Video Article

Nondestructive Monitoring of Degradable Scaffold-Based Tissue-Engineered Blood Vessel Development Using Optical Coherence Tomography

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URL: https://www.jove.com/video/58040
DOI: doi:10.3791/58040

Keywords: Bioengineering, Issue 140, Bioengineering, optical coherence tomography, vascular tissue engineering, polyglycolic acid, biodegradation, mechanical conditions

Date Published: 10/3/2018

Citation: Chen, W., Liu, S., Yang, J., Wu, Y., Ma, W., Lin, Z. Nondestructive Monitoring of Degradable Scaffold-Based Tissue-Engineered Blood Vessel Development Using Optical Coherence Tomography. *J. Vis. Exp.* (140), e58040, doi:10.3791/58040 (2018).

Abstract

Engineered vascular grafts with structural and mechanical properties similar to natural blood vessels are expected to meet the growing demand for arterial bypass. Characterization of the growth dynamics and remodeling process of degradable polymer scaffold-based tissue-engineered blood vessels (TEBVs) with pulsatile stimulation is crucial for vascular tissue engineering. Optical imaging techniques stand out as powerful tools for monitoring vascularization of engineered tissue enabling high-resolution imaging in real-time culture. This paper demonstrates a nondestructive and fast real-time imaging strategy to monitor the growth and remodeling of TEBVs in long-term culture by using optical coherence tomography (OCT). Geometric morphology is evaluated, including vascular remodeling process, wall thickness, and comparison of TEBV thickness in different culture time points and presence of pulsatile stimulation. Finally, OCT provides practical possibilities for real-time observation of the degradation of polymer in the reconstructing tissues under pulsatile stimulation or not and in each vessel segment, by compared with the assessment of polymer degradation using scanning electron microscopic(SEM) and polarized microscope.

Video Link

The video component of this article can be found at https://www.jove.com/video/58040/

Introduction

Tissue-engineered blood vessels (TEBVs) is of the most promising material as an ideal vascular graft[1]. In order to develop grafts to be clinically useful with similar structural and functional properties as native vessels, multiple techniques have been designed to maintain vascular function[2,3]. Although there have been engineered vessels with acceptable patency rates during implantation and in Phase III clinical study[4], long-term culture and high cost also show the necessity of monitoring the development of TEBVs. Understanding of extracellular matrix(ECM) growth, remodeling, and adaptation processes in TEBVs in the biomimetic chemo-mechanical environment can provide crucial information for the development of vascular tissue engineering.

The ideal strategy to track the development of small-diameter engineered vessels[5] should be nondestructive, sterile, longitudinal, three-dimensional and quantitative. TEBVs under different culture conditions could be assessed by this imaging modality, even including changes before and after vascular transplantation. Strategies to describe features of living engineered vessels are needed. Optical imaging techniques allow visualization and quantification of tissue deposition and biomaterials. Other advantages are the possibility to enable deep-tissue and label-free imaging with high resolution[6,7]. However, image-specific molecules and less easily accessible optical equipment for real-time monitoring is a significant practical obstacle, which has limited the extensive application of nonlinear optical microscopy. Optical coherence tomography (OCT) is an optical approach with intravascular imaging modality as a widely-used clinical tool to guide cardiac interventional therapy[8]. In the literature the method of OCT was reported as a way to assess the wall thickness of TEBVs[9,10], coupled with affirmative imaging modalities for vascular tissue engineering research. Whereas, the dynamics of engineered vascular growth and remodeling was not observed.

In this manuscript, we detail the preparation of biodegradable polymeric scaffold-based TEBVs for four weeks culture. Human umbilical arteries vascular smooth muscle cells (HUASMCs) are expanded and seeded into a porous degradable polyglycolic acid (PGA) scaffolds in the bioreactor. Biodegradable polymers play the role in a temporary substrate for tissue engineering and have a certain degradation rate[11]. In order to ensure an appropriate match between scaffold degradation and neo-tissue formation, ECM and PGA scaffolds are crucial factors for effective vascular remodeling. The perfusion system simulates the biomechanical microenvironment of native vessels and maintains a consistent deformation under pressure stimulation.

The aim of the presented protocol is to describe a relatively simple and nondestructive strategy for TEBVs imaging and long-term monitoring of culture. This protocol can be utilized for visualization of morphological changes and thickness measurements of engineered vessels under different culture conditions. Additionally, the analyses of polymer-based materials degradation in the tissue engineering scaffolds can be

performed for the identification. By combining methods of scanning electron microscopic(SEM) and polarized microscope used in this protocol, correlation and quantification of extracellular matrix distribution and PGA degradation can be made, which can facilitate assessing scaffold degradation combined with OCT imaging.

