Dynamic Assessment of the Endothelialization of Tissue-Engineered Blood Vessels Using an Optical Coherence Tomography Catheter-Based Fluorescence Imaging System

Abhijit Achyut Gurjarpadhye, PhD,¹ Matthew R. DeWitt, BS,¹ Yong Xu, PhD,² Ge Wang, PhD,³ Marissa Nichole Rylander, PhD,⁴ and Christopher G. Rylander, PhD⁴

Background: Lumen endothelialization of bioengineered vascular scaffolds is essential to maintain small-diameter graft patency and prevent thrombosis postimplantation. Unfortunately, nondestructive imaging methods to visualize this dynamic process are lacking, thus slowing development and clinical translation of these potential tissue-engineering approaches. To meet this need, a fluorescence imaging system utilizing a commercial optical coherence tomography (OCT) catheter was designed to visualize graft endothelialization.

Methods: C7 DragonFly™ intravascular OCT catheter was used as a channel for delivery and collection of excitation and emission spectra. Poly-DL-lactide (PDLLA) electrospun scaffolds were seeded with endothelial cells (ECs). Seeded cells were exposed to Calcein AM before imaging, causing the living cells to emit green fluorescence in response to blue laser. By positioning the catheter tip precisely over a specimen using high-fidelity electromechanical components, small regions of the specimen were excited selectively. The resulting fluorescence intensities were mapped on a two-dimensional digital grid to generate spatial distribution of fluorophores at single-cell-level resolution. Fluorescence imaging of endothelialization on glass and PDLLA scaffolds was performed using the OCT catheter-based imaging system as well as with a commercial fluorescence microscope. Cell coverage area was calculated for both image sets for quantitative comparison of imaging techniques. Tubular PDLLA scaffolds were maintained in a bioreactor on seeding with ECs, and endothelialization was monitored over 5 days using the OCT catheter-based imaging system.

Results: No significant difference was observed in images obtained using our imaging system to those acquired with the fluorescence microscope. Cell area coverage calculated using the images yielded similar values. Nondestructive imaging of endothelialization on tubular scaffolds showed cell proliferation with cell coverage area increasing from $15 \pm 4\%$ to $89 \pm 6\%$ over 5 days.

Conclusion: In this study, we showed the capability of an OCT catheter-based imaging system to obtain single-cell resolution and to quantify endothelialization in tubular electrospun scaffolds. We also compared the resulting images with traditional microscopy, showing high fidelity in image capability. This imaging system, used in conjunction with OCT, could potentially be a powerful tool for *in vitro* optimization of scaffold cellularization, ensuring long-term graft patency postimplantation.

Introduction

CARDIOVASCULAR DISEASE is a leading cause of morbidity and mortality worldwide. Each year, more than 200,000 coronary artery bypass graft procedures are performed in the United States.¹ Segments of autologous vessels, such as saphenous veins or radial arteries, have been the gold standard for revascularization procedures.² However, vessel

autografts are limited in supply and configuration and can lead to donor site morbidity, while grafts from unrelated human donors (allografts) and non-human sources (xenografts) are subject to strong immunogenic response.^{3–5} Vascular grafts using biocompatible materials such as expanded polytetrafluoroethylene (ePTFE) or Dacron (polyethylene terephthalate fiber) are useful for large-diameter (>6 mm) bypass conduits, but they are generally unsatisfactory for

¹School of Biomedical Engineering and Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

²Department of Electrical and Computer Engineering, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

³Biomedical Imaging Cluster, Rensselaer Polytechnic Institute, Troy, New York.

⁴Department of Mechanical Engineering, The University of Texas at Austin, Austin, Texas.

small-diameter grafts, due to the high frequency of thrombosis, stenosis, occlusion, and infection.^{6,7} Recent approaches to meet the demand for small-diameter vascular grafts involve tissue-engineering blood vessels using scaffolds made from biodegradable materials, which are seeded with healthy autologous cells.^{3,8–10}

Numerous techniques have been proposed as well as implemented¹¹ for developing tissue-engineered vascular grafts. While the cell types used for creating the vessel walls may vary, the lumen of these vessels is always seeded with endothelial cells (ECs), whose proliferation leads to establishment of the endothelium. Although ECs only form a monolayer of protection between the vessel wall comprising smooth muscle cells (SMC), and the blood that the vessel carries, this barrier functions as an active antithrombotic surface and is crucial for regulating the flow of blood cells as well as biologically active molecules.¹² In addition to limiting the SMC proliferation to avoid neointimal hyperplasia, the endothelium also functions as a first responder to vessel wounds by becoming procoagulant, vasoconstrictive, and proinflammatory.¹³ Therefore, it is important for a bioengineered vascular graft to have a confluent and mature monolayer of EC lining before it can be considered transplantable. Apart from chemical modification of a scaffold, confluent endothelialization of the luminal surface must succeed to achieve nonthrombogenicity, as a thrombus formation postimplantation could lead to graft failure.

