Carbon Nanotubes Fibers as Neural Biomaterial:
Nanoscale Surface and Biocompatibility

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Statement of Purpose:
When developing ideal neural biomaterial, several features should be considered: biodegradability, oriented structure to support cell migration, electrical activity, fibrilar architecture to mimic the structure of nerve fascicles and the ability to interact with cells at molecular level. Surface structure and related chemistry are critical in the design of high biocompatible materials since adsorption and adhesion of biological components are involved. These aspects become even more significant in the case of nanostructured materials such as carbon nanotubes fibers.
Carbon Nanotube (CNT) is a new form of carbon with an equivalent configuration to a two dimensional graphene sheet rolled into a tube. The geometric arrangement of graphitic sheet results in two fashions: Single Walled Carbon Nanotubes (SWNTs) and Multi Walled Carbon Nanotubes (MWNTs). The interest to use CNTs as biomaterial comes from its intrinsic characteristics [1].
In this work we present the unambiguous performances of SWNT-based fibers fabricated by Particle Coagulation Spinning (PCS) [2], an appropriate method for nanotubes integration in a hybrid system with the incorporation of a copolymer, responsible for the improvement of biocompatibility and inducer of partial biodegradability [3]. We capitalized on nanoscale surface signature of new fibers as a sensitive tool to regulate cell behaviour.
Method:
Through this investigation we evaluate the efficiency of new neural biomaterial containing SWNTs, presented as monofilament threads with a diameter ranging in 30-60 µm and variable length. We tested three types of samples: SWA, SWB and SWC. While the last one contains CNTs and polyvinyl alcohol (PVA), for both SWA and SWB fibers we incorporated the polylactic-co-glycolic acid (PLGA) with different molecular weights [2].
The nanoscale surface analysis is performed in terms of chemistry and morphology in order to determine the specific characteristics generated by the new formulation [2]. Using the Time of Flight Mass Spectroscopy (TOF-SIMS) we identify specific molecular ions and their distribution. Fiber shape, texture and structure are imaged using the Low-Vacuum Scanning Electron Microscopy (LV-SEM), and Atomic Force Microscopy (AFM) in contact mode.
From the biocompatibility point of view, we are interested to determine how the fiber shape, structure and chemistry are reflected in cells response. The answer is sustained by data provided by *in vitro* study with reference to cells proliferation, adhesion, migration and orientation. In our example, PC-12 cells are cultured, for three days, on three different substrates: SW, SWA, SWB fibers. We carried out the MTT test and the Optical Density was measured as an expression of cell viability using collagen film as the control. The migration and orientation of cells as well as their adhesion are daily monitored using an inverted optical microscope equipped with a color video camera.
Results/Discussion:
TOF-SIMS analysis diagnoses a specific signature involving copolymer molecules, SWA versus SWB fibers. Characteristic ions related to this component reveal its presence on outermost fibers surface. The homogenous distribution of molecular ions (Fig.1) demonstrates a successful interconnection between all components at the surface with effect on biocompatibility. LV-SEM and AFM images (Fig.2) display fibers morphology and structure in relation with their formulation.

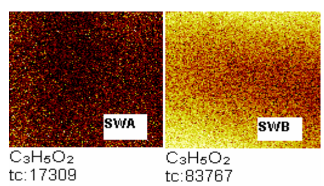

Fig.1. TOF-SIMS images of a PLGA positive ion

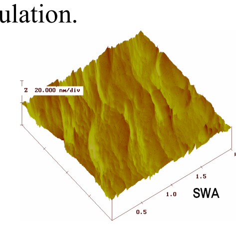

Fig. 2. AFM image: SWA fiber structure

The MTT test results demonstrate that these fibers are suitable environment for cell. In the second culture day, we observe a good migration of cells from seeded area to the opposite side. The fibers containing more CNTs generate higher migration force; SWA fibers have the best response in terms of migration. The adhesion of cells is another behaviour revealing the interaction cells-fibers. Moreover, the effect of fiber structure on cell orientation is obvious in the second day of culture (Fig.3); both SWA and SWB are positive inducer for cell alignment.

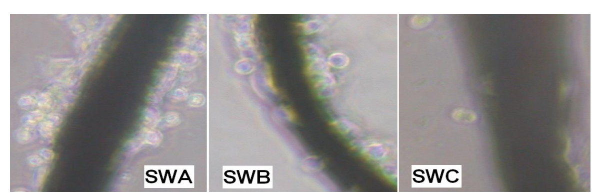

Fig. 3. Evolution of PC-12 cells as function of the substrate: day 2

Conclusion: This study confirms the biocompatibility of new SWNT-based fibers as potential substrate for nerve regeneration. The chemistry and structure of macroscopic fibers induce specific response when in contact with cells.
References: [1] S. Polizu, et al., JNN, 2006, 6, (7), 1883; [2] B. Vigolo et al., Science, 2000, Nov. 17, 290, 1331, [3] S. Polizu et al., CNT based Biomaterials for nerve regeneration, unpublished data.