Protocol

1. Degradable PGA Scaffold based Tissue-engineered Vessels Culture

1. PGA Scaffold Fabrication
    1. Sew PGA mesh (19 mm diameter and 1 mm thick) around silicone tubing sterilized by ethylene oxide (17 cm length, 5.0 mm diameter, and 0.3 mm thick) using 5-0 suture.
    2. Sew polytetrafluoroethylene (ePTFE, 1cm length) with 4-0 suture onto each end of PGA mesh and overlapped by 2 mm.
    3. Dip PGA scaffolds with the hand in 1 mol/L NaOH for 1 min to adjust the spatial structure of the mesh and soak with tissue culture grade water three times for 2 min each. Gently pat dry the scaffold with a tissue paper each time. Then dry up scaffolds in a hood with a blower for 1 h.

2. Assembly of bioreactor and the Y-junction for OCT imaging
    1. Soak the self-developed glass cylindrical bioreactor (10 cm diameter and 11.7 cm in height with four lips inside and four side-arms outside the reactor as shown in **Figure 1**), PGA scaffolds, silicone tube (external diameter 5 mm, thickness 0.3 mm), biocompatible tubes, connectors, stir bar and equipment for assembly in a 95% ethanol tank for 2 h.
    2. Pull PGA scaffold through side-arms of the bioreactor connected to one side with a connector as well as another side with the Y-junction used to deliver OCT guidewire. Assemble another PGA scaffold in the bioreactor in the same way. Please refer to **Figure 1**.
    3. Fit ePTFE to bioreactor lips by tightening with 4-0 sutures.
    4. Put the bioreactor in the ethanol tank again for 1 h and dry up overnight in hood with blower on.

3. Seeding of HUASMCs and Static Bioreactor Conditioning
    1. Isolate HUASMCs from human umbilical arteries by standard explant techniques.
    2. Expand and maintain cells in smooth muscle cell growth medium composed of DMEM medium, 20% fetal bovine serum, 2.36 mg/mL HEPES, 100 U/mL penicillin G, 50 µg/mL proline, 20 µg/mL alanine, 50 µg/mL glycine, 1.5 µg/mL CuSO4, 50 µg/mL ascorbic acid, 10 ng/mL basic fibroblast growth factor and 10 ng/mL platelet-derived growth factor.
    3. Seed HUASMCs at a concentration of 5×10^6 cells/mL in the above culture medium onto the PGA scaffolds.
    4. Put a stir bar (1.5 cm length) in the bioreactor. Insert one feeding tube (5 mm diameter, 15 cm length) and three short tubing segments (5 mm diameter, 7 cm length) for gas exchange through the silicone stopper lid.
    5. Attach PTFE 0.22 µm filters to each air change tube and one heparin cap to the feeding tube. Adjust the stir bar with a stirring speed of 13 rounds per minute. Assemble the glass bioreactor, silicone stopper lid and PGA scaffold into the culture system.
    6. Allow HUASMCs to adhere for 45 min by leaning the bioreactor every 15 min with stand, to the left and right. The reactor ports and joints are all sealed with paraffin film.
    7. Connect the Luo-Ye pump, PBS bag, the driver with biocompatible tubes as the perfusion system. Open the drive to fill the tubes with PBS.
    8. Place the overall bioreactor in a humidified incubator with 5% CO_2 at 37 °C. Fill the culture chamber with 450 mL of the HUASMCs culture medium.
    9. Press the stop button and turn off the power of the drive device. Grow the seeded scaffolds under static culture for one week.
    10. Change the culture medium every 3-4 days by aspirating half of the old medium through the feeding tube and refilling the reactor with an equivalent amount of fresh culture medium.

4. Preparation of the perfusion system for OCT imaging
    1. Pump fluids in the PBS bag to circulate through biocompatible tubes and back to the bag.
    2. Open the power of the driver and regulate pump setting with a frequency of 60 beats per minute and output systolic pressure of 120 mmHg. Adjust the mechanical parameters according to the needs of tissue engineering vascular culture.
    3. Click the run button to make the perfusion system work. Provide the above fixed pulsatile stimulation to the vessels for 3 weeks by iteratively pressurizing biocompatible tubes[10,12] after 1 week of static culture.

