Coaxial Electrospun Nanoactuating Poly(ε-caprolactone), Multi-walled Carbon Nanotubes, and Polyacrylic acid/Polyvinyl Alcohol Hydrogel Scaffolds

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Statement of Purpose:
Approximately 40% of the muscle in the human body is skeletal muscle (Guyton AC. Textbook of medical physiology. 2006. 11th ed.). The unidirectional orientation of muscle fibers allows for a large force to be generated during contraction (Bach AD. J Cell Mol Med. 2004;8(4):413-422). Once a muscle is injured and unable to contract, satellite cells are activated to help regenerate the skeletal muscle, but this process results in scar tissue formation and loss of muscle function (Bach AD. J Cell Mol Med. 2004;8(4):413-422). In this study, we created several polymer nanoactuating scaffolds consisting of poly(ε-caprolactone) (PCL), multi-walled carbon nanotubes (MWCNT), and a polyacrylic acid (PAA) and polyvinyl alcohol (PVA) hydrogel. We investigated the effects of different ratios of PAA/PVA: 83/17, 60/40, 50/50, and 40/60.

Methods:
Coaxial Electrospinning: 0.5% MWCNT in DMF (w/v) was sonicated for dispersal and then the DCM and PCL were added to make a 50% solution (w/v). PVA and PAA were dissolved in a 1:3 ratio of dIH_2O:EtOH to make the 6% (83/17, 60/40, 50/50, 40/60) (w/v) solutions. These were coaxial electrospun so that the fiber interior core was PCL-MWCNT and the outer shell was the PAA/PVA hydrogel. The fibers were collected onto a rotating mandrel (5cm, 3000 ± 200rpm). All scaffolds were cross-linked by soaking in a gluteraldehyde solution for 20 minutes at room temperature (Jegal J. J Appl Polym Sci. 1999;72(13):1755-1762). SEM was used to analyze fiber alignment, diameter (36 fibers, 6 fields), and show the fiber core/outer shell via cross-section.
Conductivity: The resulting four scaffolds were vacuum soaked in PBS for 30 minutes before being placed on an electrode (n=4). A constant voltage and current (20.00V, 1.545A) were applied to each mat, the current output measured, and the conductivity calculated.
Electroactivation Testing: Strips (1cm x 4cm) were cut from each of the four scaffolds and were vacuum soaked for 30 minutes in a 30% (w/v) saline solution. Each strip was suspended in a beaker containing 30% saline with an alligator clip attached to one end. A carbon electrode had the other alligator clip attached and was partially submerged in the beaker. The voltages applied were 5V, 10V, 15V, and 20V.

Results:
All four scaffolds displayed good fiber formation and alignment. Figure 1 shows the inner core/outer shell for all four of the scaffolds. The average scaffold fiber diameter was 0.9475 ± 0.48 µm for 83/17, 0.9075 ± 0.41 µm for 60/40, 0.9426 ± 0.29 µm for 50/50, and 0.7821 ± 0.24 µm for 40/60. The fiber diameters ranged from 0.5156-3.009 µm for 83/17, 0.4305-2.931 µm for 60/40, 0.3860-1.672 µm for 50/50, and 0.3781-1.302 µm for 40/60. Although the averages for the 83/17, 60/40, and 50/50 scaffolds are similar, the fiber diameter range for the 50/50 scaffolds is lower. Also, both the fiber diameter average and range for the 40/60 scaffolds is smaller than the other three scaffolds.

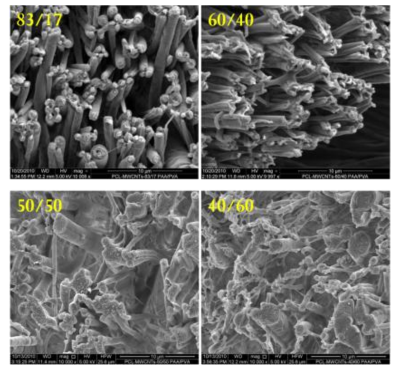

Figure 1: Cross-sectional SEM images displaying the fiber inner core/outer shell for 83/17, 60/40, 50/50, and 40/60 scaffolds.

All of the scaffolds were exposed to a constant voltage, and current and the resulting current output measured. The average current output and calculated average conductivity for each is displayed in Table 1. Current outputs for all the scaffolds are fairly similar. The scaffold conductivity increases as the amount of PVA increases; however, there is no significant difference.

Table 1: Scaffold average current output and conductivity.

Scaffold	Current Output (A)	Conductivity (S/cm)
83/17	0.013 ± 0.003	0.028 ± 0.006
60/40	0.012 ± 0.002	0.031 ± 0.003
50/50	0.011 ± 0.002	0.033 ± 0.006
40/60	0.011 ± 0.003	0.046 ± 0.014

All four of the scaffolds actuated when 10V, 15V, and 20V were applied. None of the scaffolds actuated when stimulated with 5V. However, the degree of actuation and speed of actuation varied between the different scaffold types and at different voltages.

Conclusions:
PCL, MWCNT, and several ratios of PAA/PVA (83/17, 60/40, 50/50, 40/60) were successfully coaxial electrospun as evidenced by the characteristic fiber inner core and outer shell. All four of the scaffolds were conductive and electroactive when 10V, 15V, and 20V were applied. Further analysis will involve a cell study with primary rat skeletal muscle cells, ascertaining the angle of scaffold actuation, and determining the speed of scaffold actuation.