Pore-Cast Scaffold for Vascular Tissue Engineering

Michael H. Zhang, Aimal H. Khankhel
Department of Biomedical Engineering
Boston University
Boston, United States
mhzhang@bu.edu, kaimal10@bu.edu

Kevin J. McHugh, Magali Saint-Geniez
Schepens Eye Research Institute
Boston, United States
kevin.mchugh@schepens.harvard.edu,
magali.saintgeniez@schepens.harvard.edu

***Abstract*—Transplantation of engineered vascular grafts has emerged as a potential therapeutic solution for cardiovascular disease treatment. Although engineered vascular grafts hold the potential to satisfy a large clinical need, thrombosis remains a major risk with the small-diameter grafts (<5 mm). Currently, electrospinning is considered the gold standard for scaffold construction. However, poorly controlled pore dimensions allow the migration of endothelial cells (EC) into the scaffold through large gaps, making it difficult to achieve a uniform monolayer. The aim of this study is to design and construct a thin, porous, non-thrombogenic film as a scaffold for vascular grafts. Pore dimensions will be controlled by using a casting technique, thereby reducing the problem of cell migration into the scaffold. A confluent EC monolayer can then act as a barrier preventing blood-scaffold interaction, which will reduce the risk of thrombogenesis when implanted in vivo.**

Keywords-biological scaffolds; biomaterial; blood vessel; endothelial cells; polymer scaffolds; tissue engineering; vascular graft

I. METHODS

A. Designing Scaffold Porosity

The scaffold porosity will be determined to allow solutes, particularly oxygen, to diffuse from the blood to the peripheral tissue. Oxygen diffusivity will be modeled for a native blood vessel of 5 mm in diameter such that oxygen can be detected by the surrounding tissue, which we assume to be myocardial cells. The porosity determined from the model of this native blood vessel will be applied to our engineered blood vessel scaffold. The modeling of this oxygen diffusion phenomenon can be approximated with Fick's Second Law for a steady-state system:

$$D\nabla^2\mathbf{C} = -R,$$

where D is the diffusivity constant through the given interface, $\mathbf{C}$ is the concentration profile of oxygen, and R is the oxygen consumption of the myocardial cells. The native blood vessel for this model will be simplified to three distinct regions: the lumen, the extracellular matrix (ECM)/smooth muscle cells (SMC), and the myocardial tissue. (The oxygen consumption of ECs is assumed negligible compared to that of SMCs.) With a known diffusivity value between the lumen and ECM/SMC interface, the diffusivity of the ECM/SMC to myocardial tissue interface will be adjusted until a desired oxygen concentration is attained at the peripheral myocardial tissue. The ratio of these two diffusivity values will be used to evaluate the porosity of the scaffold that is needed to mimic the same diffusion properties as this native vessel model.

B. Selecting Polymer for Scaffold Fabrication

Polycaprolactone (PCL) is a widely used polymer known to have similar mechanical properties to that of native vessels and a slow degradation rate [1]. First, a silicon wafer will be created using microfabrication techniques to etch rectangular features that are 10 μm x 1 μm when viewed from above. A PCL solution will be poured onto the silicon wafer and spun until the desired thickness is reached (~10 μm) (**fig. 1**). Removal of the polymer film will yield a thin porous membrane. The elastic modulus will be measured under cyclic and tensile strains and will be compared with previously established values for small-diameter blood vessels. The desired elastic modulus should be between 10^4 and 10^6 Pa [2].

C. Inhibiting Cell Migration into Scaffold

We will control for pore size to prohibit migration across the scaffold interface. To test for migration, human umbilical vein endothelial cells (HUVECs) will be seeded on one side of the membrane. Vascular endothelial growth factor (VEGF) will be placed in growth solution on the opposing side of the membrane as a chemoattractant. After 18 hours, cell migration will be measured by counting the cell density on the VEGF side of the scaffold. Results will be compared with a negative control that contains no VEGF and with HUVECs grown on electrospun scaffolds.

D. Inducing Cell Alignment

Previous studies have shown that cells align along ridges in the substrate [3]. To mimic this effect, pores will have rectangular dimensions of 10 x 1 μm^2 and will be aligned along the long axis. To view cell alignment, scaffolds seeded with cells will be viewed under SEM. Immunohistochemistry will be performed to view cytoskeletal organization. Measuring the angle between the cell stress fibers and the long axis of the pores will give a quantitative value for alignment.

