Enhanced Fibronectin Adsorption on Carbon Nanotubes in Polycarbonate Urethane Composites Directs Osteoblast Adhesion

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Introduction: Understanding of implant surface specific protein interactions is very important[1]. This is because such information can give us the ability to control specific cell functions on biomaterial surfaces. Previous results[2] showed increased osteoblast (bone forming cell) adhesion on higher weight ratios of carbon nanofibers (CNFs) in polycarbonate urethane (PCU). However, the fundamental mechanism was not elucidated. For this reason, the objective of the present study was to investigate surface energy and roughness relationship with adsorption of fibronectin on CNT/PCU surfaces. For these experiments, we developed a new coating method for making highly dispersed, stable and homogeneous carbon nanotube (CNT)/polymer substrates. ELISA (Enzyme-Linked-Immunosorbent- Assay) experiments were also used to observe how much fibronectin (an osteoblast adhesive protein) adsorbed on CNT/PCU surfaces.

Materials / Methods: Multi-wall carbon nanotubes (purified to 99.9% with 10nm diameter, Catalytic Materials, MA) and PCU (PC3575A, Thermedics) were used to make CNT/PCU composites. Glass coverslips (Fisher) were used as base substrates. Carbon nanotubes and PCU were highly sonicated in 1, 2-dichloroethane (Sigma) with chloroform (Sigma) and were mixed together. Specific weight ratios (CNT: PCU=1:2, 1:4, and 1:6 by weight ratio) of well dispersed CNT in PCU on 12mm diameter glass coverslips were created. After 1 day of drying in air, thinly coated CNT/PCU on glass substrates were stored in a vacuum chamber to evaporate the left over 1, 2-dichloroethane and chloroform. Also, contact angle measurements (DSA100, Kruss) were used to determine surface energies of the CNT/PCU surface. Roughness measurements were completed using multimode AFM (Nanoscope Ⅲa, Veeco: Digital Instruments Inc. Santa Barbara, CA) scan areas of 30 μm×30 μm with 3 randomly selected areas for each sample. Bovine plasma fibronectin (FN, Sigma) was used for ELISA tests. To calculate surface energies of the CNT/PCU composites, the Owens-Wendt approach was used after obtaining contact angles from 3 different solvents (PBS, Glycerol, DMSO) using contact angle measurements.

Results / Discussion: Results showed for the first time that surface energy, composed of nonpolar and polar terms, increased with higher CNT weight ratios in PCU [Figure 1]. Roughness (RMS) also increased with higher CNTs in PCU. Most importantly, ELISA results show that fibronectin adsorption increased for higher weight ratios of CNTs in PCU composites [Figure 2]. Results also showed that increased weight ratios of CNTs in PCU create higher roughness, higher surface effective area, higher surface energies and eventually higher fibronectin adsorption: such events may be helpful to explain earlier results of enhanced osteoblast adhesion on CNTs in PCU.

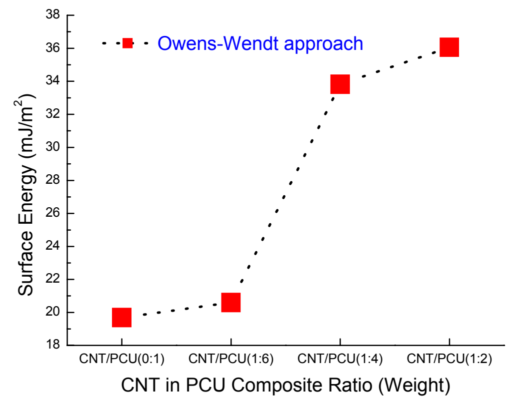

Figure 1. Increments of surface energy of CNT/PCU of various weight ratios.

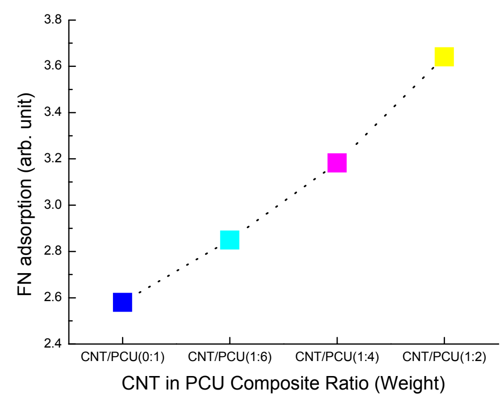

Figure 2. Increments of fibronectin adsorption on CNT/PCU of various weight ratios.

Conclusions: Surface energy and roughness were very important for mediating the adsorption of fibronectin on CNT/PCU surfaces and given previous results provide strong reasons why increased osteoblast adhesion was observed on increased CNF/PCU composite.

References: 1. Biomaterials Science (An introduction to Materials in Medicine), edited by Buddy, D. Ratner and Alla S. Hoffman *et al.,* Academic press, 1996
2. Thomas J. Webster *et al., Nanotechnology* 15 48-54 (2003)