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Intravascular Ultrasound Characterization of a Tissue-Engineered Vascular Graft in an Ovine Model

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Abstract

Patients who undergo implantation of a tissue-engineered vascular graft (TEVG) for congenital cardiac anomalies are monitored with echocardiography, followed by magnetic resonance imaging or angiography when indicated. While these methods provide data regarding the lumen, minimal information regarding neotissue formation is obtained. Intravascular ultrasound (IVUS) has previously been used in a variety of conditions to evaluate the vessel wall. The purpose of this study was to evaluate the utility of IVUS for evaluation of TEVGs in our ovine model. Eight sheep underwent implantation of TEVGs either unseeded or seeded with bone marrow-derived mononuclear cells. Angiography, IVUS, and histology were directly compared. Endothelium, media, and graft were identifiable on IVUS and histology at multiple time points. There was strong agreement between IVUS and angiography for evaluation of luminal diameter. IVUS offers

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9. Human Subjects/Animal Subjects Statement

No human studies were carried out by the authors for this article. All institutional and national guidelines for the care and use of laboratory animals were followed and approved by the appropriate institutional committees.

8. Disclosures

Support for graft production for this study was provided by Gunze Limited. Christopher Breuer, MD is also on the scientific advisory board for Cook Biomedical and is the founder of Lyst Therapeutics. The remaining authors have no conflicts of interest.

a valuable tool to evaluate the changes within TEVGs, and clinical translation of this application is warranted.

Keywords

Tissue-engineered vascular graft; TEVG; intravascular ultrasound; IVUS; neotissue; stenosis; angiography

1. Introduction

Approximately 40,000 babies born in the US are affected by congenital cardiac anomalies every year [1]. Nearly 25% of these children will require surgical interventions that can require synthetic patches or conduits [2,3]. Our lab has pioneered a biodegradable tissue-engineered vascular graft (TEVG) for use in these conditions that is composed of polyglycolic acid (PGA) fibers that are knitted into a tube and coated with a 50:50 copolymer of polycaprolactone and polylactic acid (PCLA). In contrast to typical synthetic conduits, tissue engineered scaffolds offer the potential for growth in this pediatric population, while also mitigating some of the concerning complications related to traditional vascular prostheses such as infection and chronic inflammation. The TEVG was applied in a first-in-man clinical trial in 25 children undergoing an extracardiac Fontan procedure [4,5]. Results from this trial identified that the grafts demonstrated growth, but there was a 30% incidence of stenosis and half of these patients required transcatheter interventions.

Currently, these grafts are followed in a similar fashion to other vascular conduits used in the surgical reconstruction of congenital cardiac anomalies, which typically includes routine echocardiographic imaging. If required, more invasive measures, such as computed tomography (CT), magnetic resonance imaging (MRI), angiography, or intravascular ultrasound (IVUS) may be used to evaluate complications associated with the TEVG, such as the development of stenosis or aneurysmal dilation. While these imaging modalities have been used to characterize traditional synthetic grafts in both animals and humans, only echocardiogram, MRI, pressure measurements, and angiography have routinely been used within the patients receiving TEVGs. One limitation of echocardiogram and angiography is the inability to provide definitive analysis of the vessel wall and the need to identify the lumen based on either blood flow or administration of a contrast agent. However, angiography remains the clinical gold standard for determination of TEVG stenosis and the requirement for endovascular intervention.

IVUS has more recently been utilized in a wide variety of intravascular conditions. It is most frequently described in the adult population with placement of stents for the treatment of aneurysms or atherosclerosis [6–10]. The advantages of this technique are a limitation of contrast use, a 2-dimensional view of the vessel lumen, an ability to determine true from false lumens, and accurate assessment of stent apposition to the luminal wall [11–13]. Intravascular pathology, such as thrombus, can also be more closely evaluated than with angiography, which may only show luminal occlusion or a partial filling defect [14]. In addition, IVUS can be used to evaluate pathology within the walls of vessels using radiofrequency backscatter analysis or virtual histology in processes such as coronary artery

disease or peripheral vascular disease [13,15–18]. Unlike angiography, IVUS can be used to evaluate early plaque development and remodeling of this pathology, identifying the vulnerability of these plaques based on their imaging characteristics [19,20]. Additionally, histology can correlate directly with IVUS, identifying necrotic, lipidic, fibrotic, and calcific areas within vessel walls [15–17,21].

