Dispersive Raman Spectroscopy to Assess Protein Incorporation and Cellular Remodeling of Tissue Engineered Vascular Grafts

[1]Krystal R. Kerney, [1]Andrea S. Theus, [1]Michael Fenn, [1]Chris A. Bashur
[1]Department of Biomedical Engineering, Florida Institute of Technology, Melbourne, FL 32901

Introduction: Tissue engineered vascular grafts are a promising alternative to surgery for the treatment of thrombotic occlusion of small diameter arteries. The cellular remodeling response of the graft will dictate its viability. While initial tissue deposition is needed, continued proliferation of smooth muscle cells (SMCs) and extracellular matrix (ECM) deposition can lead to intimal hyperplasia and occlusion. Real-time monitoring of remodeling would allow for efficient screening of scaffold materials. Raman spectroscopy has been used previously to characterize ECM components [1], but its use to analyze porous, 3-D scaffolds provides several challenges that we are addressing in this study. Blended scaffolds of natural and synthetic materials are used here to combine the mechanical strength of PCL and the bioactivity of natural materials such as collagen and fibrinogen. In this study, we are developing a dispersive Raman technique to verify the initial incorporation of natural materials within fibrous scaffolds, and to detect scaffold remodeling by SMCs *in vitro*.

Materials and Methods: We produced electrospun meshes with 100% poly (ε-caprolactone) (PCL) and 90% PCL/collagen using 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), and 90% PCL/fibrinogen using 90 % v/v HFIP and 10% DMEM. Scaffolds were characterized using SEM imaging. RT-PCR and immunofluorescence (IF) was performed to analyze markers of contractile SMCs (i.e. calponin) and activated SMCs (i.e. thrombospondin, osteopontin). Raman spectroscopy was performed on control meshes and meshes with SMCs incubated for 21 days. Quantification of relevant peak areas was performed using peak integration with Wire 4.1 software. Statistical analysis was performed using JMP and one way ANOVA with a Tukey-Kramer post-hoc test.

Results and Discussion: We identified protein peaks for incorporated collagen and fibrinogen (Fig. 1) (e.g., 740, 1050 cm^{-1}) that were higher than 100% PCL. For example, quantification of peak area at 1050 for electrospun PCL meshes show a significant decrease compared to PCL/collagen ($p=0.0003$) and PCL/fibrinogen ($p<0.0001$) meshes. This indicates that we can use these peaks to confirm presence of proteins even in the PCL-containing electrospun meshes. Preliminary results for the 740 cm^{-1} peak integration show an increase after 21 days of culture ($p<0.04$ for PCL/collagen and PCL/fibrinogen). In addition, RT-PCR and IF show an increase in osteopontin correlated with incorporation of collagen. IF analysis for other SMC markers (e.g., calponin) demonstrate differences in cell phenotype with scaffold composition. We are currently determining the impact of scaffold composition and remodeling after culture on the mechanical properties of the scaffolds.

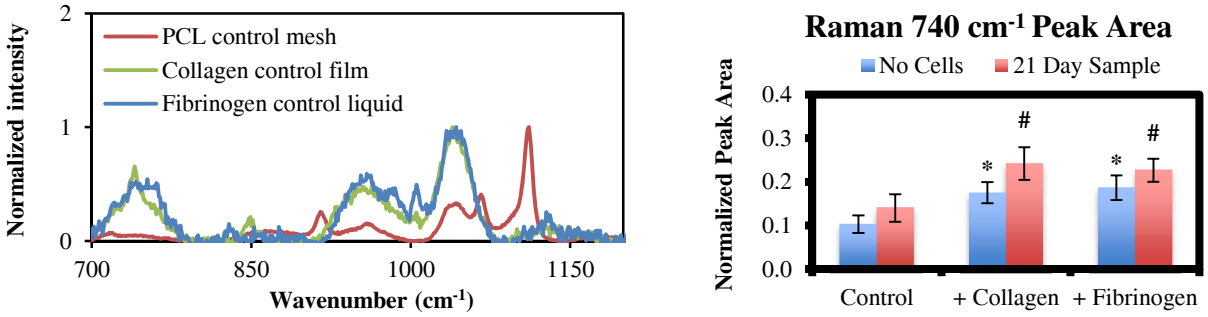

Figure 1: Raman spectra for mesh materials. Quantified 740 cm^{-1} peak area. * Significant from control. # Significant from no cells.

Conclusions: These results demonstrate that scaffold composition impacts SMC phenotype. Specifically, the incorporation of collagen is correlated with an increase in osteopontin in these SMCs. Overall, this study demonstrates the feasibility of using non-destructive Raman techniques to verify initial incorporation of protein (e.g. collagen and fibrinogen) into electrospun scaffolds. This technique also has the potential to enable the continuous monitoring of cellular remodeling of different graft materials.

References:

1. Brauchle E, Schenke-Layland K. *Biotechnology journal*. 2013.