Cytocompatibility of Electrospun PCL/CNF Scaffolds with and without Electrophoretically Deposited HA

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Statement of Purpose: Due to significant limitations of current therapies for bone fractures and diseases such as osteoporosis, tissue engineering seeks to provide next generation bone materials [1]. An alternative material should possess the favorable characteristics for cell growth along with appreciable strength. Electrospun biodegradable synthetic polymer polycaprolactone (PCL) scaffold matches the nanoscale morphology of the cells, promoting their growth [2]. A composite of this polymer with carbon nanofibers (CNFs) increases the strength of scaffold [3]. A coating of Hydoxyapatite (HA) due to its similar chemical composition as bone could bind to variety of enzymes and protein molecules [4]. A PCL-CNF composite scaffold with nanoHA coating could be required alternative material. In a previous study the current authors successfully mimicked the dimensions of extra cellular matrix in electrospun PCL tissue scaffold with nanometer fibers [5]. The CNFs (much stronger than a bone) enhanced the mechanical properties of the scaffold, thus reducing the mechanical mismatch between the scaffold and bone [5].The focus of current research was to study the cytocompatibility of the PCL-CNF composite scaffold. Mesenchymal stem cells (MSCs) were successfully grown on the scaffold, suggesting the attachment of these cells to the scaffold. Electrophoretic deposition of nano hydroxyapatite (nanoHA) on the nanofibrous scaffold was also investigated.

Methods: PCL pellets (Lactel Absorbable polymer, Pelham, AL.) dissolved in a solvent (chloroform: methanol =3:1v/v) to achieve a 10 wt% solution. The solution was electrospun at voltage of 11-13 kV and feed rate of 1-3 ml/hr in a syringe pump on to a grounded rotating collector. The collector rotating at 6000 rpm was placed 12 cm from the tip of the needle. CNFs (Pyrograph III TM grade PR-19-HT, acquired from Applied Science Incorporated) were modified as discussed in [5]. Modified CNFs were dispersed in varying amounts in the solvent by ultrasonication followed by dissolution of PCL into it. Nanofibers were spun at above mentioned specifications. For Electrophoretic deposition the nanofibrous scaffolds were exposed for 24 hours in a solution of nanoHA particles of ~ 100nm (Nanocerox Inc. Ann Arbor, MI) and isopropanol with an applied voltage of 24 volts. The coating of the nanoHA particles onto the nanofibers was confirmed by SEM (Philips SEM515). The composition of the particles was determined using the EDAX detector on the SEM, and the phase and crystal orientation was determined by XRD (Siemens D500). Human Mesenchymal Stem Cells were seeded onto the scaffolds and allowed to adhere for varying times. Cells/ scaffolds were fixed and processed for SEM using standard methods.

Results: X-ray analysis confirmed the presence of nanoHA particles present on the scaffolds. SEM images of the PCL scaffold showed the blockage of the scaffold pores by nanoHA. This blockage of porous morphology might restrict the cell growth only onto the surface. However, images from PCL-CNF showed that the nanoHA particles were coated on to the fiber surface leaving a porous network in the scaffold. This may be related to the enhanced electrical conductivity of CNFs, which helps in electrophoretic deposition. The nanoHA particles on the fiber surface may help in improving properties such as biocompatibility. Qualitative analysis showed MSCs adhered to scaffolds of both PCL and PCL-CNF composites with and without nanoHA coating on them. Cells were alive and adhered on PCL-CNF composite scaffold, even after 14days, confirming its cytocompatibility. This establishes CNF's safe usage. The inclusion of nanoHA in the scaffolds did not appear to significantly alter the initial cell adhesion or cell survival however; the potential effect of nanoHA on other MSC responses such as osteoblastic differentiation needs further research. Also, research is currently underway to investigate the effects of increased concentration of CNFs on electrophoretic deposition and cell response.

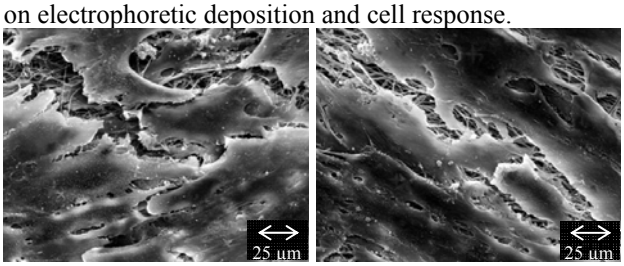

Figure 1. Adhered MSCs on PCL (left) and PCL-0.1% CNF (right) after 14 days. Cytocompatibility of PCL-0.1% CNF scaffold (right).

Conclusions: CNFs in the scaffold increased the effectiveness of the electrophoretic deposition of nanoHA. The adhesion of MSCs proved the cytocopatibility of PCL-CNF composite scaffold. PCL-CNF could be the required alternative material for scaffolds as it possesses strength and cytocompatibility.

References:

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