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DIRECTED OSTEOBLAST FUNCTIONS ON MICRO-ALIGNED PATTERNS OF CARBON NANOFIBERS ON A POLYMER MATRIX

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Abstract. Carbon nanotubes/nanofibers have generated a large amount of interest for a number of applications from solid state devices to biocomposite implantable materials. Naturally, there has been considerable curiosity to utilize carbon nanotubes/nanofibers to benefit human health. In this paper, we describe one promising application of carbon nanofibers in medicine: regeneration of the human skeleton system. More specifically, micro-patterns of carbon nanofibers were created on a polymer matrix to mimic the alignment of collagen and hydroxyapatite in bone. Results showed directed osteoblast (bone-forming cells) adhesion and deposition of calcium phosphate containing mineral on micro-patterns of carbon nanofibers; such results demonstrate that carbon nanofibers patterned on polymers should be further studied for orthopedic applications.

1. INTRODUCTION

There has been an increasing interest for self-assembled, functionalized, hybrid carbon nanotube based materials for controlling specific cell interactions [1,2]. Carbon nanotubes/nanofibers have many advantages in tissue engineering, especially in orthopedics. This is because they are light-weight, strong and, possibly more important, can mimic the nanometer structures of components of bone (such as hydroxyapatite and collagen) [3,4]. Bone has also been shown to regenerative under electrical stimuli [5], thus conductive nanophase materials (like carbon nanotubes) may play a role in promoting osteoblast (bone-forming cells) activity important for orthopedic applications. Previous studies have shown increased adhesion [6,7], viability [8] and deposition of calcium [9] by osteoblasts when cultured on carbon nanotube/nanofiber based materials. However, in all of these studies, non-aligned carbon nanotubes/nanofibers in polymers were investigated. Since long bones in the body are highly anisotropic, the objective of this in vitro study was to align micro-patterns of carbon nanofibers in a polymer matrix and determine subsequent osteoblast functions on these constructs.

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2. MATERIALS AND METHODS

2.1. Carbon nanofibers (CNFs)

Carbon nanofibers produced by carbon vapor deposition were obtained from Applied Sciences, Inc. (Cedarville, OH). These carbon nanofibers have a polynuclear aromatic hydrocarbon (PAH) layer, which is usually called a pyrolytic layer, formed during the production process. Besides hydrophobic properties, a pyrolytic insulating outer layer has a lower surface energy (approximately 25 mJ/m^2) compared to pyrolytic-free carbon nanofibers (approximately 200 mJ/m^2). Only carbon nanofibers with a pyrolytic outer layer were used in the present experiments. The diameter of each carbon nanofiber (CNF) used was 100 nm [7].

2.2. Polycarbonate urethane (PCU)

An FDA-approved polycarbonate urethane (PCU, catalog # PC-3575A, Thermedics, MA) was used as the model polymer in this study since it has a high melting temperature (above 200 °C), is FDA approved for implantation, and is non-degradable.

2.3. Micro-patterned CNF arrays on PCU

To construct micro-patterns of CNFs on PCU, a novel imprinting method was developed [10]. For CNF alignment, PCU was melted by chloroform, and was coated on a glass surface. After chloroform evaporation, a Au grid (with 20 μm width spacings) was attached. Dispersed CNFs in ethanol were then placed into the spacings of the grid, the Au grid was removed from the PCU surface, and patterned CNF arrays on PCU were subsequently made.

