Carbon Nanotube Fibers Are Compatible With Mammalian Cells and Neurons

Robert A. Dubin, Gerardo C. Callegari, Joachim Kohn, and Alexander V. Neimark*

***Abstract*—We demonstrate the biocompatibility of carbon nanotube fibers (CNFs) fabricated from single-wall carbon nanotubes. Produced by a particle-coagulation spinning process, CNFs are "hair-like" conductive microwires, which uniquely combine properties of porous nanostructured scaffolds, high-area electrodes, and permeable microfluidic conduits. We report that CNFs are nontoxic and support the attachment, spreading, and growth of mammalian cells and the extension of processes from neurons *in vitro*. Our findings suggest that CNF may be employed for an electrical interfacing of nerve cells and external devices.**

***Index Terms*—Biocompatibility, carbon nanotubes, cell adhesion, neurons.**

I. Introduction

CARBON nanotubes hold great promise for novel biomedical nanotechnologies [1]–[3]. High strength and electrical conductivity, flexibility, and potential for functionalization have stimulated significant interest in applying carbon nanotubes to the development of superior neural prosthetic implants [4], [5]. In particular, neurite extension and electrical activity are supported when neurons were grown on carbon nanotubes deposited on planar substrates [6]–[15]. In this paper, we demonstrate the biocompatibility of carbon nanotube fibers (CNFs) fabricated from single-wall carbon nanotubes (SWNTs). Produced by a particle-coagulation spinning (PCS) process [16], [17], CNFs are "hair-like" conductive and flexible microwires, which uniquely combine properties of porous nanostructured scaffolds, high-area electrodes, and permeable micro-fluidic conduits. The range of potential applications of the CNF extends from supercapacitors, electrochemical transducers, artificial muscles, and microwires, to conduits in micro- and nanofluidics devices and media packaging material [16], [18]–[23] . We report that CNFs are nontoxic and support the attachment, spreading, and growth of mammalian cells and the extension of processes from neurons *in vitro* (Table I).

CNF samples from 30 to 100 μm in diameter and up to 30 cm long in the form of threads and ribbons were produced by the PCS process [16], [18], modified as described in [17]. Fibers were spun from 0.6 wt.% dispersion of SWNTs (Nanoledge) in an aqueous solution of 1.2% of sodium dodecyl sulfate (SDS) (Aldrich) prepared in a horn sonicator. The SWNT dispersion was injected through a needle (ID = 1 mm) into a coagulation bath rotating at the rate of 33 r/min. The coagulation bath contained an aqueous solution with 5% of poly(vinyl alcohol) of the average molecular weight of 67 000 Da (Fluka). The ribbons were rinsed for 3 h prior to extraction. The fiber morphology was controlled by adding alcohol into the coagulation and extraction solutions [17]. The samples of the CNF employed in this paper were shown to be electro-conductive with

TABLE I
CNF Is not Toxic to Mammalian Cells

	Cells	Lysed cells[a]	Cells + CNF
Experiment 1	575 ± 28[b]	3147 ± 25	574 ± 37
Experiment 2	602 ± 6	4717 ± 49	627 ± 21

[a] 100% cell lysis following exposure to DMSO.
[b] Mean fluorescence (ex 544 nm / em 590 nm) ± standard deviation (n = 3).

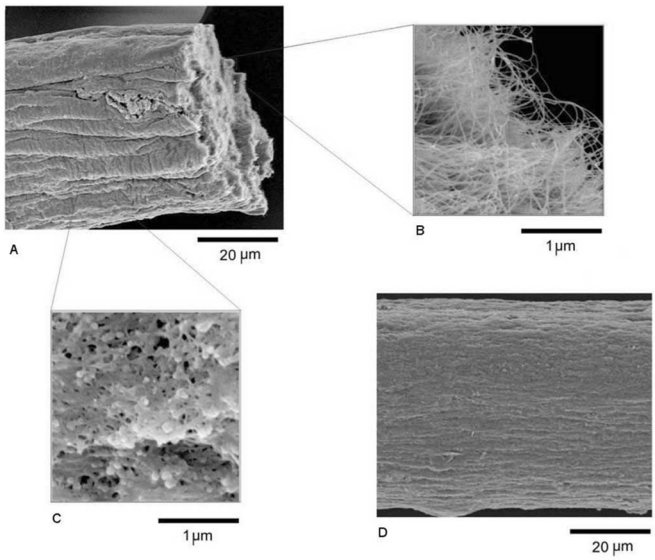

Fig. 1. Hierarchical pore structure morphology of CNF threads. (A) Tip of a CNF thread of about 50 μm in diameter produced with PVA solution. (B) Highly porous nanofelt formed of SWCN bundles of 5–30 nm in diameter. (C) Porous mesh structure on the external surface is composed of polymer-bound SWCN bundles. (D) External surface of fibers produced with PVA/ethanol solution is less corrugated.

