Acta Biomaterialia 52 (2017) 74–80

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Tropoelastin inhibits intimal hyperplasia of mouse bioresorbable arterial vascular grafts ☆

Tadahisa Sugiura [a], Riddhima Agarwal [a], Shuhei Tara [a], Tai Yi [a], Yong-Ung Lee [a], Christopher K. Breuer [a], Anthony S. Weiss [c,d,e,1], Toshiharu Shinoka [a,b,*,1]

[a] Tissue Engineering Program and Surgical Research, Nationwide Children's Hospital, Columbus, OH, USA
[b] Department of Cardiothoracic Surgery, The Heart Center, Nationwide Children's Hospital, Columbus, OH, USA
[c] School of Molecular Bioscience, University of Sydney, Sydney, NSW 2006, Australia
[d] Charles Perkins Centre, University of Sydney, Sydney, NSW 2006, Australia
[e] Bosch Institute, University of Sydney, Sydney, NSW 2006, Australia

ARTICLE INFO

Article history:
Received 21 August 2016
Received in revised form 1 December 2016
Accepted 21 December 2016
Available online 23 December 2016

Keywords:
Bioresorbable vascular grafts
Small diameter arterial grafts
Tropoelastin
Intimal hyperplasia
Smooth muscle cells

ABSTRACT

Neointimal hyperplasia, which results from the activation, proliferation and migration of vascular smooth muscle cells (SMCs), is a detrimental condition for vascular stents or vascular grafts that leads to stenosis. Preventing neointimal hyperplasia of vascular grafts is critically important for the success of arterial vascular grafts. We hypothesized that tropoelastin seeding onto the luminal surface of the graft would prevent neointimal hyperplasia through suppressing neointimal smooth muscle cell proliferation. In this study, we investigated the efficacy of tropoelastin seeding in preventing neointimal hyperplasia of bioresorbable arterial vascular grafts. Poly (glycolic acid) (PGA) fiber mesh coated with poly (L-lactic-co-ε-ca prolactone) (PLCL) scaffolds reinforced by poly (L-lactic acid) (PLA) nano-fibers were prepared as bioresorbable arterial grafts. Tropoelastin was then seeded onto the luminal surface of the grafts. Tropoelastin significantly reduced the thickness of the intimal layer. This effect was mainly due to a substantial reduction the number of cells that stained positive for SMC (α-SMA) and PCNA in the vessel walls. Mature elastin and collagen type I and III were unchanged with tropoelastin treatment. This study demonstrates that tropoelastin seeding is beneficial in preventing SMC proliferation and neointimal hyperplasia in bioresorbable arterial vascular grafts.

Statement of Significance

Small resorbable vascular grafts can block due to the over-proliferation of smooth muscle cells in neointimal hyperplasia. We show here that the proliferation of these cells is restricted in this type of graft.

This is achieved with a simple dip, non-covalent coating of tropoelastin. It is in principle amendable to other grafts and is therefore an attractive process.

This study is particularly significant because: (1) it shows that smooth muscle cell proliferation can be reduced while still accommodating the growth of endothelial cells, (2) small vascular grafts with an internal diameter of less than 1 mm are amenable to this process, and (3) this process works for resorbable grafts.

1. Introduction

Vascular grafts have been widely used in the treatment of vascular diseases, such as cardiovascular or peripheral vascular diseases. Autologous vessels are commonly recommended for this procedure, but in some cases, there are limitations in using autografts such as insufficient availability of viable tissue in patients with widespread atherosclerotic vascular disease. This lack of viable tissue necessitates the use of alternative synthetic grafts.

☆ Part of the Special Issue on Extracellular Matrix Proteins and Mimics, organized by Professor Katja Schenke-Layland.

* Corresponding author at: Cardiovascular Tissue Engineering Program, Department of Cardiothoracic Surgery, The Heart Center, Nationwide Children's Hospital, 700 Children's Drive, T2294, Columbus, OH 43205, USA.
E-mail address: toshiharu.shinoka@nationwidechildrens.org (T. Shinoka).

[1] Joint senior authors.

http://dx.doi.org/10.1016/j.actbio.2016.12.044
1742-7061/

However, current commercialized grafts composed of expanded-polytetrafluoroethylene or polyethylene terephthalate have not yet shown clinical effectiveness for small-diameter arteries (<6 mm) due to their poor patency rate [1]. To address this challenge, our group demonstrated the feasibility of novel bioresorbable small-diameter arterial grafts that restore vascular function in a mouse aortic implantation model [2,3].

Bioresorbable vascular grafts are biologically active grafts which are entirely reconstituted by host-derived cells over the course of an inflammation-mediated degradation process [4]. The application of bioresorbable vascular grafts has several advantages to the patient, such as growth potential, favorable biocompatibility, and low risk of infection. One of the most detrimental events for a vascular graft is stenosis, which is often proceeded by intimal hyperplasia. Smooth muscle cells (SMCs), the predominant cells in the arterial wall, are essential for the structural and functional integrity of the neovessel. However, excessive proliferation of SMCs leads to neointimal hyperplasia followed by graft stenosis and occlusion. Some growth factors, such as transforming growth factor-beta (TGF-β) and platelet-derived growth factor (PDGF), are understood to promote SMC recruitment and proliferation leading to neointimal hyperplasia [5,6].