The use of IVUS in pediatric conditions is less thoroughly described. However, it has been used to evaluate children with pulmonary hypertension, valvular disease, pulmonary artery stenosis, coarctation of the aorta, and Kawasaki disease. Specifically, findings of medial hypertrophy and intimal hyperplasia have been correlated with increased pulmonary hypertension [22–25]. IVUS has also been used in a fashion similar to that in adults in examining the placement of intravascular stents [6,26]. These conditions share a commonality in that they all lead to vessel wall changes, suggesting that the use of IVUS in patients receiving TEVGs would allow surveillance of growth, remodeling, and the development of pathologic lesions within the walls of the evolving neovessel, which would surpass the capabilities of traditional angiographic imaging. These changes in vessel morphology have been previously characterized histologically in both murine and ovine models, but association with IVUS could provide a potentially useful tool for monitoring TEVGs in the clinical setting. Therefore, the purpose of this study is to examine the correlation between histology and IVUS imaging and to evaluate the agreement in measures of vessel morphology between IVUS and angiography using an ovine model of TEVG implantation.

2. Methods

2.1. Animal Care/Ethics Statement

Nationwide Children's Hospital's (Columbus, OH) Institutional Animal Care and Use Committee reviewed and approved the protocol (AR13-00079). Representatives of the Animal Care staff monitored all animals both intra-operatively and during the post-operative course. Care was in accordance with humane standards published by the Public Health Service, National Institutes of Health (Bethesda, MD) in the Care and Use of Laboratory Animals (2011), as well as USDA regulations outlined in the Animal Welfare Act.

2.2. Scaffold Fabrication (Figure 1a, b)

TEVG scaffolds (12–20 mm inner diameter, 1.0 mm wall thickness, 13 cm length) were provided by Gunze Ltd. (Tokyo, Japan). Scaffolds were fabricated from seamless tubes of knitted poly-glycolic acid (PGA) fibers coated with a 50:50 copolymer solution of polycaprolactone and polylactic acid (PCLA) following GMP protocols as previously described [4]. The scaffolds used in this study are identical to those used in our current clinical trial (FDA IDE 14127).

2.3. Bone Marrow Harvest

4 of 8 sheep underwent bone marrow harvest for graft seeding. On the day before (n=2) or the morning of surgery (n=2), marrow was harvested from juvenile sheep weighing between 22–28 kg. Sheep were anesthetized using ketamine (10 mg/kg) and diazepam (0.5 mg/kg)

for induction and isoflurane (1–4%) for maintenance. Animals were placed in the lateral recumbent position, and the area overlying the iliac crest was shaved and prepped in the standard sterile fashion. A 2mm incision was made over the bone and an aspiration needle was inserted. Heparinized syringes (100 units/ml) were used to aspirate 4.6 ± 0.9 ml/kg of bone marrow.

2.4. Scaffold Seeding (Figure 1c)

Following harvest, the bone marrow was processed through Ficoll density gradient separation as previously described to isolate the bone marrow mononuclear cells (BM-MNCs) [27]. In brief, the marrow is filtered through 100 μ m cell strainers to remove clot and bone spicules. A 1:1 dilution with PBS is performed and the bone marrow is layered onto Ficoll 1077 (Sigma-Aldrich, St. Louis, MO, USA). After centrifugation, the plasma and mononuclear cell layers are isolated. The mononuclear cell layer undergoes 2 washes with PBS to yield a cell pellet that is diluted in 20 mL of PBS for the purposes of seeding the graft. The cells are vacuum-seeded onto the graft and TEVG is incubated in plasma for 1 hour (n=2) or 24 hours (n=2) at 37°C, 5% CO₂ until implantation.

2.5. Implantation

The grafts are implanted in the thoracic caudal vena cava (CaVC) as previously described. For the purposes of this paper, caudal vena cava refers to the venous structure that returns blood flow to the heart from the abdominal structures and is equivalent to the inferior vena cava in humans. The TEVG is designed for use as a vascular conduit to connect the inferior vena cava to the pulmonary artery in patients with single-ventricle anomalies undergoing Fontan surgery. As the spontaneous occurrence of single-ventricle defects is rare and surgical creation of this anomaly results in high mortality, this system is chosen to replicate the low-pressure, high-flow physiology encountered within these patients. While there are some clear differences, including a lower pressure (0–10 mmHg in the sheep versus 20–30 mmHg in Fontan circulation), the anatomic position distal to the liver allows observation of similar complications from stenosis as those that would be seen clinically, such as chronic passive congestion, cirrhosis, and ascites. Additionally, the graft is exposed to similar flow, resulting in a relevant and comparable model to patients that undergo a Fontan procedure.