E. Facilitating HUVEC Adherence

To facilitate HUVEC adherence and proliferation to the surface of the scaffold, the scaffold must be functionalized. Surface functionalization will be performed by testing gelatin and the integrin, Arg-Gly-Asp (RGD). Monolayer

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confluency will be measured after surface functionalization and cell seeding. Cells will be fixed and fluorescently stained for f-actin and E-cadherin to view tight junction formation. Fluorescence intensities will be quantified and compared to a negative control on an electrospun scaffold. To confirm tight junction formation, scaffolds will be subject to a trans-endothelial electrical resistance test (TEER).

F. Analyzing HUVEC Behavior and Thrombogenicity on Scaffold

The scaffold must be biologically compatible such that it is comparable to that of a native vessel. HUVECs will be seeded on one side of the scaffold and allowed to proliferate so a complete monolayer forms, reducing the risk of thrombus formation due to an exposed scaffold region, which can lead to platelet activation. TEER will be continually measured to evaluate the integrity of the HUVEC monolayer. Overall cell morphology will also be monitored at these selected time intervals until a complete monolayer is formed, upon which thrombogenic testing will be performed. An incomplete monolayer formation can lead to thrombus formation, leading to the expression of thrombogenic genes, so gene expression of the HUVECs will also be investigated using qPCR. The follow genes will be analyzed using this method: thrombomodulin, endothelial protein C receptor (EPCR), tissue factor (TF), tissue factor pathway inhibitor (TFPI), and Von Willebrand factor (vWF). To ensure that the ECs are functioning properly, we will observe these EC specific genes: VEGFR-1, VEGFR-2, VE-cadherin, caveolin, endothelial nitric oxide synthase (eNOS), induced nitric oxide synthase (iNOS), platelet endothelial cell adhesion molecule (PECAM-1), and intercellular adhesion molecule (ICAM).

II. Preliminary Results

HUVECs were starved for 24 hours prior to seeding onto the tops of transwell membranes of either 1 or 5 μm pore radius size. The underside of the transwell membrane was placed in the presence of either ± VEGF containing media (n=3 for all conditions). After 18 hours, cells were removed from the top of the membrane, and the bottom was stained using an H&E kit. For all 1 μm pore sizes, no cells were observed on the underside of the membrane. For the 5 μm conditions, no cells were observed on the underside of the membrane for the control group. The 5 μm condition with VEGF present resulted in cell migration through the membrane (**fig. 2**). This proved our choice of a 1 μm dimension pore width to be sufficient in preventing cell migration through the scaffold.

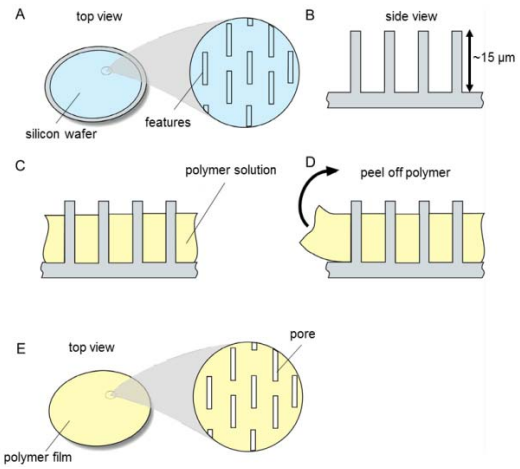

Figure 1: Schematic of silicon wafer and polymer film fabrication. (A) Top view of wafer. Features will be 10 μm by 1 μm. (B) Side view of features. Height will be 15 μm to ensure pores traverse the entire thickness of the scaffold. (C) Polymer solution is poured on. (D) After drying, the polymer is peeled off. (E) A polymer with pores that replicate the features on the wafer.

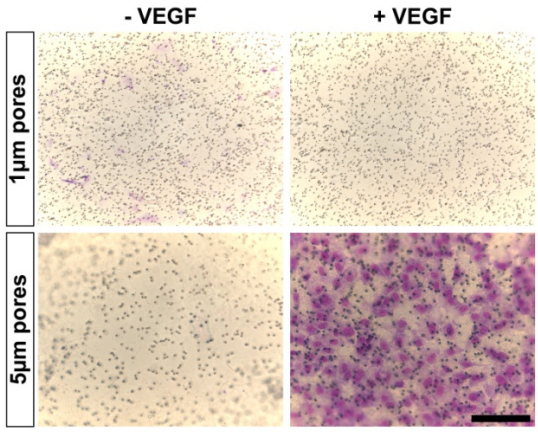

Figure 2: H&E staining of underside of transwell samples. (A) 1 μm pore diameter with no VEGF, (B) 1 μm pore diameter in the presence of VEGF, (C) 5 μm pore diameter with no VEGF, and (D) 5 μm pore diameter with VEGF. Only with a 5 μm pore size combined with the presence of VEGF did cells migrate to the underside of the membrane. Scale bar is 10 μm.

III. References

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