMed: 16513376]
22. Nerem RM. Role of mechanics in vascular tissue engineering. *Biorheology* 2003;40:281–287. [PubMed: 12454417]
23. Shepherd BR, Enis DR, Wang F, Suarez Y, Pober JS, Schechner JS. Vascularization and engraftment of a human skin substitute using circulating progenitor cell-derived endothelial cells. *FASEB J* 2006;20:1739–1741. [PubMed: 16807367]
24. Goyal A, Wang Y, Su H, Dobrucki LW, Brennan M, Fong P, Dardik A, Tellides G, Sinusas A, Pober JS, Saltzman WM, Breuer CK. Development of a model system for preliminary evaluation of tissue-engineered vascular conduits. *J Pediatr Surg* 2006;41:787–791. [PubMed: 16567194]
25. Zheng L, Gibson TF, Schechner JS, Pober JS, Bothwell AL. Bcl-2 transduction protects human endothelial cell synthetic microvessel grafts from allogeneic T cells in vivo. *J Immunol* 2004;173:3020–3026. [PubMed: 15322161]
26. Athanasiou KA, Niederauer GG, Agrawal CM. Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/ polyglycolic acid copolymers. *Biomaterials* 1996;17:93–102. [PubMed: 8624401]
27. Van der Giesson WJ, Lincoff AM, Schwartz RS, van Beusekom HM, Serruys PW, Holmes DR Jr, Ellis SG, Topol EJ. Marked inflammatory sequelae to implantation of biodegradable and nonbiodegradable polymers in porcine coronary arteries. *Circulation* 1996;94:1690–1697. [PubMed: 8840862]

Fig. 1.

Schematic of scaffold fabrication using the dual cylinder chamber system. A) Flat polyester felts were easily rolled into tubular constructs using a gradually tapered cylinder. B) P(CL/LA) solution sealed the nonwoven polyester tube by creating an interconnecting porous structure between the felt fibers. C) Cross sectional image demonstrating the configuration of the components and removal of solvents by lyophilization. D) Resultant hybrid scaffold pushed out through inlet after inner needle cylinder removed.

Fig. 2. Structural characterization of scaffolds. A) SEM of PGA-P(CL/LA) scaffold (0.9mm I.D., 150 μ m wall thickness). B) SEM of PLLA-P(CL/LA) scaffold (0.7 mm I.D., 250 μ m wall thickness). C) Pore size distribution comparing uncoated nonwoven PGA felt to PGA-P(CL/LA) scaffold. D) Pore size distribution comparing uncoated nonwoven PLLA felt to PLLA-P(CL/LA) scaffold.

Fig. 3. Biomechanical characterization of scaffolds. A) Burst pressure. B) Suture retention strength. C) Young's modulus. D) Tensile strength of PGA-P(CL/LA) and PLLA-P(CL/LA) scaffolds over 24wk time course.

Fig. 4. Scaffolds biocompatible with human cell seeding. Scanning electron photomicrographs of: A) Unseeded PGA-P(CL/LA) scaffold, B) Unseeded PLLA-P(CL/LA) scaffold, C) PGA-P(CL/LA) scaffold seeded with hAoSMC, and D) PLLA-P(CL/LA) scaffold seeded with hAoSMC. Hematoxylin and eosin stains of E) PGA-P(CL/LA) scaffold seeded with hAoSMC and F) PLLA-P(CL/LA) scaffold seeded with hAoSMC. G) MTT assay comparing hAoSMC viability on PGA-P(CL/LA), PLLA-P(CL/LA), and polystyrene shows no significant differences. Original magnification 200X (E, F).

Fig. 5. Scaffolds elicit foreign body reaction with giant cell formation (O) by postoperative wk 3 when implanted as vascular interposition grafts. Representative images of PGA-P(CL/LA) venous grafts stained with A) H&E (inner lumen), B) Mac-3 (inner lumen, brown=macrophage), C) Gomori trichrome (inner lumen, blue=collagen), and D) Gomori trichrome (outer lumen) are shown. Representative images of PLLA-P(CL/LA) arterial grafts stained with E) H&E (inner lumen), F) Mac-3 (inner lumen), G) Gomori trichrome (inner lumen), and H) Gomori trichrome (outer lumen) are shown. Original magnification 400X.

Fig. 6. Scaffolds functioning as vascular grafts at post-operative wk 3. A) MicroCT reconstruction of PGA-P(CL/LA) scaffold as IVC graft, B) MicroCT reconstruction of PLLA-P(CL/LA) scaffold as aortic graft. By 3 wks, scaffolds are fully infiltrated with cells. C) H&E of PGA-P(CL/LA) as IVC graft. D) H&E of PLLA-P(CL/LA) as aortic graft. Original magnification 100X (C,D).

Fig. 7.

Development of organized vascular neotissue by post-operative wk 6. Representative images of PGA-P(CL/LA) venous graft stained with A) vWF (endothelial layer), B) α SMA (medial layer), and C) Verhoeff-van Gieson (black=elastin, pink=collagen). Representative images of native mouse IVC stained with D) vWF, E) α SMA, F) Verhoeff-van Gieson. Representative images of PLLA-P(CL/LA) arterial graft stained with G) vWF, H) α SMA, and I) Verhoeff-van Gieson. Representative images of native mouse aorta stained with J) vWF, K) α SMA, and L) Verhoeff-van Gieson. Original magnification 400X.