Original Article

Physical and electrical characterization of TexasPEG: An electrically conductive neuronal scaffold

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Abstract

Background: Graphene and its derivatives have been shown to be biocompatible and electrically active materials upon which neurons readily grow. The fusogen poly(ethylene glycol) (PEG) has been shown to improve outcomes after cervical and dorsal spinal cord transection. The long and narrow PEGylated graphene nanoribbon stacks (PEG-GNRs) with their $5~\mu m \times 200~nm \times 10~nm$ dimensions can provide a scaffold upon which neurons can grow and fuse. We disclose here the extensive characterization data for the PEG-GNRs.

Methods: PEG-GNRs were chemically synthesized and chemically and electrically characterized.

Results: The average aspect ratio of the PEG-GNRs was determined to be ~85, which corresponds to a critical percolation value (the point where insulating material becomes conductive by addition of conductive particles) of 1%. However, there was not a sharp increase in AC conductivity at frequencies relevant to action potentials.

Conclusion: A robust characterization of PEG-GNRs is discussed, though the precise origin of efficacy in improving outcomes following spinal cord transection is not known.

Key Words: Fusogen, graphene, nanoribbons, PEG

INTRODUCTION

Graphitic structures have been shown to electrically stimulate, physically support, and organize the three-dimensional (3D) structure of neurons.[2,6,10] Many types of graphene have been used for spinal cord injury,[7,15,19] however, to our knowledge, no one has used graphene nanoribbons (GNRs), despite the literature showing that GNRs positionally inform neurons grown in cell culture.[1] In the context of the GEMINI spinal cord fusion protocol,[5] we anticipated that GNRs could act as an electrical stop-gap to transmit electrical signals across the gap produced by sharp cervical cord transection. GNRs might further act as a scaffold for regrowth of neuronal processes. The scaffold could be especially useful if the GNRs could be aligned parallel to the spinal cord across the gap by either non-contact methods, such as electric fields,[17] or teslaphoresis,[4] or by contact methods that briefly separates the cut ends and causes shear forces to align the nanoribbons.[18]

Pure poly(ethylene glycol) (PEG) can restore at least partial motor function in rodents by acting as a fusogen to seal blunt ends of neuronal processes and connect neurons across the gap.[10,12] However, PEG applied shortly after surgery is likely rapidly cleared from the area because of its low molecular weight and high water solubility. To improve outcomes, it is necessary to include a component that will persist longer at the cut site to continue to stimulate reconnection, and PEG-GNRs might also serve as a useful agent to slow the loss of the PEG.

Due to their large physical size, high molecular weight ($\sim 10^9$ g/mol) and high aspect ratio, GNRs might remain in the tissue much longer. In addition, their conductive properties might allow them to act as an electrical conduit to restore conduction through the fusion interface much more quickly.[9] As a high aspect ratio graphene, GNRs might direct anisotropic neuronal growth longitudinally with the spinal cord axis.[1] The synergy of PEG as an acute fusogen and PEG-GNRs as a long term repair scaffold might be the mechanism for the enhanced positive outcomes in spinal cord repair.[9]

This paper complements a sister paper[9] by characterizing the PEG-GNRs, the clinically relevant mixture of PEG and PEG-GNRs, in addition to providing a rationale for the formulation used.

MATERIALS AND METHODS

PEG-GNR (TexasPEG): Multi-walled carbon nanotubes (MWCNTs) were obtained from EMD Merck (produced by Mitsui & Co., lot no. 2699-64E) and were used as received. Tetrahydrofuran (THF) was dried over solid KOH for several days, degassed, and freshly distilled from sodium/benzophenone under a N_2 atmosphere. All chemicals were purchased from Sigma-Aldrich unless otherwise specified. Thermogravimetric analysis (TGA) measurements were performed on a TA instruments Q-600 Simultaneous TGA/DSC. The temperature was ramped at 10°C/min until 850°C under argon. For transmission electron microscopy (TEM, JEOL JEM 2100F) analysis, the PEG-GNRs were dispersed in water and drop cast onto a lacey carbon grid and allowed to dry for 6 h. For scanning electron microscope (SEM) analysis, the PEG-GNRs were dispersed in *o*-dichlorobenzene, briefly sonicated in a bath sonicator, and deposited on a silicon wafer at an approximate density of 1 PEG-GNR per 500 μm^2, from which the solvent was evaporated on a heat plate at <100°C. The sample was imaged by an FEI Quanta 400 ESEM FEG instrument. Thirty images were taken in a direct line across the sample starting from a random location to minimize selection bias, and the resulting images were analysed with the aid of ImageJ. Conductivity measurements were performed with a home-built copper parallel plate dip-probe connected to a Hewlett-Packard 3577a Network Analyzer. Calibrations were performed using methanol and a short-circuit liquid metal standard.

1.0 g of Mitsui MWCNTs was added to a 1 L oven-dried, nitrogen-purged, Schlenk flask; 500 mL of THF was added. 2.5 mL of eutectic NaK (1:3.3 by mass, 1:1.9 by mol) was added under nitrogen. The reaction mixture was stirred at room temperature for 3 days, until very few liquid droplets of NaK remained. The reaction was cooled in a dry ice/acetone bath to −78°C, and 30 g (0.7 mol) of gaseous ethylene oxide was added from a lecture bottle over 90 min. The mixture was slowly brought to room temperature and stirred for 3 days. A mixture of NaH (20 mmol, 0.53 g) and propargyl bromide (20 mmol, 2.4 g) suspended/dissolved in dry toluene was added to terminate the ethylene oxide polymerization. The reaction was quenched by adding 20 L of water, and the dark gray precipitate was collected *via* filtration on a 0.22 μm polyethersulfone (PES) membrane. The dark gray precipitate was filtered through a polytetrafluoroethylene (PTFE) membrane (0.45 μm), followed by crossflow filtration with a 50 kDa MWCO PES filter to remove unbound polymer. The PEG-GNRs final product (1.3 g) was collected on a PTFE membrane (0.45 μm), washed with DI water (3 × 100 mL), ethanol (3 × 100 mL), DI water (3 × 100 mL), and dried under high vacuum overnight. The propargyl units were added to some of the termini for future peptide additions if desired. Before use, the PEG-GNRs were dispersed in PEG 600 with an IKA T25 digital Ultra-Turrax machine running at 1000 rpm with an S25N-18G Dispersing element attachment. (0.5–1% (w/v) by GNR concentration). The mixture was tightly sealed in a 50 mL conical vial and was sterilized by 120°C pressurized steam for 30 min.

