Co-axial Electrospun Polycaprolactone/Polyurethane Nanofibrous Scaffolds for Myocardial Tissue Engineering

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Statement of Purpose: Tissue engineered structures are fast becoming a viable solution for the treatment of diseased cardiovascular tissue. One of the attractive polymers to use for the preparation of these scaffolds is polyurethane based on its elastic and mechanical properties. The objective of this study was to determine the ability of bicomponent electrospun scaffolds to guide differentiation of mouse embryonic stem (mES) cells as compared to previously studied[1] scaffolds made of a similar combination of polycaprolactone/gelatin. The scaffolds were also evaluated to determine their ability to withstand dynamic conditions as seen in cardiovascular tissue and biocompatibility in vitro.

Methods: Polymer solutions were made using 1,1,1,3,3,3-Hexafluoro-2-propanol (HFP, Sigma-Aldrich) as a solvent and various combinations of gelatin (Type B, Sigma), polyurethane (PU, Selectrophore®, Fluka), and polycaprolactone (PCL, Mw 10-20,000, Polysciences). Solutions were made at 5% and 10% by polymer weight of PU and PCL/gelatin combination, respectively. Once dissolved for 24hrs, the solution was used in a vertical electrospinning system[1]. The system employed the use a positively-charged needle tip in two sizes, 18G and 25G, and a grounded copper collection surface. Using a unique co-axial system, the 25G needle was inserted into 18G needle to create a bicomponent fiber. A constant power supply of 25kV and flow rate of 50µL/min were used for a collection time of approximately 15 to 20 minutes. Samples were then segregated into three groups for various testing: morphological analysis via scanning electron microscopy (SEM) and transmission electron microscopy (TEM), dynamic mechanical analysis (DMA), and in vitro biocompatibility/differentiation experiments. SEM images were collected using a JEOL JSM-6610 SEM with the following conditions: under high vacuum, an accelerating voltage of 15kV, and spot size 41. TEM images were collected with a JEOL 100CX TEM. DMA testing was done on a DMA Q800 using a strain sweep of up to 100µm at a frequency of 1Hz using a thin film clamp and a gage length of 10mm. Differentiation and biocompatibility experiments were carried out using V6.5 mES cells.

Results: Initial scaffold morphology SEM image analysis (Fig. 1) shows relatively no difference between the fiber and pore sizes of the scaffolds produced using our co-axial electrospinning process as compared to our standard one needle system. Using TEM, the presence of two polymer components was noted by incorporating gold nanoparticles (diameter size 15nm) into the gelatin/PCL solution. Morphologically, the two scaffolds are of similar fiber diameter and pore size. However, there is a significant difference between the two systems in terms of mechanical analysis. DMA experiments used a frequency of 1Hz which is
similar to the lower level of spontaneous beating[2] seen in cardiomyocytes in culture. The results of the experiments show that the bicomponent scaffolds fabricated from the co-axial system of both PU and gelatin/PCL are able to withstand the dynamic conditions needed to support cardiovascular differentiation of stem cells at higher levels of strain[2] (over 10%) as compared to scaffolds we have previously used made from our standard one needle/one polymer solution approach[1].

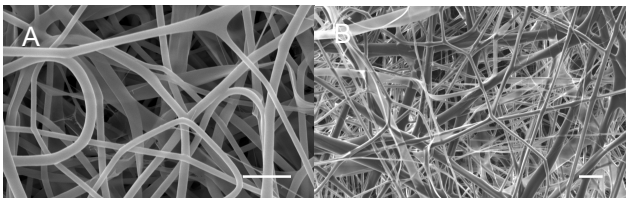

Figure 1. Representative electron micrograph of electrospun scaffolds A) 10% gelatin/PCL, one needle, 2000x B) bicomponent: 5%PU core, 10% gelatin/PCL sheath, 1000x. Scale bar is 10µm

Further, the DMA results shows the higher elastic capability of the bicomponent scaffolds based upon the higher trends of storage modulus, as compared to lower levels seen in the gelatin/PCL scaffolds. The biocompatibility results show both sets of scaffolds are able to support cellular growth for at least 28 days of culture. DAPI staining and SEM image analysis confirmed the cellular adhesion on all of the scaffolds. Initial FACS and immunostaining has confirmed the increased population of cardiac progenitor cells when cultured on a 3D scaffold vs. standard tissue culture dishes. Further analysis will examine the difference between the standard scaffolds (one needle/one polymer solution system) and the bicomponent scaffolds.

Conclusions: Overall, the initial results show promise in developing a tissue engineered myocardial patch from nanofibrous scaffolds. Thus far, we are able to produce scaffolds with a controllable diameter and pore size. We have also demonstrated the ability of the bicomponent scaffolds to withstand the dynamic control conditions we anticipate for future cardiovascular tissue engineering projects. Further study will be required to determine the ability of the scaffold to support not only cellular growth, but also differentiation of stem cells and the function of those differentiated cells.

References:

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2. Norstrom A, Exp Bio and Med, 2006:231;1753-1762