2.4. Osteoblast adhesion

Substrates were sterilized in an autoclave and were exposed to UV light for 24 hours before cell culture. Osteoblasts (CRL-11372, American Type Culture Collection, population numbers 2-5) were cultured on the different substrates under standard cell culture conditions (i.e., a 37 °C, humidified, 5% CO_2/95% air environment). Osteoblasts were cultured in Dulbecco's modified eagle medium (DMEM, Gibco), supplemented with 10% fetal bovine serum (FBS, Hyclone) and 1% penicillin/streptomycin (P/S, Hyclone), under standard cell culture conditions. Human osteoblasts were seeded at a density of 2,000 cells/cm^2 (sub-confluent) onto each substrate and were incubated under standard cell culture conditions in osteoblast growth media (DMEM, 10% FBS, and 1% P/S) for 2 days. At the end of the time period, non adherent cells were removed by rinsing in PBS while adherent cells were fixed with 4% formaldehyde (Fisher) and were stained with Rhodamine Phalloidin (R415, Molecular Probes) to visualize F-actin filaments and Hoechst dye (33258, Sigma) to visualize the nucleus.

2.5. Osteoblast calcium phosphate mineral deposition

To determine calcium phosphate mineral deposition on the micro-aligned CNFs in PCU substrates, osteoblasts were cultured (seeding density: 600,000 cells/cm^2) in DMEM supplemented with 10% FBS, 1%P/S, 10mM β-glycerophosphate (Sigma), and 50 μg/ml L-Ascorbic Acid (Sigma) under standard cell conditions for 21 days. Osteoblast growth media was replaced every other day. After that time period, cells were lysed with three freeze-thaw cycles in deionized water to leave only the calcium phosphate crystals deposited by osteoblasts. Energy dispersive spectroscopy (EDS) was used to determine deposited mineral chemistry.

2.6. Surface characterization

Images of micro-aligned CNF patterns on PCU were evaluated by fluorescence microscopy (DM IRB, Leica) and Scanning Electron Microscopy (SEM: JSM-840, JEOL). For this purpose, CNF arrays on PCU were mounted using double stick carbon tape and were sputter coated with AuPd prior to imaging at room temperature. Cell images were taken using fluorescence microscopy (DM IRB, Leica) with two different excitation wavelengths (400 nm and 550 nm) to visualize the cell nucleus and f-actin filaments, respectively.

3. RESULTS AND DISCUSSION

Aligned CNF in PCU. As expected, fluorescence microscopy results of this study showed highly aligned micro-arrays (20 μm) of CNFs successfully patterned onto PCU (Fig. 1a).

Selective adhesion of osteoblasts on micro-patterns of CNFs on PCU. The results of the present study provided evidence of selective osteoblast adhesion and alignment on CNFs compared to PCU (Figs. 1b and 1c). Specifically, more than 80% of the osteoblasts adhered on CNF arrays but less than 20% on the PCU portion of the surface.

