Graphene Nanocomposites for Neural Tissue Applications

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Statement of Purpose: Electroactive biomaterials are of particular interests to neural tissue applications, such as nerve regeneration aided by electrical stimulation and neural interfacing through recording/stimulation. Although much of the progress has been made with conducting polymer based biomaterials, the rising carbon nanomaterials such as carbon nanotubes and graphenes present promising alternatives for neural tissue applications. Here we report the development of a biocompatible and bioactive graphene nanocomposite biomaterial, as well as its interaction with neurons in vitro.

Methods: The nanocomposite material was engineered by converging electrical properties of graphene with biocompatibility and bioactivity of chitosan. A series of graphene-chitosan (G-C) nanocomposites with different G:C ratio was developed by air evaporation of precursor solutions. Both substrate-supported and free standing G-C films were prepared. The electrical resistance of the films was measured with a resistivity meter. The surface morphology, topography and roughness were characterized by scanning electron microscopy (SEM) and atomic force microscopy (AFM). Contact angle measurements were performed to characterize surface wettability. Cytotoxicity of graphene was tested on both fibroblast and neuron cell lines with a WST-1 assay. Neuronal responses to G-C films, including neuron adhesion and morphology, were evaluated by culturing neuron cell line (N2a) and primary cortical neuron on G-C films and imaged with fluorescent microscope and SEM.

Results: Chitosan, a natural polysaccharide, was selected for our composite system largely due to its biocompatibility, and more importantly, its rich presentation of primary amine groups that can improve neuron adhesion. The ratio of graphene to chitosan is important for composite preparation. As shown in Fig. 1, a 1:1 G:C led to precipitation, while solutions of the other ratios (0.5:1, 1:4, and 1:8) were stable and well dispersed, allowing preparation of uniform films. Free-standing films could be easily rolled up, indicating good flexibility (Fig. 1).

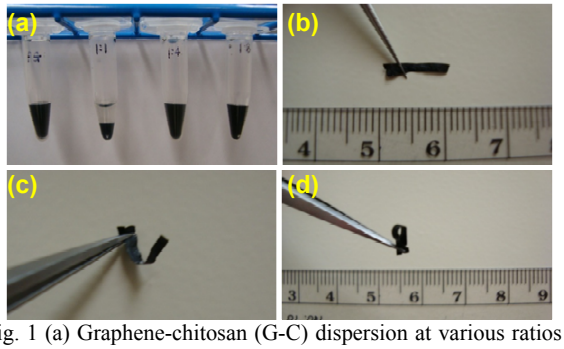

Fig. 1 (a) Graphene-chitosan (G-C) dispersion at various ratios of G to C. (b-d) A free standing G-C nanocomposite film displays flexibility and ease of handling.

The remarkable electrical conductivity of graphene is one of the main rationales behind this study. Since chitosan is non-conductive, the G-C nanocomposites showed higher resistance than plain graphene (PG) as expected. For example, G1C4 had a resistance of 260 kΩ comparing to the 1.37 kΩ of PG. Nevertheless, such resistance could be sufficient for applications in biological systems. Introduction of chitosan also affected the morphology of the film. While PG forms a smooth surface at the micro-scale, G-C composites have a relatively rough surface (Fig. 2). Furthermore, chitosan improved hydrophilicity of the film, where water contact angle decreased from 83° for PG to 69° for G1C8.

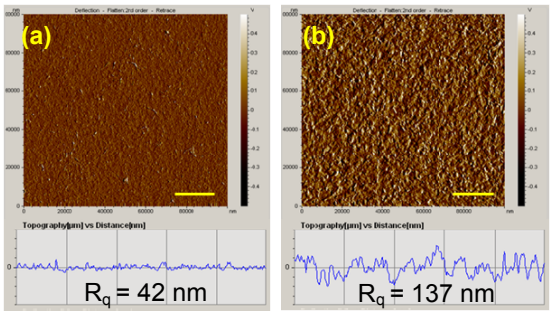

Fig. 2 AFM images showing the surface topography of (a) PG and (b) G1C8. Scale bar = 20 μm.

Cytotoxicity study showed good biocompatibility of graphene towards both fibroblast and neuron. A 93% viability was observed for both types of cells when treated with 25 μg/mL of graphene for 2 days. While plain graphene was a poor substrate for neurons, the incorporation of chitosan greatly enhanced neuron adhesion as expected. The G1C4 composition showed the most improvement in cell adhesion. Different neuron morphology was observed on plain graphene (control) and G-C composite films, which could be attributed to their different surface topography.

Conclusions: Biocompatible and electrically active nanocomposites were successfully prepared from graphene and chitosan. This new biomaterial is supportive of neuron adhesion and differentiation, and could be a promising electroactive material for interfacing with the impaired nervous systems for functional repair. Ongoing study includes electrical stimulation of PC-12 cells grown in vitro on this composite material to investigate its electroactivity.