Functionalized Multi-Walled Carbon Nanotubes Impart Electrical Conductivity to Protein Biopolymer Matrices

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Purpose: The unique electrical properties of carbon nanotubes (CNT) make them a promising material for use in conductive composites and as neural scaffolds or electrode coatings. Although simple in structure, CNT display complex relationships with their physical and biological environment. One of the biggest challenges in biomedical applications is improving CNT dispersion and biocompatibility, without altering the nanotube's native electrical properties. This study characterizes CNT functionalization and polymeric wrapping techniques for dispersing multi-walled carbon nanotubes (MWNT), with the goal of increasing the conductivity of CNT-loaded collagen and fibrin biopolymer materials.

Methods: MWNT were used either untreated (MW), or carboxylated by a 2 h acid reflux (MW2R) or by a 15 s, 2 h, or 12 h sonication in acid (MW15S, MW2S, MW12S). MWNT also were non-covalently wrapped with Pluronic F127 (MW*-P) and Gelatin (MW*-G). MWNT surface chemistry was characterized by X-ray photoelectron spectroscopy (XPS), as well as zeta potential and particle size measurements. Impedance measurements of MWNT-collagen-fibrin composites gelled at 37°C and pH~7 were obtained using a two electrode configuration at 0.01-10 kHz. Tissue constructs containing human neonatal dermal fibroblasts (HNDFb) were assessed for cell metabolic activity using a colorimetric tetrazolium salt assay (XTT). Cell morphology was visualized by confocal microscopy of the fluorescently stained actin cytoskeleton.

Results: Settling time analysis (Fig 1, inset) showed that F127-coated MWNT formed the most stable suspensions. Zeta potential measurements indicated that polymer wrapped MWNTs had a negative surface charge, but acid treated did not, compared to untreated MWNT zeta potentials. XPS spectra indicated that atomic oxygen was increased on the surface of acid treated MWNT compared to the untreated control. MWNT coated in F127 and gelatin showed a large increase in oxygen and nitrogen in comparison to acid treated and untreated samples. MWNT samples showed high purity, as evidenced by the lack of metallic contaminants in the XPS spectra. Impedance spectroscopy at 1 kHz (Fig 1) showed that control samples (no MWNT) had the highest impedance values (451.7±82.5 MΩ), while the addition of untreated MWNT decreased impedance (214.6±58.5 MΩ). Non-covalently treated MWNT further decreased the impedance of the materials, with F127-coated MWNT exhibiting the lowest impedance (1.3±2.5 MΩ). XTT analysis revealed that, relative to control constructs with no MWNT, cell metabolic activity was reduced ~60% in constructs containing 0.6 mg/ml MWNT (independent of treatment), and ~30% at a MWNT concentration of 0.075 mg/ml. MWNT wrapped with gelatin at a concentration of 0.075 mg/ml showed the smallest decrease (19%) in metabolic activity when compared to untreated MWNT constructs (59%). Confocal micrographs showed typical cell morphologies and good cell spreading across all MWNT concentrations and treatment types.

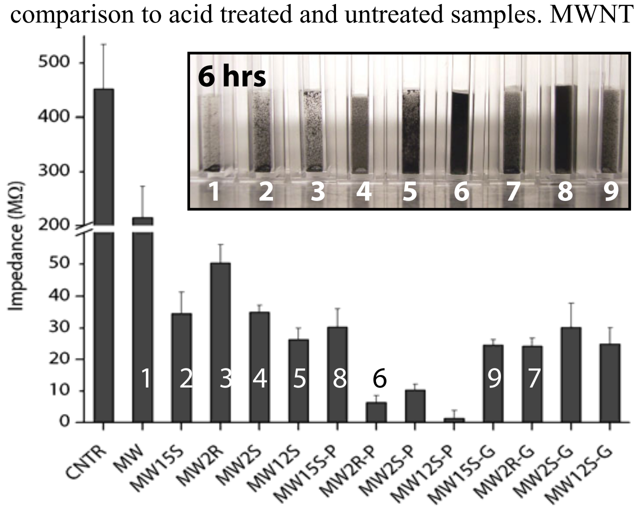

Fig. 1: Impedance values of MWNT-composites. Inset shows MWNT solutions settling after 6 h.

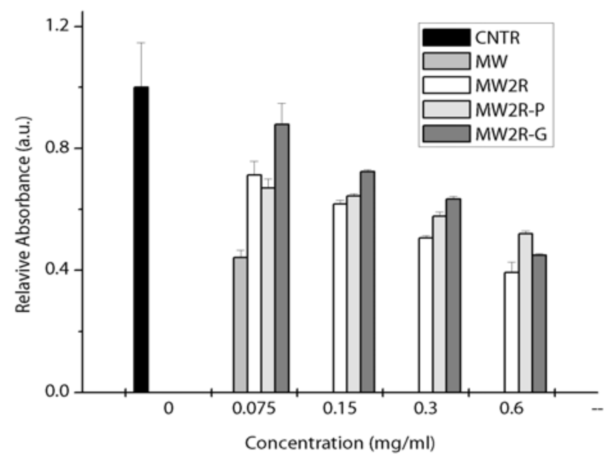

Fig. 2: XTT analysis of MWNT tissue constructs.

Conclusions: This study demonstrated that non-covalent wrapping treatments can decrease the impedance of MWNT-collagen-fibrin gels by more than two orders of magnitude compared to untreated MWNT, while still improving biocompatibility. MWNT solutions that tended to aggregate exhibited higher composite impedances because a percolating network has less probability of forming when the conductive phase is not evenly and randomly distributed. Pluronic F127-wrapped MWNT showed the best dispersion, but had higher toxicity than gelatin-wrapped MWNT. This is an interesting result because it has been suggested that nanotube aggregation leads to increased toxicity. These findings show that electrically conductive biopolymers can be created by loading with appropriately functionalized and processed MWNT. Such materials have potential application in neural engineering, biosensor development, and other areas where electrically conductive, biocompatible materials are required.