Animals (n=8) were placed in a lateral recumbent position on the operating table. Anesthesia was maintained with isoflurane. The right thorax was prepped and draped in the normal sterile fashion. An incision was made in the seventh intercostal space and the thoracic CaVC was isolated between the diaphragm and the right atrium. After proximal and distal control was obtained, the vessel was divided. No native vessel was removed. An unseeded (n=4) or seeded (n=4) tissue-engineered vascular graft was sewn in place using a running absorbable monofilament suture and covered with thrombin glue. The chest wall, overlying muscle, and skin layers were re-approximated with absorbable braided suture.

2.6. Interventional Imaging

Imaging was performed at 1, 3, and 6 months. Additional monitoring was performed as determined by the study team based on the animal's clinical condition and results of previous imaging.

2.6.1. Angiography—After sedation and intubation, the animals were placed in a left lateral recumbent position and the right cervical region was prepped in a sterile fashion. The right internal jugular vein was cannulated and a 9-French sheath (Terumo, Somerset, NJ) inserted. A wire and catheter were passed through the right atrium, into the caudal vena cava (CaVC), and across the graft. A 6-French Multi-track catheter (B. Braun, Bethlehem, PA) was then used to obtain an angiogram through instillation of ioversol 68% (Mallinckrodt Pharmaceuticals, Raleigh, NC). Diameters of vessels were measured at 7 points: abdominal CaVC, low CaVC, proximal anastomosis, midgraft, distal anastomosis, high CaVC, and the area of most severe stenosis (Figure 1d).

2.6.2. Intravascular Ultrasound (IVUS)—A 0.035 digital IVUS catheter (Volcano, San Diego, CA) was advanced past the graft. This was used to obtain images at the same 7 points measured during angiography. Longitudinal images were obtained without the use of a sled. Longitudinal images were obtained without the use of a sled. This approach generates longitudinal images that are not representative of the graft length. These were then analyzed using proprietary imaging software to obtain a cross-sectional luminal area (Figure 1e).

2.6.3. Balloon Angioplasty—Balloon angioplasty was performed based on clinical discretion.

2.7. Animal Euthanasia

At the study end point or as clinically indicated, animals were deeply sedated with ketamine (20 mg/kg) and diazepam (0.02–0.08 mg/kg), followed by induction of secondary pneumothorax. A complete autopsy was performed at time of TEVG explantation.

2.8. Tissue Processing, Histology, and Immunofluorescent Staining

TEVG explants were fixed in 10% neutral buffered formalin at 4°C, trimmed, dehydrated, and embedded in paraffin. Tissue samples, which were 4–5µm thick serial sections, were prepared and stained with hematoxylin and eosin (H&E) and Masson's Trichrome. Immunofluorescent (IF) stains were performed to identify α-smooth muscle actin (α-SMA) and von Willebrand's Factor (vWF) positive cells in the TEVG neotissue. Briefly, sections were deparaffinized, rehydrated, and antigens retrieved via the citrate buffer method (pH 6.0, 90°C). Sections were blocked for non-specific antibody binding (3% normal goat serum) and incubated overnight at 4°C with an antibody cocktail of mouse anti-human α-SMA (1:100 Dako) and rabbit anti-human vWF (1:200, Dako) which both cross-react with the ovine antigens. Antibody binding was detected by subsequent incubation with goat-anti-mouse Alexa-Fluor® 647 (1:300 Life Technologies) and goat-anti-rabbit Alexa-Fluor® 488 (1:300, Life Technologies) followed by nuclear counterstaining with DAPI (Life Technologies). Photomicrographs were acquired with a Zeiss Axio Observer Z.1 microscope with Zeiss AxioCam 503 (dark field) and 105 (bright field) digital cameras. Exposure time was informed by the appropriate negative controls.

