A Novel Metallic Scaffold that Promotes the Rapid Seeding of Endothelial Cells

Mahdis Shayan[1] and Youngjae Chun[1,2]

[1]Department of Industrial Engineering, [2]Department of Bioengineering University of Pittsburgh

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Statement of Purpose: Despite advances in pharmacologic, surgical and interventional therapies, vascular disease is the leading cause of morbidity and mortality in the industrialized world today. Bypass surgeries to restore blood flow distal to diseased vessels are common but synthetic graft materials have shown unsuccessful outcomes in small diameter (< 6 mm) applications.[1,2] Early attempts at bioprosthetic grafts (i.e., Tissue Engineered Vascular Grafts: TEVGs) focused on creating functional endothelial linings on synthetic bypass grafts. While this approach increased patency rates, the procedure to produce the grafts is complex and labor intensive, thus requires a long production time. Therefore, there is a critical need to develop a new biomaterial for TEVGs. One approach to decrease the time necessary to achieve the confluent cell deposition within scaffolds is to exploit the cells' natural electronegativity using a conductive thin metallic biomaterial, i.e., thin film nitinol. The electrical conductivity of this metallic scaffold will allow for the creation of Coulomb forces between the film's surface and the endothelial cell, substantially decreasing the required seeding time compared with other polymeric scaffolds (e.g., ePTFE or Dacron). In addition, thin film nitinol has a few micron thickness (i.e., ultra-low profile) and a great tensile strength of 500MPa with large strains (e.g., 400%). This study demonstrated the rapid electrostatic seeding of endothelial cells on the thin film nitinol after growing a superhydrophilic oxide layer (i.e., insulating layer) showing a novel scaffold for TEVGs.

Materials and Methods: A 6µm thick thin film nitinol was produced by a hot-sputter deposition method. Surface oxide layer (i.e., insulating layer) was grown by a superhydrophilic surface treatment using Hydrogen peroxide. Surface-treated thin film nitinol with dimensions of 9×6.5mm were used as a scaffold for the electrostatic endothelial cell seeding. Figure 1 shows the experimental set-up used for electrostatic endothelial cell seeding. The thin film nitinol was deployed in a 3mm diameter silicone tube, then a nitinol wire (NDC, CA, USA) was placed in the middle of the lumen of the tube (i.e., similar to a cylindrical capacitor). The electrical charge was controlled through changing the applied voltages (i.e., 1V, 5V, 15V and 30V).

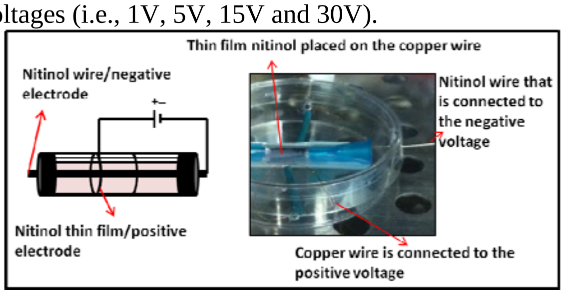

Figure1. An experimental set-up for the electrostatic endothelial cell seeding on thin film nitinol scaffold

The silicone tube was filled with 200µl Endothelial Cell Basal Medium (EBM)-2 (Lonza, NJ, USA) containing EGM-2 SingleQuot Kit Suppl. & Growth Factors with suspended BAEC (Bovine aortic endothelial cells, Lonza, NJ, USA). All experiment was conducted at 37°C, 5% CO_2. After 30min, all samples were rinsed in PBS and stained with Calcein AM (life technologies™, NY, USA), then fluorescence images were taken by fluorescent microscopy (Olympus BX43 PA, USA). The number of attached cells was counted and the morphology and proliferation of adhered cells were characterized using SEM (JEOL JSM-6610LV, Japan).

Results: Using the cylindrical capacitor model, the accumulated electrical charges on the surface were calculated using Gauess' law: 0.136×10^{-6}µC, 0.68×10^{-6}µC, 2.04×10^{-6}µC, 4.08×10^{-6}µC for 1, 5, 15, and 30V, respectively. SEM images showed a few number of cells attached on the surface without speadout in control and 1V samples. The attached cells, however, began spread out after 5V was applied showing completely flat and well spread out morphology (Figure 2-3). In higher voltages (i.e., 15V and 30V), the morphology of cells are completely spread out similar to the 5V condition, but the number of cells were increased. The average number of attached cells counted per mm^2 for each condition include: control (109±50 cells/mm^2), 1V (170±140 cells/mm^2), 5V (161±29 cells/mm^2), 15V (251±144 cells/mm^2) and 30V (436±250 cells/mm^2) (n=5), without generating any damage on the cells. The results demonstrated the higher electrical field attracted more cells up to 30V in 30min.

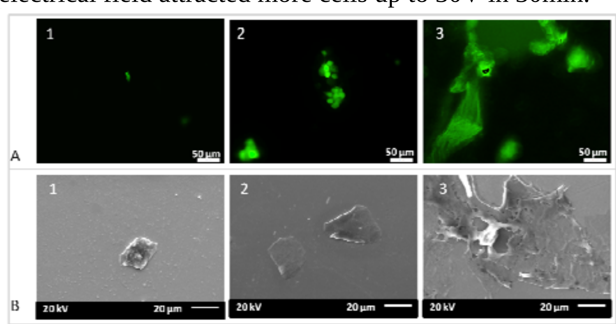

Figure2. A) Fluorescent microscope images and B) SEM images of thin film nitinol scaffold after 30 min under 1) 0V, 2) 1V, 3) 5V.

Conclusions: This study demonstrated that a novel metallic scaffold, thin film nitinol, could rapidly increase the number of cells attached on the sufrace via electrostatic cell seeding. Thin film nitinol reduced the complexity of the cell seeding apparatus enhancing the cell attachment. Thin film nitinol could be potentially used for a tissue engineering vascular graft, specifically for treating small vascular dieseases.

References:

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