It is crucial to monitor the progress of bioengineered vascular grafts *in vitro*, specifically those with small diameter (<6 mm), to ensure their structural integrity and proper development. More importantly, monitoring the EC proliferation and formation of confluent monolayer *in vitro* is a vital part of creating a viable, implantable vascular graft and must be taken into account when developing vascular grafts within bioreactors. However, monitoring endothelialization in these grafts in a bioreactor setting can be very challenging. Current technology provides only limited means to assess the tissue development either *in vitro* or *in vivo*. Most available assessment methods are static, requiring the sacrifice at fixed time points of individual tissue constructs or experimental animals, necessitating high sample counts to follow graft endothelialization over these multiple time points. Therefore, there is a great need for a system to monitor the process of tissue regeneration, specifically graft endothelialization, noninvasively and nondestructively at a high resolution.

Optical coherence tomography (OCT) is a light-based, high-resolution imaging modality that provides structural details of a specimen nondestructively. Several groups have demonstrated the use of OCT in imaging native physiological and pathological blood vessels^{14,15} or bioengineered blood vessel mimics,¹⁶ taking advantage of its high axial resolution and image contrast. In recent years, intravascular catheters have been developed for intravascular OCT imaging to monitor the state of coronary as well as peripheral arteries and to evaluate microstructure of plaques.^{17,18} OCT can be used to monitor the development of a bioengineered vascular graft through high-resolution imaging as well as through extraction of its geometrical and optical properties, and thus can aid with improving the design of engineered tissues. We recently demonstrated the ability of OCT to image a developing tissue-engineered blood vessel within a

bioreactor using either free-space OCT imaging or a vascular OCT endoscope.¹⁹ We were able to observe changes in the wall thickness and optical properties of the vessel over 4 weeks. However, one of the major limitations of OCT is its inability to extract cellular and molecular signatures specifically and sensitively. In addition, the monolayer of the ECs forming the intima of blood vessels is very thin—typically <10 μm —and is therefore unable to be resolved by OCT. The ability to monitor dynamic cellular processes such as cell proliferation and migration in addition to the structural and optical properties of tissue engineered blood vessels will greatly enhance our potential to oversee the maturation of bioengineered vascular grafts in a bioreactor, resulting in optimized blood vessels before implantation.

In this study, we therefore demonstrated that a commercially available intravascular OCT catheter could be used for fluorescent imaging of bioengineered vascular grafts. We utilized the OCT catheter for visualizing distribution of ECs seeded on the lumen of scaffolds made from biocompatible material, and monitored cellular proliferation, leading to endothelialization of scaffold lumen over 5 days in a non-destructive manner. A nondestructive imaging tool such as the system described in this study can be useful for the development of novel tissue engineering constructs in the future, leading to optimized therapeutic options for small-diameter graft implantations.

Materials and Methods

Fluorescence imaging with C7 DragonFly™ OCT catheter

Fluorescence imaging was performed using a commercial OCT catheter (C7 DragonFly™ intravascular imaging catheter; St. Jude Medical, Inc.) as shown in Figure 1. The proximal end of the catheter was connected to a blue ($\lambda=473\text{ nm}$) excitation laser source through a fiber-optic rotary joint (FORJ) (MJP-131-28-FA; Princetel, Inc.). The catheter contains a single-mode fiber, which acts as a few-mode fiber for the blue excitation light. The tip of the catheter contains lens formed by fusion splicing segments of coreless fiber to graded-index fiber.²⁰ The outer diameter of the catheter tip measures 0.9 mm. At the excitation wavelength, the catheter has a working distance of $\sim 1\text{ mm}$. The $1/e$ waist size of the excitation beam at the focal distance of 1 mm from the catheter tip was measured to be $\sim 25\text{ }\mu\text{m}$ using the knife-edge method.²¹ Although the FORJ was designed and optimized for 1310 nm wavelength, the excitation beam spot profile remained unchanged at the focal point of the catheter over a full 360° rotation of the FORJ rotor. This rotational uniformity ensured that the fluorescence quality of the fluorescence signal is not altered by the angular position of the rotary joint. The distal end, which includes the focusing optics, is placed over the region of interest (ROI) with the excitation beam orthogonal to the surface of the specimen. In the case of tubular scaffolds mounted inside the bioreactor, the catheter was inserted through a port in the bioreactor and was positioned over the ROI on the scaffold. During imaging, the catheter delivers blue excitation light and collects fluorescent emission from fluorophores, which is then filtered by the dichroic mirror (DMLP505; Thorlabs, Inc.), and the bandpass filter (CW=525 nm, BW=39 nm, MF525-39; Thorlabs, Inc.). A plano-convex lens ($f=65\text{ mm}$; Thorlabs, Inc.) was