2.9. Statistical Analysis

Lumen diameter measurements from angiography were compared to lumen diameters calculated from IVUS luminal area measurements ($Diameter = \frac{Area}{\pi}$). Similarly, lumen area

measurements from IVUS were compared to lumen areas calculated from angiographic

luminal diameter measurements $\left(Area = \pi \left(\frac{Diameter}{2} \right)^2 \right)$. The degree of TEVG stenosis (%) at each time point was calculated by dividing the change in angiographic lumen diameter or IVUS lumen area by the corresponding implantation measurement. IVUS and angiographic data were considered paired if they were derived from the same level of the TEVG or native CaVC at the same time point. Statistical analyses were performed using GraphPad Prism 7 (GraphPad Software, Inc., CA). Type I error was controlled at $\alpha = 0.05$ and two-sided p values < 0.05 were considered statistically significant. Associations among variables were evaluated using Pearson correlation coefficients, and significantly correlated variables were further subjected to Bland-Altman agreement analysis.

3. Results

3.1. Animal Outcomes

A total of 8 animals underwent implantation (4 unseeded, 4 seeded). Of these animals 75% (n=6) did not reach the end point of 6 months. *In vivo* complications included stenosis (n=4), graft dilation (n=1), thrombosis (n=4), wound infection (n=1), pericardial tamponade (n=1), pneumonia (n=1), and renal insufficiency (n=3).

3.2. IVUS Characterization of Neovessel Formation

3.2.1. IVUS Characterization Over Time (Figure 2)—Intravascular ultrasound identified the presence (Figure 2a) or absence of luminal fibrin and platelet deposition at the time of implantation. There was a paucity of tissue formation within the intima, media, or adventitia of the graft. At approximately 1-month post-implantation, the neointima had developed and was lined by an endothelium and neomedia inside the lumen of the graft (represented by a hyperechoic laminar layer) (Figure 2b). The endothelial lining became more clearly defined as the tissue organized and remodeled between 3 and 6 months (Figure 2c). At 6 months, as the graft degraded, the hyperechoic polymeric layers became less clearly defined on IVUS (Figure 2d).

3.2.2. IVUS Findings over the Length of the Graft (Figure 3)—IVUS findings in the low CaVC illustrated a thin-walled vessel consistent with histologic findings. At the proximal and distal anastomosis (Figure 3b and d), thickening of the neointimal and neomedial layers was present in both the IVUS and histologic images. The hyperechoic inner layer seen on IVUS correlated with the neointima on both Masson's Trichrome and IF analyses. The neomedia was hypoechoic on IVUS and characterized by a higher density extracellular matrix (including collagen) as seen on trichrome and vascular smooth muscle cells as seen with IF. The midgraft section (Figure 3c) was noted to have a thinner layer in this area, which correlated to a thinner neointima and neomedia.

3.3. Comparison of Angiography and IVUS by Quantification of Lumen Geometry

Lumen diameter approximations from IVUS imaging were significantly correlated with angiographic measurements (Fig. 4a, Pearson $r = 0.8669$, $p < 0.0001$). Conversely, calculated luminal area derived from angiographic imaging were not significantly correlated

with IVUS area measurement (Fig. 4b, Pearson $r = 0.2886$, $p = 0.0832$). When the degree of stenosis at each time point was calculated based on the corresponding measurements from the implant, a significant correlation between IVUS and angiography was discovered (Fig. 4c, Pearson $r = 0.6952$, $p < 0.0001$). Significant correlations prompted quantification of the agreement between IVUS and angiography by Bland-Altman analysis to investigate whether these modalities could be used interchangeably for the determination of lumen diameter and degree of stenosis. With regards to approximation of lumen diameter, the mean difference between IVUS and angiography was 0.739 ± 2.055 mm (Fig. 4d, 95% Confidence Interval: 0.077 to 1.401 mm, 95% Limits of Agreement: -3.288 to 4.767 mm). While quantification of stenosis was significantly correlated between the two modalities, the agreement between IVUS and angiography was not robust (Fig. 4e, Bias: $1.863 \pm 36.84\%$, 95% Confidence Interval -5.749 to 9.475% , 95% Limits of Agreement: -70.35 to 74.08%).

