Supplementary Information

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Carbon Nanotube Fibers are Compatible with Mammalian Cells and Neurons

Robert A.Dubin[1], Gerardo C.Callegari[2], Joachim Kohn[1], and Alexander V. Neimark[*,3]

[1]The New Jersey Center for Biomaterials, Rutgers, The State University of New Jersey, Piscataway, NJ 08854
[2]Center for Modeling and Characterization of Nanoporous Materials, TRI/Princeton, Princeton, NJ 08542
[3]Department of Chemical and Biochemical Engineering, Rutgers, The State University of New Jersey, Piscataway, NJ 08854

[*]Corresponding Author,
Email: aneimark@rutgers.edu

Details of experimental studies and microscopy of cell culture

Cell Culture. L929 mouse fibroblasts (a gift from F.W. Pierson, Virginia Tech) were propagated in DMEM:F12 (1:1) supplemented with 10% horse serum. Human KB (ATCC, Manassas, VA), mouse NIH3T3 fibroblasts (ATCC), and primary, neonatal, Green Fluorescent Protein (GFP)-expressing rat dermal fibroblasts (a gift from D. Schreiber, Rutgers University) were propagated in DMEM supplemented with 10% fetal bovine serum (FBS). Saos-2 human osteoblasts (a gift from D. Denhardt, Rutgers University) were grown in F12H media supplemented with 10% FBS and were transfected with pEGFP-actin (Clontech, Mountain View, CA) using Lipofectamine (Invitrogen; Carlsbad, CA); transfected cells were enriched by selection in 0.5 mg/ml G-418. PC12 cells (ATCC) were propagated on collagen-coated CNF in DMEM containing 10% heat-inactivated horse serum (HIHS) and 5% FBS, and induced to extend processes in DMEM supplemented with 1% HIHS and 66 ng/ml nerve growth factor (NGF). Primary rat hippocampal neuronal cells (Cambrex Bioscience; Walkersville, MD) were grown on CNF pre-adsorbed with laminin and poly-D-lysine in neurobasal medium supplemented with 2% B27. All growth media was further supplemented with 2 mM glutamine, 100 U/ml penicillin, and 0.1 mg/ml streptomycin, and cells were grown at $37^{o}C$ in a humidified atmosphere containing 5% CO_2. CNF placed into wells of tissue culture plates were sterilized by exposure to UV for 1 hour in a tissue culture laminar flow hood. In some cases, fresh nanofelt was exposed immediately prior to UV sterilization by brief exposure of CNF to liquid nitrogen and subsequent breaking of the thread with forceps. DMEM:F12, DMEM, F12H, Dulbecco's modified phosphate-

buffered saline (D-PBS), glutamine, antibiotics, human plasma fibronectin, poly-D-lysine, mouse submaxillary gland NGF (2.5S fragment), rat-tail collagen, and Hoechst 33342 were from Sigma (St. Louis, MO). Neurobasal medium, B27 supplement, and mouse laminin were from Invitrogen.

Cytotoxicity. Released glucose-6-phosphate was quantified using the Vybrant Cytotoxicity Assay Kit (Molecular Probes, Eugene, OR) following conditions described by the manufacturer. Fluorescence measurements were acquired with a Spectra Max Gemini fluorescent plate reader (Molecular Devices, Sunnyvale, CA).

Microscopy. Cells were observed in visible and fluorescent light using the Nikon Eclipse TE 2000-S inverted microscope and digital photomicrographs obtained using Image Pro-Plus software (Media Cybernetics, Silver Spring, MD). Confocal microscopy was performed with the TCS SP2 laser-scanning, spectral confocal microscope and associated imaging software (Leica; Exton, PA). For scanning electron microscopy (SEM) of cells on CNF, preparations were dehydrated in ethanol, critical point dried with CO_2 (CPD 020 Critical Point Drier; Balzars, Lichtenstein), sputter-coated with gold palladium (SCD 004 Sputter Coating Unit; Balzars), and visualized using the Amray 1830 I (Bedford, MA). Cells processed for immuncytochemistry, SEM, and with phalloidin were fixed in 4% paraformaldehyde / D-PBS. Rat hippocampal neurons were permeabilized with 0.2% Triton-X 100 / D-PBS, blocked with 10% FBS / D-PBS, and reacted with a 1:500 dilution of anti-neuronal class III β-tubulin monoclonal IgG (Covance; Berkely, CA) in 2% FBS / D-PBS. FITC-phalloidin and Alexa Fluor 594-conjugated wheat germ agglutinin were obtained from Invitrogen and used following conditions suggested by the manufacturer.