ANALYSIS OF CARBON NANOFIBERS AND POROUS SILICON FOR NEURAL APPLICATIONS

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Abstract

Chronic neural implants are usually made from silicon materials and are subject to scar tissue formation at the tissue/implant interface, which interferes with their functionality. Carbon nanofibers are an example of a material that may improve neural implant interactions with native cell populations since these nanofibers have promising cytocompatibility, mechanical, and electrical properties. Neural implants may achieve better tissue interactions simply by incorporating carbon nanofibers into a silicon matrix. The objective of the present in vitro study was to determine cytocompatibility properties of carbon nanofibers and porous silicon materials. Carbon fiber substrates were prepared from carbon fibers with either nanoscale or micron scale diameters, and both high and low surface energy fibers were investigated. Porous silicon was prepared by treatments resulting in mesoscale pores with nanoscale roughness between pores. Astrocytes (glial scar tissue-forming cells) were seeded separately onto the carbon fiber and silicon substrates. Astrocytes preferentially adhered on the largest diameter carbon fiber with the lower surface energy and preferred the silicon sample with the greatest porosity. These results indicate that nanoscale surface roughness may deter astrocyte adhesion. Controlling carbon fiber diameter and silicon porosity may be approaches for increasing implant contact with neurons and decreasing scar tissue formation.

Key Words
Carbon nanofibers, porous silicon, astrocytes

1. Introduction

Biomaterials for neural applications have been implemented in many ways including tissue bridges and probes. Since such implants require unique biocompatibility properties to function successfully in the presence of native tissue, new formulations of biomaterials are currently being investigated to customize materials for these neural applications. A key design parameter is the reduction of scar tissue interference at the site of the implant. This gliotic response is mediated largely by astrocytes in the central nervous system [1,2]. Design of synthetic biomaterials that mimic the properties of natural tissues is a promising method to minimize adverse reactions such as the foreign body response and scar tissue formation. Cells of the body are accustomed to interacting with surfaces with a large degree of nanostructured surface roughness due to the size of proteins that compose the extracellular matrices in vivo. In vitro studies with nanophase biomaterials have indeed shown that cells respond differently to materials with nanoscale roughness when compared to those with micron-sized roughness [3,4].

Carbon fibers have been shown to be compatible with physiological tissues, and nano-dimensioned fibers have excellent conductivity and high strength to weight ratios [3,5,6,7]. The size of carbon nanofibers contributes to their strength and high conductivity, but since their size is also in the nanometer regime, they are on the same scale as physiological proteins. Conceivably these nanofibers can be integrated with conventional neural implant materials such as silicon to improve cell interactions with these implant materials. The objective of this present in vitro study was to explore the cytocompatibility properties of carbon nanofibers and porous silicon separately with astrocytes cells to facilitate neural biomaterial design.

2. Body of Paper

2.1 Substrates

Two type of porous silicon were obtained from Spire Biomedical (Bedford, MA) and are shown in Figures 1 and 2. The first silicon used was n type, with orientation <100> and resistivity of 1.0-10.0 ohm cm (Figure 1). It was anodized in a 2:1:1 ethanol/HF/H2O solution, using a Pt cathode for 60 min with 300 mA of current. While anodizing, the back side of the Si wafer was illuminated with ultraviolet light (term low porosity silicon). The second silicon sample shown in Figure 2 was treated the same as above except that 400 mA was used, and instead of the back side being illuminated, the front side of the
wafer (the same side that was being etched) was illuminated by ultraviolet light (termed high porosity silicon). The low porosity silicon had nanoscale roughness and mesoscale porosity, including some nanoscale pores. The high porosity silicon displayed nanoscale roughness and relatively homogeneous mesoscale pores. The UV backlit illumination method produced much less porosity than the UV illumination on the front side. The pores in the low porosity silicon were periodic clusters separated by many microns of nonporous silicon with nanoscale roughness whereas the high porosity silicon surface was highly porous with very little separation between pores.

Multiwalled carbon fibers (CF) with four different diameters (from 60 to 200 nm) that had been synthesized using catalytic and chemical vapor deposition were acquired from Applied Sciences, Inc./Pyrograf Products, Inc. (Cedarville, OH) and are shown in Figure 3. The fibers were separated into two groups, those considered to be conventional (with diameters greater than 100 nm, specifically 125 and 200 nm), and those classified as nanophase (with diameters of 100 nm or less, specifically 60 and 100 nm). In each group of fibers a high surface energy (SE; 125-140 mJ/m$^2$) and low surface energy (25-50 mJ/m$^2$) fiber was represented. The low surface energy fiber was left as grown, and the high surface energy fiber was obtained by pyrolytic stripping the carbon fiber to remove the outer hydrocarbon layer. Each type of carbon fiber was uniaxially pressed using a steel-tool die at 4000 psi for 3 minutes at room temperature to obtain a compact disc (1.327 cm$^2$ surface area) for cytocompatibility studies. Etched glass coverslips were used as reference material.

2.2 Cell culture

Rat astrocytes were obtained from American Type Culture Collection (CRL-2005) and cultured in Dulbecco’s Modified Eagle Medium (DMEM;Gibco), supplemented with 10% fetal bovine serum (FBS;Hyclone), and 1% penicillin/streptomycin (P/S;Hyclone) in a standard cell culture environment (37°C, humidified, 5% CO$_2$/95% air). These cells were seeded at a density of 3,500 cells/cm$^2$ onto the substrates and were cultured for one hour. Cells were then rinsed with phosphate buffered saline (PBS) to remove nonadherent cells, fixed with formaldehyde (Fisher Scientific), and stained with Hoechst 33258 dye (Sigma). The visible cell nuclei were counted using fluorescence microscopy (365 excitation; 400 nm emission). The average cell count was recorded per square cm of sample substrate area. Experiments were run in triplicate and completed at least three different times. Statistical analysis based on the method of ANOVA was performed. p < 0.1 was used as statistically significant as commonly reported by other studies [8-10].

2.3 Results

Astrocyte adhesion was the greatest on the micron scale, low surface energy carbon fiber, and this result was significant (p < 0.083) when compared to all of the other substrates except the high porosity silicon sample (see Figure 4). The number of astrocytes that adhered to high surface energy carbon fibers of both the nanophase and conventional size regimes was similar; these results were also comparable to the low porosity silicon adhesion. The cells may have responded similarly to surfaces with nanoscale roughness, although it appears as though the surface energy also influenced adhesion. It is interesting to note that the highly porous silicon had greater adhesion than the silicon with less porosity and more surface area for cell interactions.
3. Conclusion

Astrocytes appear to favor micron scale diameter and low surface energy carbon fibers in vitro. Increased nanoscale surface roughness (the silicon with the least porosity and hence the greatest nanoscale roughness) seemed to reduce astrocyte adhesion. On the other hand, silicon with nanoscale roughness has been shown to favorably affect adhesion and viability of neural cells [11]. Neurite outgrowth and branching has also been shown on functionalized carbon nanotubes [12]. These findings indicate that perhaps using a nanophase carbon fiber, and/or porous silicon with a substantial amount of nanoscale roughness for a neural biomaterial formulation would limit gliotic scar tissue formation (mediated largely by astrocytes) and perhaps maintain positive interactions with surrounding neurons. In order to gain the beneficial bulk mechanical properties of a supporting matrix, composites can be formed with carbon nanofibers. Composite carbon nanofiber materials show promise for neural biomaterial applications with tunable material properties [13]. Formulation of silicon and carbon nanofiber material combinations may produce enhanced cytocompatibility when compared with conventional silicon implant materials.

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References