3.4. Characterization of Anomalies

3.4.1. Strengths of IVUS—Characteristics of the graft that were clearly identifiable on angiography included anastomotic narrowing, stenosis, dilation, and compression (Figure 5). These problems are easily identified with IVUS, and correlated with angiographic imaging. While IVUS demonstrated these changes, it may be less likely to identify some of the sequelae of the pathology, such as the collateralization visualized with severe stenosis (Figure 5b). However, angiography may not be able to readily identify narrowing or other pathology that occurs due to its single axis view. An example of this pathology is seen in Figure 5d, which shows the narrowing of the vessel in the anterior/posterior direction as it traverses diaphragm. This anomaly may be less readily identified with angiography when only one view is acquired. In addition, IVUS can specifically identify and characterize intimal depositions, such as fibrin and platelet deposition (Figure 6a). In this comparison, the relationship between the graft and fibrin and platelet deposition was more specifically characterized by IVUS than angiography where only the luminal narrowing could be identified. Following balloon dilation, IVUS was also able to identify therapeutic tears (Figure 6b). While this change was apparent on angiography, its spatial relationship to the graft, neotissue, and surrounding tissues could not be evaluated. A tear through the neotissue and graft, as well as containment within the surrounding tissue was clearly identifiable both on IVUS and histology (Figure 6b).

3.4.2. Weaknesses of IVUS—Histopathologic assessment offers a greater subgross resolution of neotissue formation, extracellular matrix composition, and polymeric graft degradation in comparison to IVUS. Important findings specific to histology, not identified by IVUS include mineralization or microcalcification. Additionally, with IVUS, as the graft matured and the scaffold degraded, the tissue layers were less readily discerned. This finding is highlighted at the 180 day time point shown in Figure 2d.

4. Discussion

Currently, patients who receive TEVGs are closely monitored clinically and with serial echocardiogram. If indicated, these children may also undergo MRI or angiography. While these studies can identify dilation, stenosis, or changes in intravascular flow, they are less

informative regarding the changes that are occurring within the wall of the neovessel. Data interpretation generated by these studies is limited and correlation with histologic findings is in the clinical setting is also limited. Our previous work with murine models has characterized the natural time course of neotissue formation that occur following TEVG implantation, as it progresses to a neovessel that has similar characteristics to that of native vein [28,29]. Time-course studies over 6 months have characterized both *in vivo* changes by ultrasound, as well as *ex vivo* with histology and biomechanical testing. As with echocardiography in the clinic, this technique is useful to determine patency versus stenosis, and provides some limited information regarding flow. However, histology continues to provide the majority of data for analysis of changes within the vessel wall in preclinical settings.

While histologic analysis has been the gold standard in both our murine and ovine models, IVUS offers the potential to evaluate the progression of changes within our ovine model over time *in vivo* within the same animals, obviating the need for serial explantation. The results described in this study highlight the capacity of IVUS to follow changes that occur within the wall of the graft over time, including acute and chronic responses to angioplasty. In comparison to histology, the progressive changes in the endothelium, underlying neotissue, and graft degradation clearly correlate with IVUS findings. In the current study, IVUS is able to demonstrate all relevant graft components at early time points, but by 180 days these findings are no longer present. These results suggest that IVUS will be able to monitor not only neotissue development, but the degradation of the graft over time.

The IVUS analysis over the length of the graft also provides support for the use of the modality for graft analysis. Our previous work has shown that the cells seeded onto the graft modulate the innate humoral and cell-mediated inflammatory response, stimulating ingrowth of cells from the adjacent native vessel [28]. IVUS successfully captures the differences in thickness of the neovessel wall over the length of the graft, and these changes can be correlated with histologic images. These results suggest that IVUS can be used to follow the formation of an endothelial lining, the development of a neomedia comprised of vascular smooth muscle cells, and the eventual maturation of the neotissue. As IVUS correlates with angiographic and histologic findings, further evaluation of this technique within the ovine model may allow characterization of changes within these tissue planes that could be used to identify grafts that will progress to stenosis or, alternatively, stenotic segments that will resolve without need for angiographic intervention. One limitation may be the availability of this modality and clinicians that are experienced with IVUS for evaluation of this specific indication.

