Plasma Surface Modification of Electrospun Poly(ε-Caprolactone) Nanofibers for Potential Tissue Engineering Scaffold Applications

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Statement of Purpose: The nanoscale dimension and slow degradation rate of electrospun Poly(ε-caprolactone) (PCL) nanofibers make them suitable candidates for tissue-engineered scaffolds.[1,2] Unfortunately, PCL nanofibers have low surface wettabilities that could impair cell binding. To overcome this problem, plasma surface treatment of PCL nanofibers using a radio frequency (RF) glow discharge (RFGD) has been proposed. When a nitrogen-containing gas is used to sustain the RFGD, hydrophilic groups will be incorporated onto the surface, thereby enhancing the cell-binding capability of the PCL.

Methods: A 9 wt% PCL solution was prepared by dissolving PCL (Aldrich Chemical Co., Milwaukee, WI) in a 3:1 by volume mixture of chloroform and methanol. The PCL was allowed to dissolve for 24 h and was then drawn into a 10 ml plastic syringe fitted with a 22 gauge blunt-end stainless steel needle. A high power supply was used to deliver 20 kV to the needle. A syringe pump was used to deliver the polymer solution at a pump rate of 0.01 ml/s. Randomly arranged nanofibers were deposited onto an electrically-grounded 15 cm x 10 cm aluminum panel set 17 cm from the tip of the needle. The total collection time was 1 h. The PCL mats were then immersed in deionized water for 3 days to remove residual solvent. Samples were then dried overnight at 25°C.

Following drying, PCL nanofiber mats were placed inside a bell jar-type plasma reactor with dimensions of 46 cm in height and 44.5 cm in diameter. The sample was placed between two titanium electrodes of dimensions 17.9 cm x 17.9 cm x 0.08 cm. The reactor was pumped down to less than 1 mTorr (0.133 Pa) pressure. The treatment gases were then introduced to the reactor and the pressure was allowed to stabilize at 100 mTorr (13.3 Pa). In this study, four gas mixtures were used: NH_3, N_2, 1:1 N_2/H_2, and 2:1 N_2/H_2, respectively. A 13.56 MHz RF plasma power source was then used to excite and sustain the RFGD at powers of 20 and 30 W (RF) for a period of 10 min.

Surface wettabilities were assessed by measuring the contact angle of a 1 µl sessile deionized water droplet deposited onto the nanofiber surfaces with a contact angle measuring system (AST Products Inc., Billerica, MA). Measurements were taken immediately after treatment.

Cell culture of the PCL nanofiber mats was performed with the mouse osteoblast MC3T3-E1cell line acquired from American Type Culture Collection (ATCC). Cells were grown on 75 cm^2 culture flasks at 37°C in 5% CO_2 modified α-MEM lacking ascorbic acid (GIBCO), supplemented with 10% fetal bovine serum and antibiotics. The cell medium was changed every 3 days until 90-95% confluence was reached. Cells were passaged with 0.05 wt% trypsin/EDTA (Invitrogen Corp., Carlsbad, CA). Three ml of the cell solution was seeded onto 2 cm x 2 cm nanofiber mats, and the final cell concentration was 5 x 10^4 cells ml^{-1}. An MTT assay was used to assess proliferation and vitality.

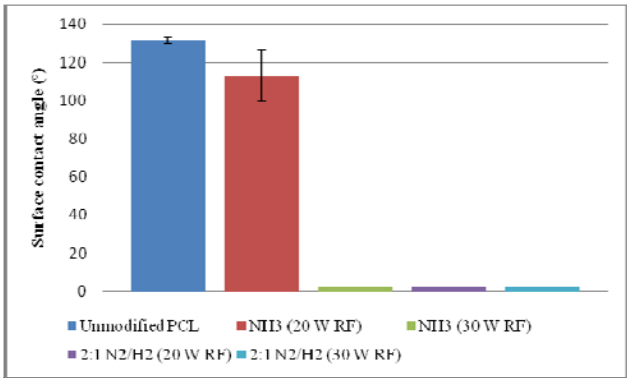

Figure 1. Surface contact angle measurements of RFGD-modified PCL nanofibers at 20 or 30 W (RF). Treatment time was 10 min.

Results: Representative surface wettabilities of plasma treated PCL fiber mats and their untreated controls are shown in Figure 1. Most nitrogen plasma-treated samples exhibited higher wettabilities after the treatments. The major exception to wettability enhancement was the 20 W (RF) NH_3 treatment.

FTIR transmittance spectra of the plasma treated samples showed new peaks in the 2:1 N_2/H_2 surface modified samples. Particularly, several small peaks appear between 1160 and 1260 cm^{-1} indicating C-N bond stretching.

The MTT assay is being conducted to evaluate osteoblast proliferation on the plasma modified samples compared to the unmodified PCL controls.

Conclusions: The nitrogen-containing plasmas used in this study produced more wettable PCL surfaces capable of sustaining enhanced cell growth. Further studies should take into account the hydrophobic recovery of the nanofibers surfaces over prolonged periods of time (i.e., months). Additionally, cell culture on aligned nanofibers could be done as they may provide directionality and more extensive proliferation of the osteoblasts.

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References:

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