FIG. 1. Schematic of the setup used for fluorescence imaging using LightLab™ C7 DragonFly™ optical coherence tomography (OCT) catheter. The position of the catheter tip is precisely controlled over the region of interest (ROI) of the specimen using the stepper motor and the linear translation stage. The catheter serves as a delivery and collection channel for both the *blue* excitation and *green* emission spectra. The *green* fluorescence emitted by fluorophores in response to *blue* laser excitation is recorded by the EMCCD camera. Color images available online at www.liebertpub.com/tec

used to focus the filtered emission light on the EMCCD camera (iXon Ultra 897; Andor Technologies, Inc.). The power of the excitation laser beam at the stator end of the FORJ was measured to be $470\ \mu\text{W}$ and that at the tip of the catheter was $300\ \mu\text{W}$, providing the light delivery efficiency through the catheter and the FORJ together to be $\sim 64\%$. A high-precision stepper motor (T21NRLH-LDN-NS-00; National Instruments) was used to rotate the tip of the catheter to provide scanning in one dimension. A linear translation stage (Newport UTM100CC.1; Newport Corp.) was used to precisely move the specimen orthogonally to the rotational scan, thus providing scanning in a second dimension. The acquired fluorescent emission intensities are mapped on a digital grid to generate two-dimensional distribution of fluorescent cells over the scanned area of the specimen.

Scaffold fabrication

The scaffolds used in this study were fabricated by electrospinning bioabsorbable poly-DL-lactide (PDLLA).²² Electrospinning was conducted using a typical system, which includes a high voltage power supply (Spellman High Voltage), a syringe pump (Medfusion 2001; Medex, Inc.) and a rotating mandrel (Custom Design & Fabrication; 4.8 mm diameter and 12.5 mm length) with a rotation rate of $\sim 60\text{ rpm}$. A 22% w/v PDLLA ($M_w = 80,000\text{ g/mol}$) (Sur Modics Pharmaceuticals) solution was prepared in a 3:1 ratio of tetrahydrofuran:dimethylformamide (Fisher Scientific) under gentle stirring for 4 h. A 5 mL syringe with an 18 G blunt tip needle was filled with the solution and delivered at a flow rate of 5 mL/h. A 16 kV potential was applied between the needle tip and an aluminum mandrel. The distance between the needle and the mandrel was set at 20 cm. Electrospun scaffolds with an approximate thickness of $300\ \mu\text{m}$ were fabricated and placed in a desiccator overnight to remove the residual solvent.

Phantoms for imaging system validation

Green fluorescent microspheres ($30\ \mu\text{m}$ diameter) (Thermo Fisher Scientific, Inc.) were used as fluorescent phantoms to validate the OCT catheter-based fluorescence imaging system. The microspheres were stored in DI-water suspension. A few drops of the suspension were placed on a glass slide, which was then set on a hot plate at 40°C for $\sim 5\text{ min}$. Evaporation of water due to heat ensured that the microspheres did not form aggregates and were distributed uniformly on the slide. Microspheres were also embedded within the PDLLA scaffold wall to mimic cellularization of the scaffolds with ECs. To accomplish this, a thin layer of PDLLA scaffold was spun on the mandrel, followed by approximately even placement of a few drops of microsphere-DI water suspension before continuing the spinning process to form the scaffold with $\sim 300\ \mu\text{m}$ wall thickness. Embedding the microspheres in this way ensured that they are located close to the lumen and do not get displaced when the lumen is filled with media.

Cell culture and cell seeding

A human time immortalized microvascular EC line (American Type Culture Collection) between passages 32 and 35 was used for all experiments. The ECs were maintained in an incubator at 37°C , 5% CO_2 and cultured in endothelial growth medium-2 (EGM-2) media with necessary supplements (Lonza Biomedical).

To demonstrate the ability to fluorescently image on a nonfluorescent background, ECs were seeded on glass slides, as glass is not autofluorescent and thus minimizes background noise (Fig. 2a). The microscope glass slides were cut $\sim 1'' \times 1''$, sterilized with 70% ethanol, and washed with sterile phosphate-buffered saline (PBS) to remove residual ethanol. The slides were then kept immersed in EGM-2 media for 24 h to enhance cell adhesion. The cells were

FIG. 2. Schematics of the configurations used for fluorescence imaging of (a) endothelial cells (ECs) on a glass slide, (b) ECs on a flat poly-DL-lactide (PDLLA) scaffold, and (c) ECs on the lumen of tubular PDLLA scaffold. Subset in (c) shows the schematic of the PDLLA scaffold mounted within the bioreactor. The fluorinated ethylene propylene (FEP) tubes provide mechanical support for the scaffold, whereas the silicone tubes link the tissue-engineered blood vessel (TEBV) with the dispensing needles of the bioreactor. Color images available online at www.liebertpub.com/tec

seeded on the slides at 8000 cells/cm^2 , and the specimens were then individually stored in EGM-2 media in the incubator. A total of nine such specimens were used for validation studies involving cells. Imaging of these specimens was performed on days 1, 3, and 5 postseeding using three specimens at each time point. In addition, three separate random sites were scanned on each specimen. Approximately similar regions were also scanned using the fluorescence microscope (Leica DMI6000-B; Leica Microsystems) for control imaging, which were later compared with the remapped images resulting from our catheter-based imaging system. The media was replaced every 24 h.

