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CYTOCOMPATIBILITY OF CARBON NANOFIBERS FOR USE AS A NEURAL BIOMATERIAL

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Abstract – **Carbon nanofibers possess excellent conductivity properties, which may be beneficial in the design of more effective neural prostheses. The objective of the present *in vitro* study was to determine cytocompatibility properties of carbon nanofibers pertinent for neuronal prosthetic applications. Carbon fibers were compacted in a manual hydraulic press *via* a uniaxial loading cycle. The substrates were prepared from four different types of carbon fibers, two with nanoscale diameters and two with microscale diameters. In these two categories, both a high and a low surface energy fiber were investigated. Astrocytes (glial scar tissue-forming cells) were seeded onto the substrates for adhesion, proliferation, and long-term function studies. Astrocytes preferentially adhered, proliferated, and showed the greatest alkaline phosphatase activity on the carbon fiber that had the largest diameter and the lowest surface energy. For this reason, carbon fibers in the nanometer regime with high surface energies may limit astrocyte interactions. Positive interactions with neurons, and, at the same time, limited astrocyte functions leading to scar tissue formation are essential for increased neuronal implant efficacy.**

Keywords – **Carbon nanofibers, neural biomaterial, cytocompatibility, astrocytes, neurons**

I. INTRODUCTION

Neural prostheses provide a means for monitoring and applying electrical signals to neural tissue. The biomaterials used in neural prostheses need to be able to transmit and receive electrical signals while supporting and enhancing nerve cell viability and neurite extension at the implant region. At an implant site, a cellular process known as gliotic response partitions the implant from surrounding tissue by scar formation. This scar tissue development is largely due to the activity of astrocyte cells, and is thought to interfere with the long-term operation of implanted neural prosthetic devices [1,2]. The development of biomaterials that enhance nerve cell interactions and deter astrocyte formation of scar tissue provides an attractive alternative to conventional neural prosthetic materials.

Recent studies confirm that nanoscale biomaterials achieve better cytocompatibility with certain types of cells than their conventionally sized counterparts [3,4]. Carbon nanofibers possess excellent electrical properties, which may be desirable in the area of neural prostheses. The objective of the present *in vitro* study was to determine cytocompatibility properties of carbon nanofibers pertinent to neural prosthetic biomaterial formulations.

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II. METHODOLOGY

A. Substrates

Carbon samples with a surface area of 1.327 cm^2 were prepared by compacting carbon nanofibers (Applied Sciences, Inc./Pyrograf Products, Inc.) in a manual hydraulic press *via* a uniaxial loading cycle (4000 psi for a three minute period at room temperature) and then were sterilized by exposure to ultraviolet radiation. The samples were made from four different types of carbon nanofibers, PR-1-AG (AG stands for as grown), PR-19-AG, PR-23-PS (PS stands for pyrolitically stripped), and PR-24-PS that were initially formed by catalytic and chemical vapor deposition. PR-1-AG and PR-19-AG (diameters of 100 and 200 nm, respectively) have surface energies of 25-50 mJ/m^2 due to hydrocarbon coatings. PR-23-PS and PR-24-PS (diameters of 125 and 60 nm, respectively) have surface energies of 125-140 mJ/m^2 due to lack of hydrocarbon coatings. Borosilicate glass coverslips etched in 1 N NaOH, then autoclaved, were used as reference substrates and prepared for cell adhesion experiments according to standard protocols [5].

B. Cell Cultures

Rat astrocytes were obtained from ATCC (No. CRL-2005) and cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco), supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin (P/S; Hyclone) in a standard cell culture environment (37° C, humidified, 5% CO_2 / 95% air). PC-12 cells (ATCC No. CRL-1721) were cultured in RPMI medium (Sigma) supplemented with 10% horse serum (Gibco), 5% FBS, and in a standard cell culture environment.

C. Cell Adhesion, Proliferation, and Neurite Extension

1) *Astrocytes*: Cells were seeded at 3,500 cells/cm^2 onto the substrates and allowed to adhere for one hour. For proliferation studies, astrocytes were seeded in a similar manner and were cultured for three and five days. After the appropriate time period, astrocytes on carbon nanofiber substrates and glass surfaces were fixed with 4% formaldehyde (Fisher Scientific) and stained with Hoescht (No. 33258; Sigma). By counting the stained nuclei using fluorescence microscopy, and averaging the number of cells in five random fields per substrate, cell density was determined.

2) *PC-12 cells*: A seeding density of 1500 cells/cm^2 was used and then 1 hour was allowed for adhesion. Neurite

extension was performed in a similar manner to the PC-12 adhesion and cultured for time periods of three and five days, then the cells were fixed with 4% formaldehyde and stained with β3-tubulin and DAPI nuclear dye (Molecular Probes). Counting stained nuclei and averaging five random fields determined cell density for adhesion. Neurite length of the longest extension of 50 cells was measured using ImagePro software.