2. Performing Optical Imaging with OCT

1. Use a light source to ensure the axial resolution of 10-20µm and the image depth of 1-2 mm to identify the structure of TEBV based on the frequency-domain OCT intravascular imaging system[9].
2. Turn on the power switch and open the image capture software.
3. Connect the fiber optic imaging catheter to the drive-motor and optical controller (DOC) with catheter automatic retreat function.
4. Set parameters of image acquisition rate to 10 frames per second with an automatic pullback speed of 10 mm/s.
5. Attach imaging catheter to Y-junction via heparin cap with a 18G needle.
6. Place the catheter into the silicone tube and identify the suture tightness of PGA mesh before loading PGA scaffold onto the bioreactor.
7. Place the catheter tip over the region of interest. Adjust the pullback device and check for the image quality[8].
8. Acquire images at 1, 4, 7, 10, 14, 17, 21, 28 days in culture for each individual TEBV and save sequentially with real time observation of TEBV microstructure, including surface morphology, internal structure and composition.
9. Repeat the measurement for 3 times to get reliable measurement of engineered vessels each time. Capture a series of images throughout testing using the image capture software.

3. Imaging Analysis

1. Use image analysis software to measure TEBV wall thickness. Select the image to be analyzed. Click the tracking tool to identify the inner side of TEBV by the software automatically and manually sketch the outer side. A diagram of thickness will appear on the screen.
2. Repeat the measurement for 5 times to get reliable measurement of the constructs. The OCT analysis was performed by two independent investigators blinded to the obtained information.

4. Harvest of TEBV and Tissue Processing

1. Open the silicone stopper lid placed over the bioreactor when the culture is finished and discard the culture medium. Loosen ePTFE from bioreactor lips and cut the silicone tubes from the outer side of ePTFE with scissors. Harvest TEBVs from the bioreactor and cut into sections for scanning electron microscopy examination.
2. Take out the rest of TEBVs and cut into 4 μm thick sections. Pull out the supporting silicone tube and fix sections with 4% paraformaldehyde. Perform routine histological staining of Masson's trichrome and Sirius red to examine the morphology of collagen and PGA[10,13,14].
3. To assess PGA content and collagen component, observe histologic samples with Sirius red staining by a polarized microscope. PGA remnants are clearly demarcated through birefringence and the remnant area can be quantified based on the cross-sectional area[10].

Representative Results

The three-dimensional culture system consisted of a culture chamber in the bioreactor and the perfusion system with a closed fluid cycle[10,13] (**Figure 1**). The OCT imaging catheter was inserted into the distal end of the Y-junction and pulled back in the silicone tube for imaging. OCT imaging was first used to delineate the structural characterization of biodegradable polymeric scaffold-based TEBVs during bioreactor cultivation.

Figure 2 showed the process of engineered vascular remodeling through these cross-sectional imaging of tissue microstructure in real time. Geometric morphology was evaluated, including wall thickness, degradable PGA content, and comparison of TEBV thickness in different culture time points as well as presence of pulsatile stimulation. A trend of decreasing thickness and dramatically changes of engineered tissue within the first two weeks of culture was seen, suggesting signal-rich PGA gradual degradation and the structure of new tissue from loose to tight. At 21 days in culture, the vasculature had formed a smooth structure with extracellular matrix evenly distributed and high signal components mostly dissipated. The wall thickness of TEBVs with even signal increased gradually after three weeks of culture. This remodeling occurred earlier and the morphological changes manifested more obviously in the dynamic group (**Figure 3**). Thereby OCT enables imaging of engineered vascular morphology to be visualized in situ and in real time in the course of long-running culture.

Figure 4 compared OCT images with histopathological finds of TEBV after 4 weeks of culture. Masson's trichrome staining demonstrates collagen fibers distributed in a certain direction along with PGA remnants in media layer of engineered vessels (**Figure 4B**). Sirius red staining revealed PGA remnants and collagen component by using a polarized microscope (**Figure 4C**). Scanning electron micrographs of engineered vessels with compact microstructure were compared with histological assessment (**Figure 4D**). Taken together, OCT images showed PGA was with different sizes and porous network structure. The structure of PGA scaffold has no obvious change and swollen by direct contact with culture medium in early stage of culture. But signal intensity of PGA was reduced. PGA components was disintegrated and replaced with cells and extracellular matrix. Fewer fragments were seen over four-week period. SEM images of cross-sectional engineered vessels demonstrated fiber rupture to the extension of incubation time. Material and extracellular matrix composites were in honeycomb-like structure with more compact and less transparency.

