Cell Growth on Single Wall Carbon Nanotube Fibers

Robert A. Dubin[1], Gerardo C. Callegari[2], John Kuppler[2],
Konstantin G. Kornev[2], Sigrid Ruetsch[2], Alexander V. Neimark[2], and Joachim Kohn[1].
[1]The New Jersey Center for Biomaterials*, Rutgers, The State University of New Jersey.
[2]Center for Modeling and Characterization of Nanoporous Materials, TRI/Princeton University and

Statement of Purpose: Recent discoveries in nanofiber technologies open up new avenues for fabrication of permeable and conductive supports from carbon nanotube-polymers composites. Carbon Nanotube Fibrous Substrates (CNFS) with designed hierarchical pore structure architecture may provide a suitable environment for tissue engineering and, especially, for generating electrically conductive interfaces with neuronal tissue. In this study, we evaluate the ability of our CNSF to support the attachment and proliferation of mammalian cells.

Methods: CNFS are produced from an aqueous dispersion of single wall carbon nanotubes (SWCNT) injected in a poly(vinyl alcohol) solution (PVA) with subsequent washing and drying [1,2]. SWCNT fibers are electrically conductive and posses a hierarchical pore structure with the specific surface area as high as $160 m^2/g$, variation of the pore sizes from 5 nm to 5 μm and electrical conductivity from 0.1 to 1 S/cm. Cell lines NIH3T3 and KB were obtained from ATCC. Primary, GFP-expressing, rat dermal fibroblasts (passage 25) were a gift from D. Shreiber (Rutgers University). Cells were propagated in DMEM supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin and 0.1 mg/ml streptomycin. 10 to 20 mm long CNFS were placed in wells of a 12-well tissue culture plate, sterilized by exposure to UV, and incubated in the presence of NIH3T3, KB, and dermal fibroblast cells at $37^oC/5\%$ CO_2 for 24 – 48 hours to encourage cell attachment. Using sterile forceps, fibers were transferred to a new well containing fresh growth medium, incubated for an additional 5 – 6 days, and photographed using the Eclipse TE2000-S microscope (Nikon). Cytotoxicity was assessed as the release of G6PDH using the Vybrant Cytotoxicity Assay (Molecular Probes) following 15 - 16 hours growth of L929 cells exposed to fiber fragments of 5mm or 8mm length in a 96 well plate. L929 was grown in DMEM:F12Hams (1:1) containing 10% horse serum and antibiotics.

Results / Discussion: We have implemented and modified a technique of particle coagulation spinning to fabricate one-dimensional CNFS in the form of "hair-like" fibers of thickness from 20 to 50 μm and length up to 50 cm (Fig. 1 a, b). Fibers were incubated with KB, NIH3T3, and rat dermal fibroblasts. Microscopic observation at 24 hours revealed that while the majority of KB, NIH3T3, and rat dermal fibroblasts attached and spread over the tissue culture plastic, some cells of each cell type attached to the CNFS fibers. In most cases, a significant number of cells were observed growing along the fiber shafts following 6 – 7 days incubation (Fig. 2a). In general, only a few cells were observed on the fiber tips. However, in some cases, the apparent exposure of CNFS at the fiber tip strongly stimulated cell attachment and growth (Fig. 2b). Essentially no cytotoxicity was observed in L929 cells that were grown in the presence of the CNFS (data not shown). Studies examining the compatibility of CNFS with mammalian neural cell attachment, survival, and process extension are in progress.

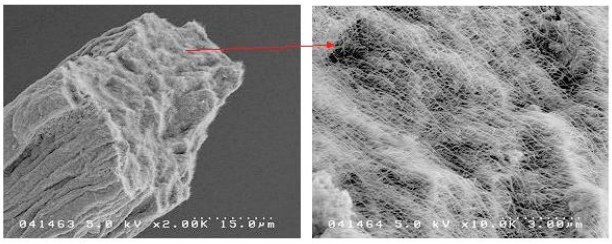

Figure 1. Nanoscale morphology of SWCNT fibers. a) fiber is made of bundles of by particle coagulation spinning, b) detail of a).

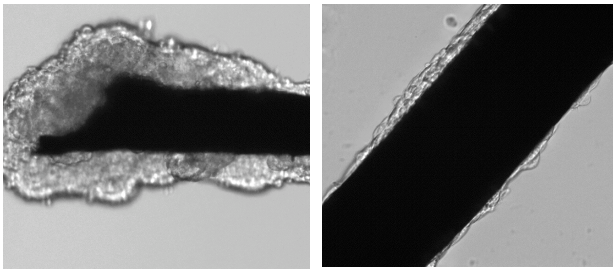

Figure 2. Cell growth on CNFS. KB cells growing on a) fiber shaft (200x) and b) fiber tip (100x);

Conclusion: We have demonstrated that CNFS produced by particle coagulation spinning, which are electrically conductive, highly porous and have a high specific surface area, are non-toxic to primary mammalian cells and cell lines and are compatible with cell attachment and growth. These findings make it possible to consider the use of these particular fibers in future studies aimed at the development of electrically conductive interfaces with neuronal tissue.

References:

[1] Vigolo B et al. Science 290 (5495): 1331-1334 (2000).
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