PDLLA scaffolds were used for additional cellular imaging experiments to investigate the effect of autofluorescent background noise. Flat pieces of PDLLA scaffolds ($1 \times 1 \text{ cm}$) were used for these experiments, as such geometry allows for control images to be taken using the fluorescence microscope. The scaffolds were glued to microscope glass slides using medical adhesive (Silastic® medical adhesive type A; Dow Corning Corp.) (Fig. 2b). After 24 h of adhesive curing, the scaffolds were sterilized in 70% ethanol followed by two washes with sterile PBS to remove residual ethanol. Next, the scaffolds were allowed to immerse in EGM-2 media for 24 h to enhance adhesion of ECs. The cells were seeded on the scaffolds at 8000 cells/cm^2 , and the specimens were then individually stored in EGM-2 media in the incubator. Three scaffolds were scanned at each time point, at days 1, 3, and 5 postseeding, using our catheter-based imaging system to

monitor endothelialization. Within each specimen, three random isolated regions were imaged. Control images of the same regions were also obtained with the Leica fluorescence microscope. The media was replaced every 24 h.

A tubular bioreactor as described earlier¹⁹ was used for EC imaging of tubular PDLLA scaffolds. Short segments ($\sim 1 \text{ cm}$) of thin fluorinated ethylene propylene (FEP) tube (OD=3 mm) were glued to the two ends of the PDLLA scaffolds using medical-grade silicone adhesive (Silastic medical adhesive type A; Dow Corning Corp.). A small segment ($\sim 1 \text{ cm}$) of silicone tube (ID=3.5 mm, wall thickness=0.8 mm) was used to create a bridge between the dispensing needle and the FEP tube. PDLLA scaffolds were sterilized and prepared for seeding in the same manner as previously described. After 24-h immersion in EGM-2 media, the tubular scaffolds were seeded with ECs. The cell solution was injected into the scaffold lumen, and the scaffolds were rotated at 1 rpm for 2 h to provide even seeding of ECs along the lumen. The scaffolds were then maintained stationary and immersed in media for 24 h and then mounted into the bioreactors (Fig. 2c). The bioreactors were filled with media, which was replaced once every 24 h. Three such bioreactors were maintained over 5 days and were imaged at days 1, 3, and 5 postseeding. No control images were obtained for tubular scaffold imaging.

Cell staining

Calcein AM (CaAM) (C3100MP; Life Technologies Corp.) is a nontoxic cell-permeant nonfluorescent dye that is converted to green-fluorescent calcein when enzymatically changed in live cells.²³ Thirty minutes before imaging, the specimens seeded with ECs were first washed with PBS to remove any dead cells. CaAM was reconstituted with dimethyl sulfoxide and added to phenol-red-free media ($2 \mu\text{L/mL}$ of media). Next, the specimens were incubated at 37°C in media containing CaAM for 30 min. The media containing dye was aspirated, and the specimens were washed again twice with PBS and placed in EGM-2 media.

Image acquisition and remapping

The image acquisition methodology and remapping algorithm is explained graphically in Figure 3. Briefly, the catheter is placed over the ROI such that the excitation beam is orthogonal to the surface of the specimen. The catheter scans the specimen in a stepwise manner, laterally as well as longitudinally, using a stepper motor and translation stage, as shown in Figure 1. The region to be scanned is divided into a pixel grid, with each pixel approximately the size of $4 \times 4 \mu\text{m}$. At each pixel location, the catheter delivers blue excitation light and captures green fluorescence emission resulting from the fluorophore (Fig. 3a). The catheter is rotated for lateral scanning over 40° (20° on either side of the initial orthogonal position of the beam) in 180 steps using a high-precision stepper motor (T21NRLH-LDN-NS-00; National Instruments). At the end of each lateral scan, the specimen is translated away from the catheter tip by $4 \mu\text{m}$ using a linear translation stage (Newport UTM100CC.1; Newport Corp.) (Fig. 3b). Thus, the pixel grid representing the ROI will contain pixels measuring $4 \times 4 \mu\text{m}$ each. For flat specimens, scanning of areas located further away from the longitudinal axis may result in slightly skewed pixels.