With regards to the capacity of IVUS to quantify vessel geometry, there was a significant correlation between lumen diameters approximated from IVUS measurements and angiographic measures of lumen diameters at the same level of the graft and there existed a clear agreement between these two modalities. This indicates that IVUS is able to determine the measurements obtained by both the one-dimensional angiography while also providing a two-dimensional area, which one-plane angiography was unable to approximate. We propose that IVUS is the best modality to assess the asymmetrical lumens of the ovine vena cava at physiological pressure, and is therefore better suited to evaluate TEVG growth and

remodeling in this model. Further, traditional angiography cannot be used with confidence to approximate a luminal area when compared to IVUS. Graft lumen area may better approximate IVUS measurements when 3D angiography is employed, but this requires a significant increase in both contrast and radiation. Taken along with the advantages of IVUS evaluation of vascular neotissue described herein, the lack of strong agreement with regards to percent stenosis determinations between IVUS and angiography further supports the use of IVUS for longitudinal surveillance of TEVGs *in vivo*. Clinically, the ability of IVUS to obtain an accurate luminal area and diameter at physiologic conditions should allow a significant reduction of contrast and radiation exposure, an especially important concern in the pediatric population undergoing multiple cardiac catheterizations. Further research in our animal model and in the clinic will be needed to further assess this benefit.

The ability of IVUS to identify abnormalities has previously been documented including its ability to demonstrate vessel interfaces with stent use [6,26] and to clearly identify false lumens [11]. These strengths are similar to those we have characterized above when evaluating TEVGs. Examples include the ability to monitor the degradation of the graft and the ability to identify therapeutic tears and the resultant contained fluid collections in the surrounding tissue. While IVUS has many strengths, there are several weaknesses when compared to histologic techniques. While previous studies have been able to view calcific plaques within the vessel wall [18], two of the specimens demonstrated microcalcifications that were not identified on either angiography or IVUS (data not shown). Additionally, once the graft degrades and tissue maturation has occurred at 6 months, tissue planes are not as easily identified on IVUS. However, the findings at this point are similar to those in the native vena cava, and are confirmed by histologic similarity between native and tissue-engineered vena cava at late time points. While commonly utilized imaging techniques such as angiography, CT, MRI, or echocardiogram are unable to visualize these tissue planes, optical coherence tomography (OCT) has been used experimentally to evaluate coronary plaques. This technique uses infrared light and has a high resolution of 10–15µm, but has a limited penetration into tissue (1.5 mm) [30]. While this technique might provide a more detailed picture of tissue closest to the luminal surface, the depth of penetration would limit its application for imaging of the TEVG.

Data from the current study demonstrates that serial IVUS can be used safely *in vivo* in animals that have undergone implantation of a TEVG in the thoracic vena cava. The use of this technology complements angiography and provides a more detailed examination of the neovessel wall, including neotissue development. With longitudinal studies involving the same animals, a detailed analysis of neotissue formation on the graft with associated luminal narrowing, as well as the remodeling of this tissue can be more closely monitored. Additionally, IVUS is able to identify common abnormalities from graft implantation and their spatial relationship to the graft material. Extending this technology using backscatter analysis or virtual histology would allow more detailed examination of the TEVG *in vivo*, which may inform the use of interventions such as angioplasty or stenting in the clinic. Our data suggests that the measurements obtained by IVUS agree with angiographic measurements and provide superior quantification of neovessel geometry, which could benefit patients in the clinical setting by decreasing contrast and radiation exposure. Finally,

IVUS offers the potential to follow both animal models and patients with TEVGs longitudinally, allowing direct correlative data from one subject over time.

5. Conclusion

IVUS identification of neotissue and graft lumen morphometry correlates closely with both angiography and histology, and can provide further analysis of the maturation of tissue-engineered vascular grafts.

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Abbreviations

°C	degrees Celsius
CI	confidence interval
α-SMA	alpha smooth muscle actin
CaVC	caudal vena cava
CT	computed tomography
IF	immunofluorescence
IVUS	Intravascular Ultrasound
kg	kilogram
MRI	magnetic resonance imaging
mg	milligram
mm	millimeter
μm	micrometer
PBS	phosphate buffered saline
PCLA	polycaprolactone and polylactic acid
PGA	polyglycolic acid

TEVG	Tissue Engineered Vascular Graft
vWF	von Willebrand's factor

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Figure 1. TEVG Seeding and Imaging Methods

(a, b) Tissue-engineered vascular graft prior to seeding. (c) Seeding of graft with BM-MNC. (d) A representative angiographic image with sites of measurement; abdominal CaVC (line), low CaVC (dotted line), proximal anastomosis (arrow head), midgraft (dashed line), distal anastomosis (arrow), and high CaVC (double line). (e) Comparison of histology and IVUS showing concentric circles of probe, lumen, endothelium, neotissue, graft, and surrounding connective tissue.