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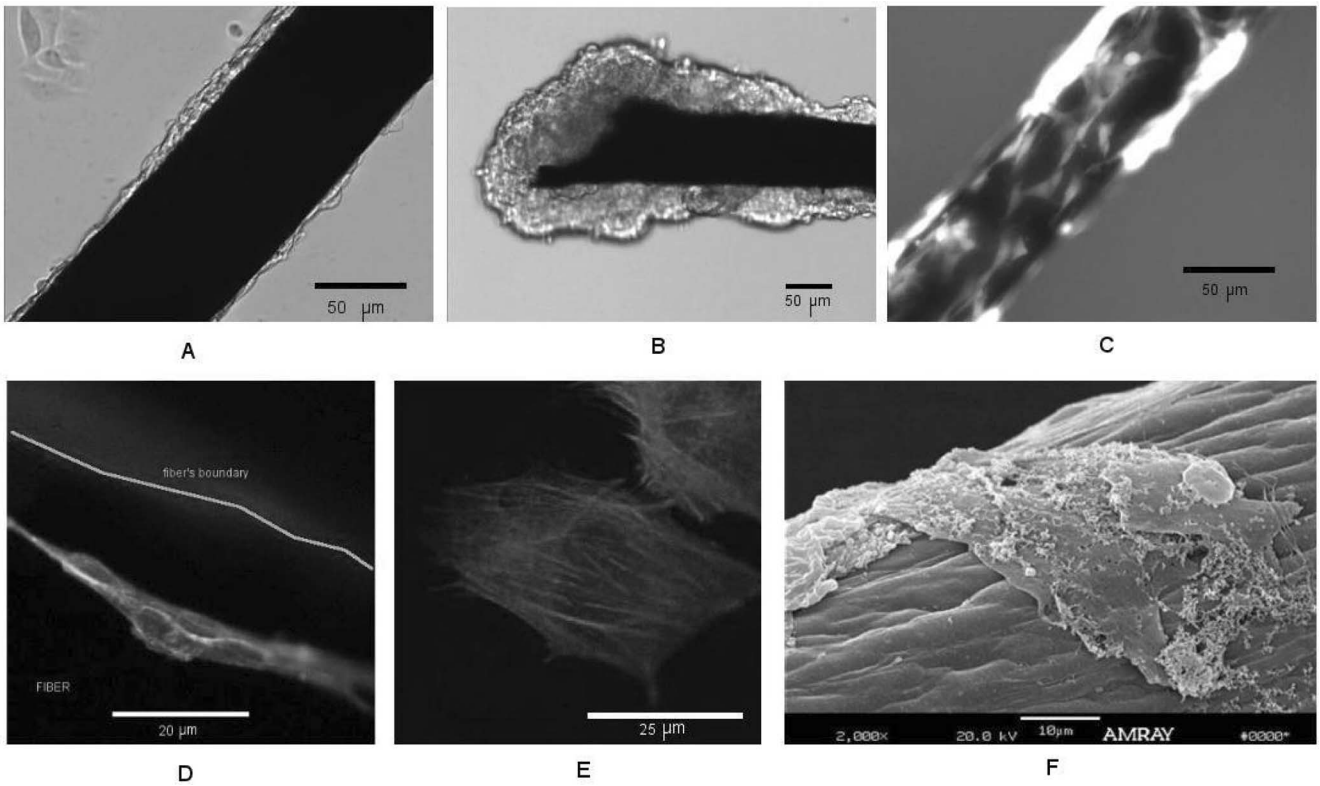

Fig. 2. Cell attachment, growth, and spreading are compatible with CNF. (A) KB cells growing along the shaft of CNF. (B) Excessive KB cell growth on the region of freshly exposed nanofelt at CNF tip. (C) Rat GFP-expressing, dermal fibroblasts grown on the CNF, and imaged by fluorescent microscopy. (D) NIH3T3 cells on CNF and stained with FITC-phalloidin. (E) Confocal micrograph of Saos-2 osteoblasts expressing GFP-Actin on CNF. (F) SEM images of rat, GFP-expressing, dermal fibroblasts grown on CNF preadsorbed with fibronectin. Cells were grown on CNF for 6–9 days prior to imaging.