FIG. 3. Schematic presentation of fluorescence image acquisition and intensity remapping methodology. (a) Shows the OCT catheter delivering *blue* excitation light to the fluorophores, such as microsphere or Calcein AM-stained ECs. The resulting fluorescence is captured by the catheter. (b) The region of interest (ROI) of the specimen is divided into certain number of slices, each $\sim 4\ \mu\text{m}$ apart, and each slice is scanned laterally over 40° in 180 steps. (c) Remapping pixel intensities thus acquired yields a two-dimensional distribution of fluorescence over the ROI of the scanned specimen. Color images available online at www.liebertpub.com/tec

However, this effect may be considered negligible for very small scan angles. The EMCCD camera captures a frame at each position of the catheter during the acquisition process. Pixel intensities within each frame are summed to obtain a single fluorescence intensity value. This value is then assigned to a specific pixel of the digital grid representing the precise location of the catheter tip over the ROI (Fig. 3c). The image acquisition and processing algorithm was written in LabVIEW[®] (National Instruments Corp.). The exposure time at each pixel was adjusted depending on the sample (from 0.06 to 0.2 s), and the acquisition time for a $700 \times 700\ \mu\text{m}$ region varied accordingly from ~ 1 to 3.5 h. Signal-to-noise ratio (SNR) was determined as a ratio of the intensity of signal obtained from a fluorophore, such as fluorescent microsphere or cells to the background signal obtained from the substrate only.

Quantification of cell coverage area and statistical analysis

Specimens such as glass slides or PDLLA scaffolds seeded with ECs were imaged on days 1, 3, and 5 postseeding using our imaging system. Area of the scanned portion of the specimen occupied by ECs was assessed to quantify endothelialization. More specifically, a constant intensity threshold was applied to all the images obtained to convert them to binary images, from which fractional area occupied

by cells is readily quantified. A custom MATLAB[™] program was developed to process all the images for cell coverage area quantification.

The results of cell coverage were expressed as percent mean $\pm$ SD. Two-tailed Student's *t*-test was used to test the difference in cell coverage area measurements obtained using control and remapped images. The difference was considered statistically significant when $p < .05$.

Results

Fluorescence imaging using microsphere-based phantoms

A glass slide with a sparse distribution of $30\ \mu\text{m}$ -diameter green microspheres was scanned using the OCT catheter-based fluorescence imaging system. Figure 4a suggests that the remapped image obtained using our OCT catheter-based system matches closely with the control image obtained using Leica fluorescence microscope. This result validated the image acquisition as well as the image-remapping algorithm. In addition, the scanning parameters such as the lateral and longitudinal step size produce an image that offers acceptable resolution. Imaging system and scanning parameters were also tested using a phantom created by embedding the $30\ \mu\text{m}$ -diameter green microspheres within the PDLLA scaffold near the luminal surface. Scanning this phantom yielded a remapped image that was comparable to the control image obtained using the Leica fluorescence microscope (Fig. 4b). The exposure time at each pixel was

FIG. 4. Fluorescence imaging using microsphere-based phantoms. Imaging using Leica fluorescence microscope and using OCT catheter-based imaging system, of $30\text{-}\mu\text{m}$ green microspheres (a) on glass slide, and (b) within flat PDLLA scaffold. Color images available online at www.liebertpub.com/tec

0.06 s, and the acquisition time for a $700 \times 700 \mu\text{m}$ region was ~ 1 h. The SNR observed during OCT catheter-based imaging of microspheres was typically between 8 and 10.

Imaging and assessment of endothelialization on glass slide

The ECs seeded on the glass slides were imaged at three different sites at each time point. OCT catheter-based fluorescence images in the panel (Fig. 5a) are comparable with those obtained using the Leica fluorescence microscope. Images acquired using the fluorescence microscope indicated that the cell coverage area increased from $7 \pm 2.2\%$ to $69.6 \pm 5.5\%$ over 5 days, whereas those acquired using our imaging system showed the cell coverage area increasing from $10.1 \pm 3.7\%$ to $73.6 \pm 6.1\%$ over the same duration (Fig. 5b). No significant difference in area measurement was observed at any time point between images obtained using the Leica microscope and OCT catheter-based imaging system. The exposure time at each pixel was 0.2 s, and the acquisition time for a $700 \times 700 \mu\text{m}$ region was ~ 3.5 h. The SNR was observed to be between 3 and 4.

Imaging and assessment of endothelialization on flat PDLLA scaffold

Endothelialization on flat PDLLA scaffolds was monitored over 5 days using our OCT catheter-based fluores-

cence imaging system (Fig. 6a). Control images of same scaffolds were obtained using the Leica fluorescence microscope. An increase in EC coverage area was observed using both modalities. Images acquired using the fluorescence microscope indicated that the cell coverage area increased from $15.6 \pm 6.5\%$ to $92 \pm 3.5\%$ over 5 days, whereas those acquired using our imaging system showed the cell coverage area increasing from $12.3 \pm 2.8\%$ to $94.6 \pm 4.8\%$ over the same duration (Fig. 6b). No statistically significant difference ($p > .1$) in EC coverage area assessment between images obtained using the Leica microscope and OCT catheter-based imaging system was observed (Fig. 6b). The exposure time at each pixel was 0.2 s, and the acquisition time for a $700 \times 700 \mu\text{m}$ region was ~ 3.5 h. The SNR achieved by the OCT catheter-based fluorescence imaging system with CaAM-stained ECs on the PDLLA scaffolds was ~ 2 .