Elastin is an integral part of the vasculature as a major component of the internal elastic lamina and in arteries in alternating elastic lamellae [7]. Elastin also regulates the phenotypic modulation, proliferation and migration of SMCs and is a crucial signaling molecule that directly controls SMC biology and stabilizes arterial structure [8]. On this basis, in an undamaged artery SMCs are sandwiched between elastic lamellae. Elastin is predominantly comprised of the 60–70 kDa protein monomer tropoelastin, which is crosslinked and organized into elastin polymers that form elastic fibers around the arterial lumen which provide the compliance that arteries need to absorb and transmit hemodynamic forces [9,10]. We hypothesize that an abundant presence of tropoelastin will inhibit the migration and proliferation of SMCs into the bioresorbable vascular grafts. The purpose of the present study is to use a murine aortic implantation model to confirm whether a tropoelastin seeded bioresorbable arterial vascular graft inhibits SMC proliferation and thus prevents neointimal hyperplasia.

2. Materials and methods

2.1. Bioresorbable arterial vascular graft preparation

Bioresorbable arterial vascular grafts were constructed using a dual cylinder chamber molding system from a nonwoven poly (glycolic acid) (PGA) fiber mesh (Biomedical Structures, Warwick, RI) coated with a 50:50 copolymer sealant solution of poly(L-lactic-co-ε-caprolactone) (PLCL) (Gunze, Kyoto, Japan), as previously described [11]. These scaffolds were reinforced by electrospinning poly (L-lactic acid) (PLA) nano-fiber (30 μm thickness) onto the outer side of the PLCL scaffold to endure the high pressure arterial environment; the wall thickness of the grafts in all groups was around 65 μm, and the luminal diameter of each graft was around 620 μm (Fig. 1A, B, C). All grafts were provided by Gunze Ltd (Kyoto, Japan).

2.2. Tropoelastin coating

Grafts were incubated under aseptic conditions with a sterile phosphate-buffered saline solution of 1 mg/ml tropoelastin for at 1 h at room temperature, corresponding to the most common 60 kDa mature form of the secreted protein after removal of the signal peptide, which generates a monolayer coating of physisorbed tropoelastin molecules as described previously [12].

2.3. Animal model and surgical implantation

All animals received humane care in compliance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME). Bioresorbable vascular grafts of 3 mm in length were implanted in 8–10 week old female mice (n = 10 for each group) as infra-renal aortic interposition conduits using standard microsurgical techniques (Fig. 1D) [13]. Low pressure vascular clamps (Fine Science Tools, Foster City, CA) were used for cross clamping and 400 U/kg of heparin was injected intramuscularly 5 min before unclamping. Animals were followed for 8 weeks to evaluate neotissue formation. An aspirin-mixed diet (0.1% of diet) was fed to mice in each group 3 days before and 3 days after surgery to prevent acute thrombosis.

2.4. Histology, immunohistochemistry, and immunofluorescence

Explanted grafts at 8 weeks after implantation were fixed in 4% *para*-formaldehyde, embedded in paraffin, sliced (5 μm thick sections), and stained with Hematoxylin and Eosin (H&E), Hart's, and Picrosirius Red. Outer and luminal perimeters of the grafts were manually measured from H&E staining with Image J software (NIH, Bethesda, MD) to obtain luminal diameter and wall thickness measurements. Wall thickness of the graft was calculated by the following formula: (Wall thickness) = (Graft outer circumference)/2π − (Graft inner circumference)/2π. The area fraction, positively stained using Hart's stain, was measured with Image J software to obtain area fraction of elastin deposition within the scaffold. Polarized microscopic images of Picrosirius Red staining were used to measure the area fraction of collagen type I and III deposition with Image J software.

Identification of SMCs, proliferating-cell nuclear antigens (PCNA), and macrophages was done by immunohistochemical staining of paraffin-embedded explant sections with anti- α-smooth muscle actin (α-SMA) antibody (1:500, DAKO, Carpinteria, CA), anti- PCNA antibody (1:1000, Abcam, Cambridge, MA), and anti-MAC3 antibody (1:75, DAKO), respectively. Primary antibody binding was detected using biotinylated IgG (Vector, Burlingame, CA), and was followed by the binding of streptavidin-horseradish peroxidase and color development with 3,3-diaminobenzidine.

SMCs identified by α-SMA expression, PCNA positive cells in the neointimal layer, and MAC3 positive macrophages were quantified by manual counting. One representative section from each explant was stained and imaged. Low magnification (5×) images were divided into nine sections (3 × 3). Four of these regions (upper middle, center, lower right, and lower left) were selected to obtain high magnification (40×) images. All positively stained nuclei were counted from high magnification images. Averages of these four regions provided the number of positive cells in each section.

Immunofluorescent staining for CD31 as a marker of endothelial cells and for α-SMA was performed using rabbit anti-CD31 primary antibody (1:50, Abcam) and mouse anti-α-SMA primary antibody (1:500, DAKO), followed by Alexa Fluor 488 anti-rabbit IgG secondary antibody (1:300, Invitrogen, Carlsbad, CA), and Alexa Fluor 647 anti-mouse IgG secondary antibody (1:300, Invitrogen), respectively. Fluorescence images were obtained with an Olympus IX51 microscope (Olympus, Tokyo, Japan).

2.5. Statistical analysis

Numeric values are listed as mean ± standard deviation. The number of experiments is shown in each case. Data of continuous variables with normal distribution were evaluated by student's *t* test. *P* values less than 0.05 indicated statistical significance.