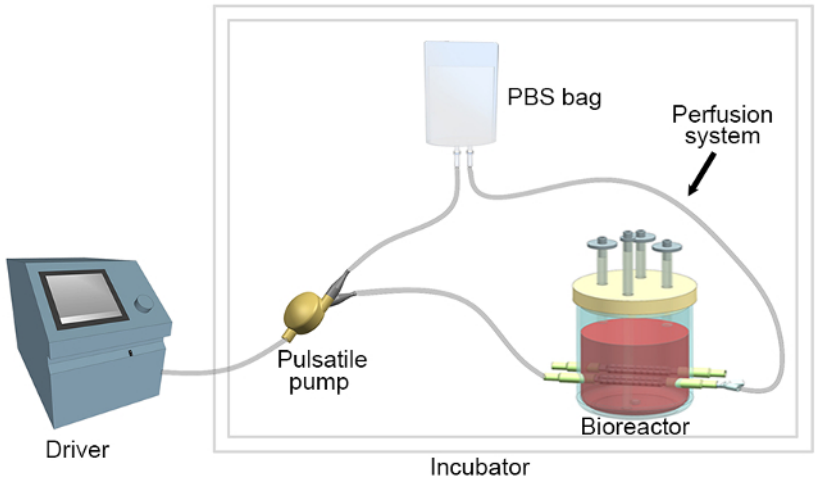

Figure 1. Schematic of tissue engineering vascular culture system, which consisted of a culture chamber in the bioreactor and the perfusion system for OCT imaging. The pulsatile pump provided a stable fluid flow simulating the biomechanical microenvironment. OCT imaging catheter was pulled back in the silicone tube in the culture chamber. Please click here to view a larger version of this figure.

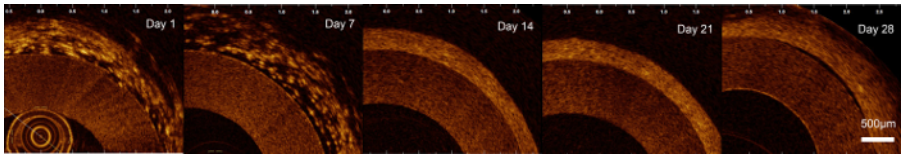

Figure 2. The microstructure of tissue engineered blood vessels during culture. Over culture time, signal-rich PGA gradually degraded and the structure of new tissue was from loose to tight. TEBVs had a smooth surface and abundant extracellular matrix evenly distributed after four weeks of culture. It showed the process of engineered vascular remodeling through cross-sectional images in real time. This figure has been modified from Chen, W. *et al*.[10] The thickness of silicone tube used here is 0.8mm. Please click here to view a larger version of this figure.

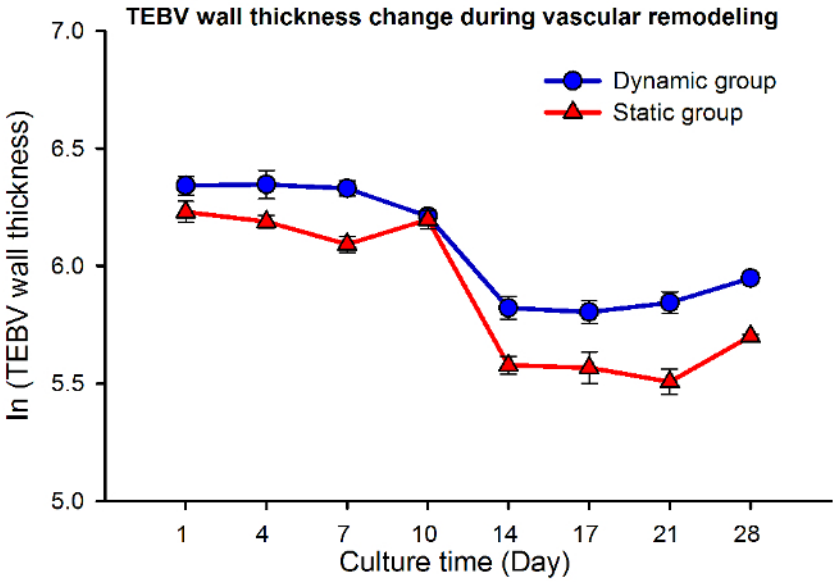

Figure 3. Comparison of TEBV wall thickness change during vascular remodeling in dynamic and static groups obtained from OCT measurements. Error bars indicate standard error. This figure has been modified from Chen, W. *et al*.[10] Please click here to view a larger version of this figure.