Imaging and assessment of tubular PDLLA scaffold endothelialization

Endothelialization of the tubular scaffold was observed using the OCT catheter-based imaging system to image and assess the cell coverage area (Fig. 7a). Images acquired using the setup indicated that the cell coverage area increased from $15 \pm 4\%$ to $89 \pm 6\%$ over 5 days (Fig. 7b). Results could not be compared with Leica microscope-produced images due to

FIG. 5. Endothelialization on glass slides. (a) Shows images of the ECs seeded on glass slide using fluorescence microscope and OCT catheter-based imaging. (b) Plot shows increase in cell coverage area over 5 days. No statistically significant difference was observed in area measurements assessed using images obtained from the fluorescence microscope versus OCT catheter-based imaging system ($n=9$). Color images available online at www.liebertpub.com/tec

FIG. 6. Endothelialization of flat PDLLA scaffolds. (a) Panel shows images of endothelialization of flat PDLLA scaffolds, obtained using Leica microscope and OCT catheter-based system, at days 1, 3, and 5. (b) Plot shows increase in cell coverage area over 5 days. No statistically significant difference was observed in area measurements assessed using images obtained from Leica microscope and OCT catheter-based system ($n=9$). Color images available online at www.liebertpub.com/tec

FIG. 7. Endothelialization of tubular PDLLA scaffolds. (a) Panel shows OCT catheter-based fluorescence images of endothelialization of tubular PDLLA maintained within bioreactors at days 1, 3, and 5. (b) Plot shows increase in cell coverage area over 5 days ($n=9$). Color images available online at www.liebertpub.com/tec

the three-dimensional tubular geometry, which does not allow microscopic imaging. The results from this study validate the ability of the OCT catheter-based system to visualize cell dynamics in a nondestructive manner inside a bioreactor. The exposure time at each pixel was 0.2 s, and the acquisition time for a $700 \times 700 \mu\text{m}$ region was ~ 3.5 h. The SNR achieved by the OCT catheter-based fluorescence imaging system with CaAM-stained ECs on the PDLLA scaffolds was ~ 2 .

Discussion

Imaging instrumentation

Numerous fiber-optic-based probes have been developed in recent years for endoscopic fluorescence imaging of tissues and cells that are inaccessible to conventional free-space optical imaging techniques.²⁴ We developed a custom fluorescence imaging system using a commercial OCT catheter and demonstrated its utility to image ECs and to monitor endothelialization of bioengineered vascular grafts at single-cell-level resolution over time. Phantom imaging using fluorescent microspheres confirmed the utility of OCT catheter for fluorescence imaging and validated the image remapping algorithm as well as scanning parameters. The signal intensity arising from CaAM-stained ECs fluorescence was observed to be about 2.5 times less than that from the microspheres. However, the staining provided sufficient contrast for the cells to be detected when seeded on glass as well as PDLLA scaffold. The scanning step size was set to be $\sim 4 \mu\text{m}$, which is significantly less than the excitation beam waist size ($\sim 25 \mu\text{m}$ diameter). Such small beam displacements enabled the system to achieve single-cell-level resolution. In addition, cellular structures were clearly observed as the ECs approach confluence as shown in Figures 6 and 7. The catheter has a working distance of ~ 1 mm; therefore, the vessel with minimum diameter of ~ 3 mm

could be imaged without compromising the spatial resolution provided the catheter can be roughly placed in the center of the vessel. To image vessels with diameter larger than ~ 2 mm, the entire vessel may be rotated around a stationary catheter, as our group previously demonstrated.¹⁹

Monitoring the endothelialization

In the tubular PDLLA scaffolds, the cell coverage area was observed to be only 90% by day 5. These scaffolds were mounted in a tubular bioreactor and may have experienced different stresses during media replacement and CaAM staining procedures. These differences may have contributed to the slightly reduced proliferation rate observed in tubular scaffolds compared with that in the flat PDLLA scaffolds (95%) in cell culture dish.

Images acquired by the microscope after the OCT catheter-based imaging was performed also suggest that exposure to room temperature for the 3.5 h duration of OCT catheter-based imaging may not have had effect on cell mortality. In case of tubular specimens, repeated imaging of same specimens suggests the same minimal effect. However, a change in surrounding temperature for longer durations could adversely affect the functional characteristics of ECs. To monitor the endothelialization of larger areas of tubular vascular grafts, which may require longer acquisition time, the imaging could be performed in a temperature-controlled environment or even in the incubator to avoid any change in surrounding temperature.

There is a trade-off between the area of region scanned, the spatial resolution of the system, and the duration of imaging. In an attempt to achieve single-cell-level resolution, our imaging system scans a very small ROI ($700 \times 700 \mu\text{m}$) over a long duration (over 3 h). Larger areas may be scanned by increasing the distance at which the stepper motor and the translation stage move the catheter tip and the specimen, thus reducing the spatial resolution. Imaging time can be reduced by either scanning smaller ROIs or scanning the same ROI at a lower spatial resolution.