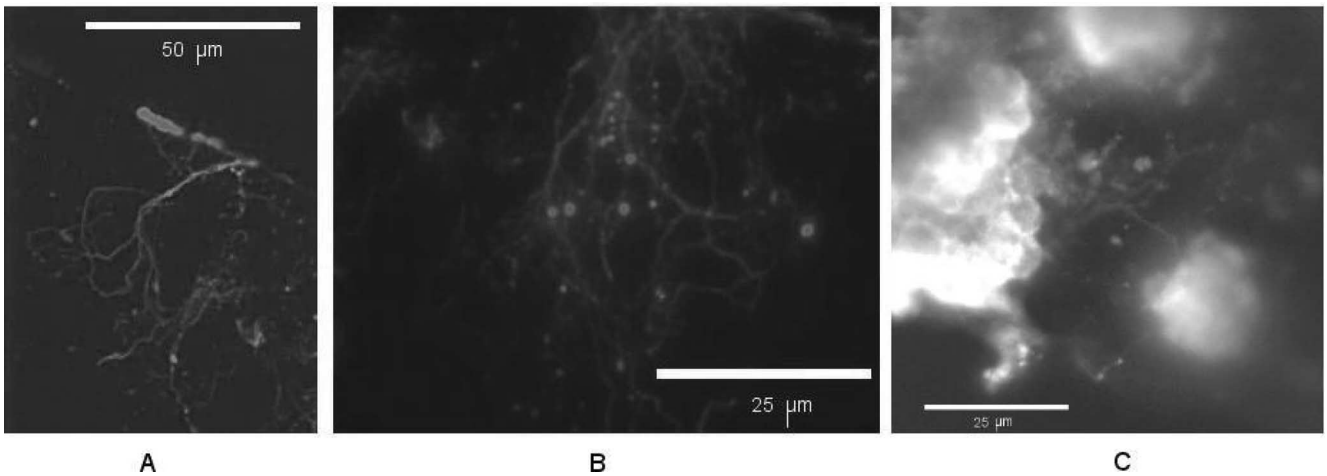

Fig. 3. Neurite extensions on CNF. (A) and (B) Fluorescent images of rat hippocampal neural extensions on CNF following immunocytochemical staining for neural-specific β-tubulin. The edge of CNF is clearly visible. (C) Nerve growth factor-induced processes extending from PC12 cells grown on CNF and stained with Alexa Fluor 594-conjugated wheat germ agglutinin.

the specific conductivity of about 2×10^3 S/m, which corresponded to the fiber resistance per unit length of the order of 1–2 kΩ/cm. The fact that the fiber is conductive confirms that the nanotubes are not buried in the polymer. The fibers exhibited robust mechanical properties with Young's modules of the order of 1–3 GPa that is comparable to human hair. The permeability of the CNF was demonstrated in our earlier work [24].

Mechanical, transport, and adhesion properties of the CNF are determined by their hierarchical pore structure [24]. As presented by a series of field emission SEM images (FESEM S-4500, Hitachi) in Fig. 1, CNFs of round cylindrical shape [Fig. 1(A)] are composed of SWNT bundles of diameter 5–30 nm, which form a highly disordered nanofelt [Fig. 1(B)]. The fiber external surface represents a porous mesh of SWNT bundles bonded by polymer [Fig. 1(C)]. It is corrugated with

pronounced longitudinal grooves. The specific surface area of CNF samples was 150–250 m^2/g, as measured by the nitrogen adsorption technique [24], corresponding to 3–10 nm pores within the nanofelt. The CNF porosity and surface morphology can be controlled by varying the composition of coagulation and extraction solutions [17]. For example, CNF samples spun into a coagulation solution with ethanol had a less corrugated surface [Fig. 1(D)] than those produced without alcohol additives [Fig. 1(A)]. Details of the CNT hierarchical structure were discussed elsewhere [17], [24].