D. *Alkaline Phosphatase Activity*

Astrocytes were seeded and cultured (as above) on substrates for 7, 14, and 21 days. The media was then replaced with distilled water and exposed to three freeze-thaw cycles. Total intracellular protein content was then assessed spectrophotometrically with a BCA Protein Assay Kit (Pierce Chemical Company) by following the instructions. The protein concentration was determined from a standard curve obtained by running known albumin concentrations in parallel with the samples.

Alkaline phosphatase activity was determined using an alkaline phosphatase assay in conjunction with the total intracellular protein assay. Sigma Diagnostic Kit 104 was used for the alkaline phosphatase assay. A standard curve was used to correlate the absorbance to sigma units. The sigma units were divided by the relevant total intracellular protein and surface area.

E. *Statistical Analysis*

Experiments were run in triplicate and repeated at three different times. Statistical analysis was performed on the data sets using the student t-test.

III. RESULTS

Scanning electron micrographs (SEM) were taken to confirm the nanoscale dimension of each of the carbon fiber compacts used for in vitro cell studies (Fig. 1). The astrocyte cytocompatibility studies indicated a trend of increasing astrocyte adhesion and proliferation on all carbon nanofiber substrates when compared to borosilicate glass coverslips. More importantly, astrocytes preferentially adhered and proliferated on the PR-19-AG substrate, which has the largest diameter of the carbon nanofibers used (200 nm), and one of the lowest surface energies (25-50 mJ/m2), at the one hour, three, and five day time points. These results were statistically significant for the one hour and five day studies ($p < 0.1$ at one hour; $p < 0.05$ at five days).

IV. DISCUSSION AND CONCLUSIONS

Lastly, preliminary alkaline phosphatase studies indicate increased extracellular matrix protein deposition on PR-19-AG. These studies indicate that astrocytes prefer larger carbon nanofibers with lower surface energies. By minimizing astrocyte functions, carbon fibers with high surface energies and nanometer dimensions may be the next generation of neural prostheses with improved efficacy. Positive interactions with neurons, and, at the same time, limited astrocyte functions leading to scar tissue formation are essential for increased neuronal implant efficacy.

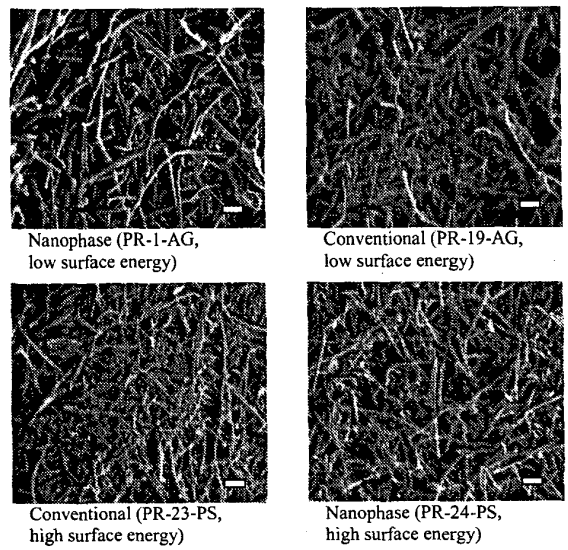

Fig. 1. SEM of carbon nanofiber compacts at 10000X magnification. Scale bar represents 1 micron.

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REFERENCES

[1] L. Kam, W. Shain, J.N. Turner, R. Bizios, "Correlation of astroglial cell function on micro-patterned surfaces with specific geometric parameters," *Biomat.*, vol. 20, pp. 23432350, 1999.

[2] C. Xinyan, V.A. Lee, Y. Raphael, J.A. Wiler, J.F. Hetke, D.J. Anderson, D.C. Martin, "Surface modification of neural recording electrodes with conducting polymer/biomolecule blends," *J. Biomed. Mat. Res.*, vol. 56, pp. 261-722, 2001.

[3] T.J. Webster, R.W. Siegel, R. Bizios, "Osteoblast adhesion on nanophase ceramics," *Biomat.*, vol. 20, pp. 1221-1227, 1999.

[4] T.J. Webster, C. Ergun, R.H. Doremus, R.W. Siegel, R. Bizios, "Enhanced functions of osteoblasts on nanophase ceramics," *Biomat.*, vol. 21, pp. 1803-1810, 2000.

[5] D.A. Puleo, L.A. Holleran, R.H. Doremus, R. Bizios, "Osteoblast responses to orthopaedic implant materials in vitro. *J. Biomed. Mat. Res.* vol. 25, pp. 711-723, 1991.