Comparison with other imaging techniques

Current methods to assess presence of ECs and degree of endothelialization are histology based and are thus static and sample destructive. Our microendoscopic approach introduces a commercially available OCT catheter to gain direct line-of-sight access to the developing lumen of the vascular graft and can monitor the cellular proliferation. In addition, the insertion and removal of the OCT catheter during the imaging process did not seem to detach the seeded ECs by abrasion. Thus, the procedure appears safe for EC proliferation and endothelialization. As our demonstrated technique is not sample destructive, the progress of the same specimen can be monitored repeatedly over time, requiring fewer samples for each study, thus reducing the material costs to prepare and culture bioengineered tissue. Since the same sample can be monitored over time, it eliminates the possibility of inter-sample variations and thus offers better process control. In addition, our imaging system could be potentially used for fluorescent-based immune-staining, which could provide additional spatial information for tracking endothelialization, although this would require sample fixation and destruction.

Recently, Whited *et al.*²⁵ showed dynamic monitoring of endothelium development in a bioengineered vessel using a fiber-optic-based imaging system. However, their imaging technique relies on localized delivery of excitation light using micro-imaging channels embedded within the scaffold, and collection of diffusely scattered fluorescence by optics external to the specimen. OCT catheter-based imaging relies on direct line-of-sight approach and hence requires neither an embedded channel for delivery of excitation light nor separate light collection optics. In our novel system, delivery of excitation wavelength and collection of fluorescence is achieved with the same catheter, resulting in a less obtrusive monitoring system.

Significant research has been done in recent years to make novel endoscopes for simultaneous transport of the fluorescence signal and OCT signal. Tumlinson *et al.*²⁶ fabricated an endoscope that comprises separate fibers for transporting the OCT and fluorescence spectra, whereas other researchers^{27,28} have utilized double-clad fibers and wavelength-division multiplexer (WDM) to combine the two spectra in the same fiber such that the single-mode core transmits and receives the OCT light and a multi-mode light-guiding inner cladding that transmits the excitation and receives the emitted fluorescence light. However, these probes are difficult to fabricate and were not commercially available when this study was performed. We designed an imaging system that utilizes a catheter, which has been optimized for OCT imaging and is commercially available. Our work demonstrates for the first time that a commercially available OCT catheter can be customized for fluorescence-based functional imaging of developing tissue-engineered constructs. The current setup allows the catheter to be used for one modality at a time—OCT or fluorescence. The catheter is connected to the fluorescence imaging optics discussed in this study for visualizing distribution of cells and for dynamic monitoring of endothelium. It can be connected to the OCT engine as described elsewhere,¹⁹ for structural imaging of the specimen. However, in the future with a custom-designed WDM,²⁹ and modified acquisition algorithm, both modalities may be simultaneously operated for co-registered structural and cellular imaging of the developing vascular graft.

Acknowledgments

The authors thank Timothy Ball, MD, for generous donation of C7 DragonFly™ Intravascular OCT imaging catheters and Dr. Gladys H. Samuel for assistance in cell staining and imaging experiments. This research was funded by a grant from the National Institutes of Health (NIBIB HL098912).

Disclosure Statement

No competing financial interests exist.