II. Results

We have characterized the cellular response to the CNF *in vitro*. Details of experimental protocols and microscopy techniques are given in supplementary information.[1] Initial experiments demonstrated the absence from CNF preparations of leachable, cytotoxic compounds (surfactants and additives) used in the CNF fabrication. Approximately 10 000 L929 cells were incubated in the presence of a 5 or 8 mm fragment of CNF thread in wells of a 96-well tissue culture plate. After 14–18 h, the extent of acute cytotoxicity was quantified as the concentration of cytosolic glucose-6-phosphate dehydrogenase released into the growth medium. Results from two independent experiments using CNF from a single preparation reveal that the short-term exposure to CNF elicited no significant, acute, and cytotoxic effect. Cytotoxic studies performed with two additional, independent CNF preparations showed similar results (data not shown).

Long-term studies demonstrated the compatibility of the CNF with cell attachment and growth. By 24 h, immortal KB and NIH3T3 cells had attached to the CNF. Microscopic examination over the course of one week revealed that these cells were proliferating [Fig. 2(A)]. Interestingly, significant cell growth was observed on surfaces of freshly exposed nanofelt [Fig. 2(B)]. An explanation for this observation remains unclear; however, it is likely that cell adhesion was enhanced due to the high area of nanotube bundles on the fiber tip [Fig. 1(B)]. An on-edge imaging was inadequate to demonstrate cell spreading in living cells. To overcome this problem, we took advantage of the natural fluorescence of primary, neonatal, rat dermal fibroblasts engineered to express green fluorescent protein (GFP). As shown in [Fig. 2(C)], these cells exhibited clear evidence of spreading over the CNF surface. By visualizing cytoskeleton components of cells on the CNF, the ability of additional cell types to spread over this material was confirmed. Organized actin stress fibers were observed in both NIH3T3 and Saos-2 cells growing on the CNF [Fig. 2(D) and (E)]. Upon a detailed examination of cells spread over the CNF by SEM, the cells and the elementary filaments of the CNF can be distinguished. Intimate association between the two is observed [Fig. 2(F)]. Together, these results demonstrate the CNF compatibility with the attachment, growth, and spreading of immortal and primary cells.

[1]Supplementary Information "Details of experimental studies and microscopy of cell culture" can be viewed and downloaded at http://sol.rutgers.edu/~aneimark/PDFs/ Dubin Et Al IEEE NanoBio 2008 SI.pdf.

To determine whether SWNT organized as CNF are compatible with neurons, primary, rat hippocampal neural cells were grown on the CNF ribbon that had been preadsorbed with poly-D-lysine and laminin. After one week of growth, preparations were fixed and immunostained to detect neuronal class III β-tubulin, a neural-specific marker; it is worth noting that this anti-class III β-tubulin antibody does not cross-react with β-tubulin expressed in glial cells. Cells plated on tissue culture plastic were organized as aggregations of four to eight cell bodies from which numerous processes extended (data not shown). By contrast, no cell bodies were observed on the CNF. However, well-formed, branching neurites extended onto the CNF material at its edges [Fig. 3(A) and (B)]. This observation suggests that cell bodies on an adjacent tissue culture plastic extended processes onto the CNF material. Similar results were observed for rat PC12 cells, a clonal cell line exhibiting features of sympathetic neurons that include neurite outgrowth in response to nerve growth factor (NGF) [25]. Following one week of growth on the collagen-coated CNF and an additional week exposed to NGF, living PC12 cells were imaged immediately, following brief incubation with the vital stain Alexa Fluor 594-conjugated wheat germ agglutinin. In this case, PC12 cell bodies and extended neurite-like processes were observed on the CNF material [Fig. 3(C)]. Together, these results clearly demonstrate that the CNF is permissive to the neural cell extension.

III. Conclusion

In conclusion, electrical conductivity and cell compatibility suggest that the CNF may be a suitable substrate for electrically conductive tissue, including neurons. High surface area of the CNF may facilitate enhanced adhesion with cells and neurites. The possibility for covalent modification of the CNF offers hope that CNF electrodes may be fabricated so as to ameliorate the reactive gliosis that is associated with current neural electrode designs [26]. CNF may also serve as porous permeable conduits for drug, nutrients, and growth factors delivery to cells. The "one-dimensional" hair-like geometry of the CNF may offer novel opportunities, compared to carbon nanotubes deposited on planar substrates [8]–[12], [14], for the design of implantable electrodes and microwires connecting neural tissue with external devices needed for improving sensory and stimulatory prosthetic devices for spinal cord injuries and other diseases of the nervous system.

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