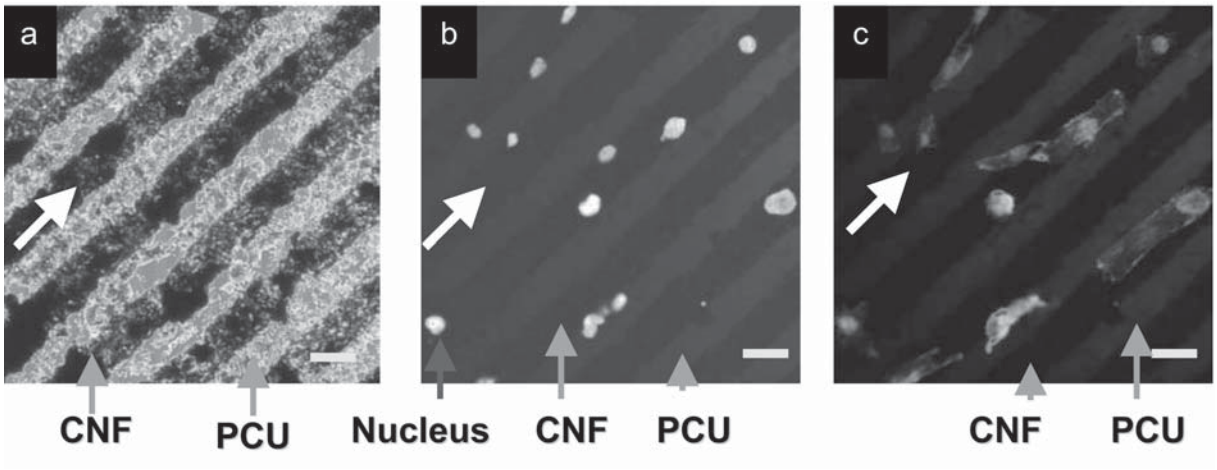

Fig. 1. (a) Micro-patterned CNFs on PCU; (b) Selective adhesion of osteoblasts on aligned micro-patterns of CNFs on PCU; (c) Selective adhesion of osteoblasts on aligned micro-patterns of CNFs on PCU. All scale bars are 20 μm. All arrows show aligned direction of CNF micro-arrays.

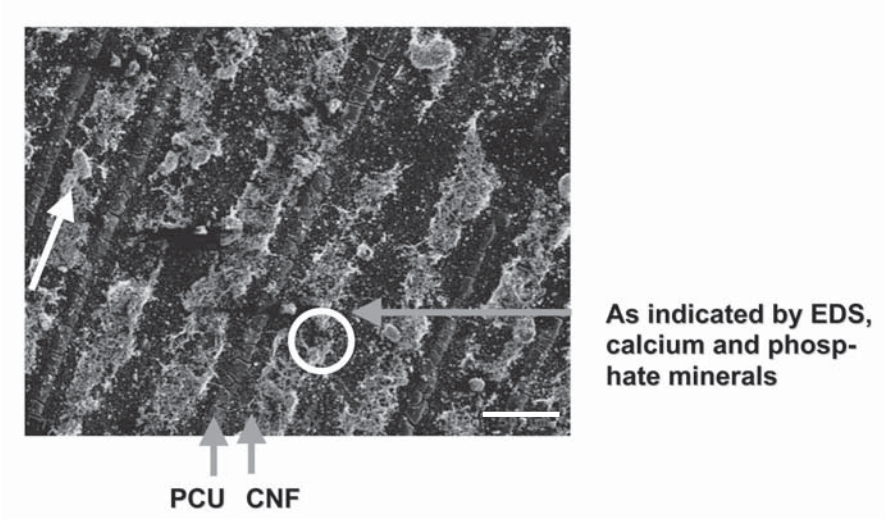

Fig. 2. Directed calcium phosphate mineral deposition on aligned micro-patterns of CNF on PCU. Osteoblast culture time = 21 days. EDS confirmed that these minerals are composed of calcium and phosphate. Scale bar = 50 μm. Arrow shows aligned direction of CNF micro-arrays.

Selective deposition of calcium phosphate minerals on micro-patterns of CNFs on PCU. After 21 days of osteoblast culture, directed deposition of calcium phosphate minerals were observed on CNF compared to PCU micro-patterns using SEM (Fig. 2) and EDS. Thus, the results of this study showed the ability to mimic the alignment of hydroxyapatite in bone on micro-patterned CNFs on PCU. Moreover, since this occurred on the conductive region of the substrate (CNFs), it is possible that future studies could use applied voltages to further improve osteoblast function. Lastly, since adhesion is a prerequisite for osteoblasts to deposit calcium, it was expected that the preferred attachment of osteoblasts on CNF over PCU regions would translate into preferred calcium phosphate mineral deposition directed on CNF micro-patterns.

4. CONCLUSIONS

In conclusion, we observed selective osteoblast adhesion on aligned patterns of carbon nanofibers (CNFs) on a polymer (PCU) matrix. Moreover, we observed enhanced calcium phosphate mineral deposition by osteoblasts along CNF micro-patterns on PCU matrices. These results demonstrated the optimal interactions osteoblasts have with CNFs. Their ability to increase osteoblast function may be used as novel implant nanophase materials in bone tissue engineering. Lastly, these results strongly

suggest that CNF micro-patterns in PCU should be further studied for orthopedic applications.

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