References

- Yodwut, C., Weinert, L., Klas, B., Lang, R.M., and Mor-Avi, V. Effects of frame rate on three-dimensional speckle-tracking-based measurements of myocardial deformation. *J Am Soc Echocardiogr* **25**, 978, 2012.
- Cleary, M.A., Geiger, E., Grady, C., Best, C., Naito, Y., and Breuer, C. Vascular tissue engineering: the next generation. *Trends Mol Med* **18**, 394, 2012.
- Gong, Z., and Niklason, L.E. Blood vessels engineered from human cells. *Trends Cardiovasc Med* **16**, 153, 2006.
- Bujan, J., Garcia-Hondurilla, N., and Bellon, J.M. Engineering conduits to resemble natural vascular tissue. *Biotechnol Appl Biochem* **39**, 17, 2004.
- Edelman, E.R. Vascular tissue engineering: designer arteries. *Circ Res* **85**, 1115, 1999.
- Greenwald, S.E., and Berry, C.L. Improving vascular grafts: the importance of mechanical and haemodynamic properties. *J Pathol* **190**, 292, 2000.
- Guidoin, R., Chakfe, N., Maurel, S., How, T., Batt, M., Marois, M., and Gosselin, C. Expanded polytetrafluoroethylene arterial prostheses in humans: histopathological study of 298 surgically excised grafts. *Biomaterials* **14**, 678, 1993.
- Kassem, M., Burns, J., and Abd-Allah, B. Blood vessels engineered from human cells. *Lancet* **366**, 891, 2005.
- L'Heureux, N., Dusserre, N., Konig, G., Victor, B., Keire, P., Wight, T.N., Chronos, N.A., Kyles, A.E., Gregory, C.R., Hoyt, G., Robbins, R.C., and McAllister, T.N. Human tissue-engineered blood vessels for adult arterial revascularization. *Nat Med* **12**, 361, 2006.
- Torensma, R. Blood vessels engineered from human cells. *Lancet* **366**, 892, 2005; author reply 892.
- Huang, A.H., and Niklason, L.E. Engineering of arteries in vitro. *Cell Mol Life Sci* **71**, 2103, 2014.
- Pries, A.R., Secomb, T.W., and Gaetgens, P. The endothelial surface layer. *Pflügers Arch* **440**, 653, 2000.
- Cines, D.B., Pollak, E.S., Buck, C.A., Loscalzo, J., Zimmerman, G.A., McEver, R.P., Pober, J.S., Wick, T.M., Konkle, B.A., Schwartz, B.S., Barnathan, E.S., McCrae, K.R., Hug, B.A., Schmidt, A.M., and Stern, D.M. Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* **91**, 3527, 1998.
- Karanasos, A., Lighthart, J., Witberg, K., van Soest, G., Bruining, N., and Regar, E. Optical coherence tomography: potential clinical applications. *Curr Cardiovasc Imaging Rep* **5**, 206, 2012.
- Jaguszewski, M., and Landmesser, U. Optical coherence tomography imaging: novel insights into the vascular response after coronary stent implantation. *Curr Cardiovasc Imaging Rep* **5**, 231, 2012.
- Bonnema, G.T., Cardinal, K.O., McNally, J.B., Williams, S.K., and Barton, J.K. Assessment of blood vessel mimics with optical coherence tomography. *J Biomed Opt* **12**, 024018, 2007.
- Reimers, B., Nikas, D., Stabile, E., Favero, L., Sacca, S., Cremonesi, A., and Rubino, P. Preliminary experience with optical coherence tomography imaging to evaluate carotid artery stents: safety, feasibility and techniques. *Euro-Intervention* **7**, 98, 2011.
- Yonetsu, T., Bouma, B.E., Kato, K., Fujimoto, J.G., and Jang, I.K. Optical coherence tomography—15 years in cardiology. *Circ J* **77**, 1933, 2013.
- Gurjarpadhye, A.A., Whited, B.M., Sampson, A., Niu, G., Sharma, K.S., Vogt, W.C., Wang, G., Xu, Y., Soker, S., Rylander, M.N., and Rylander, C.G. Imaging and characterization of bioengineered blood vessels within a bioreactor using free-space and catheter-based OCT. *Lasers Surg Med* **45**, 391, 2013.
- Adler, D.C., Wieser, W., Trepanier, F., Schmitt, J.M., and Huber, R.A. Extended coherence length Fourier domain mode locked lasers at 1310 nm. *Opt Express* **19**, 20930, 2011.

21. Khosroffian, J.M., and Garetz, B.A. Measurement of a Gaussian laser beam diameter through the direct inversion of knife-edge data. *Appl Opt* **22**, 3406, 1983.
22. Pham, Q.P., Sharma, U., and Mikos, A.G. Electrospinning of polymeric nanofibers for tissue engineering applications: a review. *Tissue Eng* **12**, 1197, 2006.
23. Zaitsev, V.Y., Matveev, L.A., Matveyev, A.L., Gelikonov, G.V., and Gelikonov, V.M. Elastographic mapping in optical coherence tomography using an unconventional approach based on correlation stability. *J Biomed Opt* **19**, 21107, 2014.
24. Flusberg, B.A., Cocker, E.D., Piyawattanametha, W., Jung, J.C., Cheung, E.L., and Schnitzer, M.J. Fiber-optic fluorescence imaging. *Nat Methods* **2**, 941, 2005.
25. Whited, B.M., Hofmann, M.C., Lu, P., Xu, Y., Rylander, C.G., Wang, G., Sapoznik, E., Criswell, T., Lee, S.J., Soker, S., and Rylander, M.N. Dynamic, nondestructive imaging of a bioengineered vascular graft endothelium. *PLoS One* **8**, e61275, 2013.
26. Tumlinson, A.R., Hariri, L.P., Utzinger, U., and Barton, J.K. Miniature endoscope for simultaneous optical coherence tomography and laser-induced fluorescence measurement. *Appl Opt* **43**, 113, 2004.
27. Schmitt, J. OCT elastography: imaging microscopic deformation and strain of tissue. *Opt Express* **3**, 199, 1998.
28. Rogowska, J., Patel, N.A., Fujimoto, J.G., and Brezinski, M.E. Optical coherence tomographic elastography technique for measuring deformation and strain of atherosclerotic tissues. *Heart* **90**, 556, 2004.
29. Mavadia, J., Xi, J., Chen, Y., and Li, X. An all-fiber-optic endoscopy platform for simultaneous OCT and fluorescence imaging. *Biomed Opt Express* **3**, 2851, 2012.

Address correspondence to:
Christopher G. Rylander, PhD
Department of Mechanical Engineering
The University of Texas at Austin
Austin, TX 78712

E-mail: cgr@austin.utexas.edu

Received: June 9, 2014

Accepted: December 23, 2014

Online Publication Date: January 27, 2015