Figure 2. IVUS and Histology Over Time

A comparison of representative IVUS and histochemical stains at 0 (a), 30 (b), 60–90 (c), and 180 (d) days showing the evolution of the neotissue development and graft degradation. (a, b, c, d.1) IVUS at the level of the midgraft. (a, b, c, d.2) H&E photomicrographs (2.5x) of the TEVG. (a, b, c, d.3) Photomicrographs of Masson's Trichrome stains (2.5x) of the TEVG. (a, b, c, d.4) Immunofluorescent images of TEVG immunolabelled for smooth muscle (alpha-SMA: red) and endothelium (von Willebrand's Factor: green) (10x). Neovessel layers are outlined as followed: dotted line – luminal interface; dashed line – graft polymer; solid line – adventitia; L - Lumen. IVUS and histologic images are from Day 0 are different, but correlative animals.

Figure 3. IVUS and Histology Along the Graft Length

TEVGs at measured points along the length of the graft 74 days post-implantation. The top panel demonstrates the longitudinal IVUS images with points of interest annotated during manual manipulation of the catheter; (a.1–3): proximal CaVC; (b1–3): proximal anastomosis; (c.1–3): midgraft; (d.1–3): distal anastomosis; (e.1–3): distal CaVC. (a, b, c, d. 2) As sled was not used, there is no correlation to the length of graft. Photomicrographs of TEVG over the length of the graft (Masson's Trichrome). (a, b, c, d.3): Immunofluorescent images of TEVG immunolabelled for vWF (green) and α SMA (red). Neovessel layers are outlined as followed: dotted line – lumen; dashed – line graft polymer; solid line – adventitia; asterisk – suture; L - lumen. While an endothelial lining is present along the length of the graft, differential vascular smooth muscle cell labelling along the length of the graft can be appreciated.

Figure 4. Luminal Geometry: IVUS vs. Angiographic Quantification

A significant correlation between direct measurement of lumen diameter from angiography and calculated lumen diameter from IVUS was discovered (a, Pearson $r = 0.8669$, 95% CI: 0.7549 to 0.928mm, $R^2 = 0.7515$, $p < 0.0001$). The correlation between direct measurement of lumen area from IVUS and calculated lumen area from angiography was not significant (b, Pearson $r = 0.2886$, 95% CI: -0.03903 to 0.5603mm², $R^2 = 0.08331$, $p = 0.0832$). In addition, a significant correlation between IVUS and angiography was found for determination of TEVG stenosis (c, Pearson $r = 0.6952$, 95% CI: 0.5703 to 0.7888%, $R^2 = 0.4834$, $p < 0.0001$). Negative values indicate cases of dilation, which may confirm growth of the TEVG over time. Bland-Altman agreement analysis (difference versus mean) between IVUS and angiography revealed a strong agreement between the two modalities in measurement of lumen diameter (d, Bias: 0.739 ± 2.055 mm, 95% CI: 0.077 to 1.401mm, 95% Limits of Agreement: -3.288 to 4.767mm), however, the agreement between IVUS and angiography for determination of stenosis was not as robust (e, Bias: 1.863 ± 36.84 %, 95% CI: -5.749 to 9.475%, 95% Limits of Agreement: -70.35 to 74.08%). Dashed horizontal lines identify the limits of agreement between the two modalities.

Figure 5. Abnormalities Encountered in Model: IVUS versus Angiography

Several pathologic conditions are better evaluated with imaging techniques. Narrowing is visible at anastomotic site on angiography, but can also be seen using the transverse and longitudinal images on IVUS (a.1–3). Similar results are seen with evaluation of stenosis (b. 1–3), however the IVUS images demonstrate neotissue development (arrow head) at this site. Additionally, collateralization can be seen (arrow). While rare, dilation can occur, and is equally visible with angiography and IVUS (c.1–3). External compression may or may not be seen with angiography (d.1), while the combined views of IVUS (d.2–3) allow for identification of compression as the VC traverses the diaphragm.

Figure 6. Correlation of IVUS Findings to Ex Vivo Pathology

Angiography, IVUS, and H&E comparison of fibrin and platelet deposition (a.1–3) and therapeutic tear (b.1–3) following balloon dilation. Fibrin and platelet deposition can be visualized with both imaging formats and by histology (Hematoxylin and eosin; 5x). The shadow of the contained leak can be seen on angiography, but the relationship to the neotissue and graft material is better appreciated with IVUS and histology. The line on angiography denotes correlative position of IVUS and histologic images.