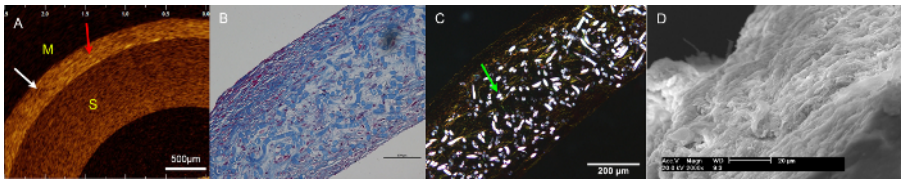

Figure 4. Imaging of biodegradable polymer-based tissue-engineered blood vessels. (A) OCT image of TEBV after four weeks of culture. M: culture medium; S: silicone tube; the thickness of silicone tube used here is 0.8mm. White arrow indicated TEBV. Red arrow indicated PGA fragment. (B) Masson's trichrome staining demonstrated well-organized collagen fibers along with the residual content of PGA in media layer of engineered vessels. Scale bar=100 μm. (C) Sirius red staining revealed PGA remnants by using a polarized microscope. Green arrow indicates PGA fragment. Scale bar: 100 μm. (D) Scanning electron micrographs of engineered vessel with compact microstructure were showed to compare with histological assessment. Scale bar=50 μm. Please click here to view a larger version of this figure.

Discussion

To generate engineered vessels with structural and mechanical properties similar to those of native blood vessels can lead to shorten the time for clinical use and is the ultimate goal of vascular engineering. Optical imaging techniques permit the visualization of tissue engineered vascular specific components, which cannot monitor individual constructs throughout culture and exposure grafts to a culture environment without compromising sterility[7]. In this article, the culture chamber is separated from the perfusion system. The relatively independent perfusion system guarantees the decrease risk of pollution during culture and the placement of OCT guidewire. Meanwhile this intraluminal imaging modality adopted easy and safety monitoring of TEBVs in situs with high resolution approaching that of histopathology, which made the assessment of TEBV growth status more practical and was even expected to be used before or after implant placement.

The current protocol indicates a readily available, fast real-time and nondestructive imaging strategy to evaluate degradable polymer-based engineered vessel development using catheter-based OCT. Through observation of the dynamic process, some main factors affecting vascular engineering, such as contamination or unmatched cell-material interaction led to tissue loss, can be distinguished with early detection. Critical steps to ensure the efficacy of the protocol include the fabrication of NaOH-modified PGA scaffold, successful seeding of HUASMCs in the scaffold, separation sterile culture system from the monitoring system, fast and skilled catheter operation process.

This technique can be utilized to assess degradation states and complex structure of PGA scaffolds blended in with new tissue. The polymeric scaffold with porous network structure degrades gradually and dominates the process of vascular remodeling in the first three weeks, which are important for cell adhesion and extracellular matrix deposition with a three-dimensioned structure for nutrient exchange and as a signal carrier[15,16]. For quantification of PGA remnants in engineered vessels clearly identified by Sirius red-stained images, the use of polarized microscopes[17] in degradable scaffold-based vascular engineering has the potential to become the standard evaluation after cultivation. Hence OCT imaging combined with polarized microscope might serve as qualitative and quantitative methods for assessing PGA degradation in vascular engineering.

A limitation of this technique is the resolution limit to assess cell proliferation, distribution, cell-cell and cell-ECM interaction during engineered vascular remodeling. We hope to find suitable method to investigate TEBVs microstructure at cellular or subcellular level[18] and quantify growth kinetics. With quantitative analysis of average optical signals of OCT imaging, we might be more aware of the mechanism of material degradation in vascular engineering. Such experiments are being considered for our future studies.

Overall, our results show that OCT is a readily available, fast real-time and nondestructive imaging strategy to monitor the growth and remodeling of TEBVs. It is utilized to characterize structural architectural features and the long-term remodeling process of engineered vessels. The application of polarized microscope which provided supplementary evidence for the quantification of polymeric remnants in engineered vessels might be useful for assessing scaffold degradation combined with OCT imaging. Taken together, the current protocol holds promising value of OCT for its application in vascular tissue engineering.

Disclosures

The authors declare they have no competing financial interests.

Acknowledgements

We would like to acknowledge the Science and Technology Planning Project of the Guangdong Province of China (2016B070701007) for supporting this work.

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