Response of chitosan/PCL nanofibers with airway epithelial cells

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Statement of Purpose: Polymer scaffolds and metallic stents have emerged as viable options for airway obstructions including such as stenosis. Stents are often coated with tissue engineered scaffolds to enhance biocompatibility, repair and regeneration of several different complex tissues such as trachea, cartilage, and heart valves. Ideally, the scaffold should mimic the target environment and maintain the required tissue-specific mechanical properties. Chitosan (CS), a naturally occurring polymer, is biorenewable, biodegradable, biocompatible, and bio-functional. Recent studies have shown that CS promotes cell attachment, differentiation and tissue growth, but alone, has weak mechanical strength. Polycaprolactone (PCL) is a synthetic biodegradable polymer that is mechanically stronger. CS based composites will demonstrate favorable performance in airway tissue engineering applications. In our research, we developed a bio-friendly technique to develop electrospun nanofiberous scaffold of chitosan/PCL. We also studied cellular response of these nanofibers with airway epithelial cells by utilizing liquid-air interface cell seeding technique [2, 3].

Methods: Chitosan/PCL nanofibers were prepared by using the elctrospinning technique. Chitosan was first depolymerized by using sodium nitrite ($NaNO_2$) and then dissolved with DI water. PCL solution is prepared in trifluoroethanol. Chitosan and PCL solutions were combined at different ratios and applied for electrospinning [1]. The high voltage source was set to 20 kV, a syringe containing solution was positioned at -50°, and the distance between the syringe and rotating collector was approximately 11 cm. The nanofibers produced were then subjected to study the microstructure analysis, chemical structure analysis and mechanical strength analysis by using scanning electron microscopy (SEM), Fourier transform Infrared (FT-IR) spectroscopy and Shimadzu Tensile Machine, respectively . Nanofiber membranes were cut into rectangular shapes (~30 mm^2) and prepared for cellular exposure followed by sterilization technique that included a repeated ethanol wash. Sterilized nanofibers membrane were placed on apical surface of fully differentiated normal human bronchial epithelial (NHBE) cells for 24 hours. Cellular responses were evaluated via measuring inflammatory modulators, lactate dehydrogenase (LDH), and mucin secretion levels.

Results: Aqueous system based chitosan solution was successfully mixed with various weight ratios of PCL solution (chitosan/PCL: 50/50, 40/60, 30/70, 20/80 and 10/90). Mixed solution produced nanofibers successfully by using popular electrospinning technique. Several methods were used to analyze the physical and chemical properties of the composite nanofiber material. Under the SEM analysis, as synthesized nanofibers were observed in the sizes range from 100 to 800 nanometers. Mechanical testing proved that the 90/10 ratio of PCL/CS was the strongest composition and the most elastic fiber composition was a result of the 70/30 ratio. The mechanical and FTIR test results correlate with similar test data seen published literatures. X-ray diffraction (XRD) confirmed the diminishing characteristics of crystalline phage of PCL due to the presence of chitosan in the fibers. LDH assay was able to determine cytotoxicity levels in response to each composition of nanofiber. Cytotoxicity levels for all fibers were incredibly low. Also, as the composition of chitosan increased in the fibers, cytotoxicity levels decreased at the 24 hour time point. Mucus secretion levels in Fig. 1 show that there is seldom change between the control (cells only) and the levels seen from nanofiber interaction.

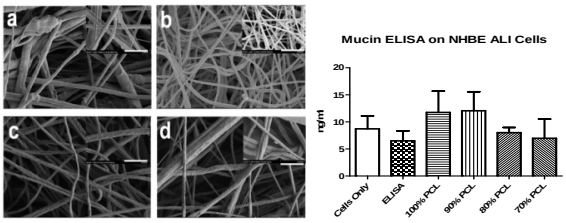

Figure 1. SEM images of CS/PCL nanofibers: (a) 100% PCL, (b) 10/90, (c) 20/80, (d) 30/70. Chart displays a statistical average of the amount of mucus gathered from cells after 24h time period in each nanofiber composition.

Conclusions: PCL and depolymerized chitosan solution were mixed together in a continuous phase and successfully created fibers in the nanoscale range. Our study suggests that there are no major issues with normal human bronchial epithelial cells when coming in contact with the PCL/CS nanofibers. These results showed potential application of chtosan/PCL nanofiber to repair and regeneration of bronchial tissues.

References:

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