RESULTS AND DISCUSSION

Characterization of relevant physical and electrical properties was performed. First, to assess the amount of polymer covalently bound to the GNRs, TGA was performed. PEG decomposes fully by 400°C, while the GNRs are stable under the temperatures tested. The PEG-GNRs are composed of 30% PEG, while GNRs comprise the remaining 70% [Figure 1].

By examination of the SEM and TEM images in Figure 2, one can see that the MWCNTs were indeed split into GNRs, displaying wavy patterns characteristic of GNRs and a much smaller persistence length than MWCNTs. The GNRs are in triangular stacks that result from unzipping several nanotubes in a MWCNT, where the largest tube makes the widest ribbon at the bottom, and the smaller tubes make increasingly thinner GNRs, stacked in order on top of each other [Figure 2d]. The GNRs are not individuals, but staked structures that do not easily exfoliate.

The critical percolation concentration is determined by the aspect ratio of the conductive structures. This was calculated by measuring the lengths of individually dispersed PEG-GNRs on a silicon substrate of every PEG-GNR longer than 1 μm [Figure 2a]. The average width and height of PEG-GNRs was measured at higher magnification under TEM. Dividing the distribution of lengths over the average height and width resulted in a histogram [Figure 3] that fit a normal logarithm plot with a standard deviation of 35, and a number average of the aspect ratio was 85. This average aspect ratio corresponds to a critical void fraction necessary for percolation of 1% in order for a conductive path to form.[8]

The percolation conductivity was measured from a concentration of 0.003% to 100% of PEG-GNRs in PEG at 1 kHz, as this is approximately the frequency of neuronal signals [Figure 4]. While there was not a dramatic change in conductivity at ~1% as the average aspect ratio predicted, the conductivity was an order of magnitude higher than the pure PEG. However, in a biological system, the PEG might diffuse away rapidly, leaving the PEG-GNRs behind in much higher concentration, allowing for the effective conductivity, and thus efficacy, of the PEG-GNR solution to rise over time.

CONCLUSION

In the sister paper,[9] we have shown that the addition of PEG-GNRs to PEG dramatically increases the favourability of the outcomes following complete cervical spinal cord transection. In this paper, we have characterized the material as used. We anticipate further enhancements to the outcomes by ameliorating traumatically caused oxidative stress with our high-capacity, fast-acting antioxidants.[3,12,13,15] For full
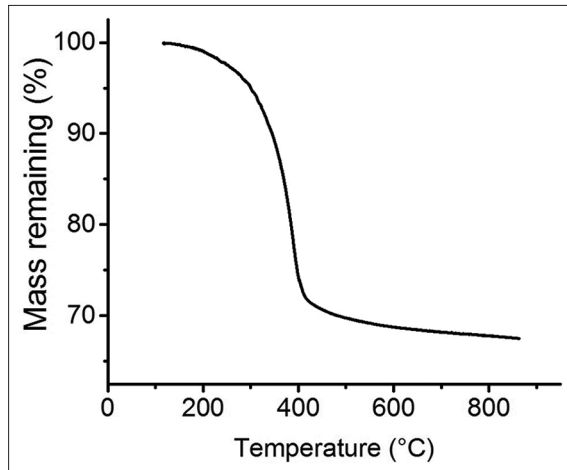

Figure 2: SEM and TEM analysis revealed the ribbon-like structure of the PEG-GNRs. (a) Individualized graphene nanoribbon stacks. Scale = 10 μm. (b) Open end of a large GNR structure. Scale = 200 nm; (c) End of thin GNR. Scale = 100 nm; (d) floppy end of a GNR stack, showing the triangular stack of GNRs. The white arrow shows the stack increasing in thickness. Scale = 10 nm

Figure 1: Thermogravimetric analysis of PEG-GNRs under argon at a ramp 10°C/min, where PEG has completely decomposed before 400°C, and the GNRs are stable past 800°C
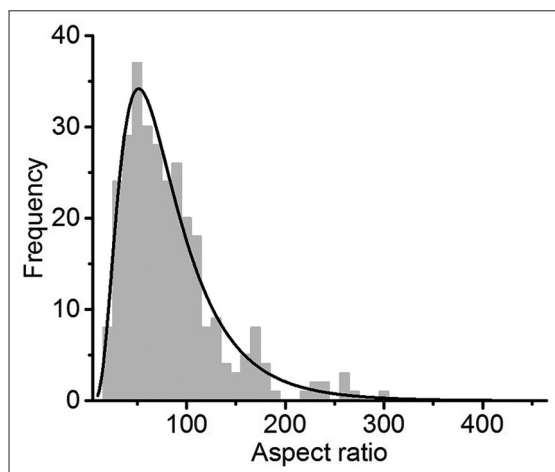

Figure 3: Measured histogram of aspect ratio of 300 PEG-GNRs as measured by TEM. Log normal fit gives a standard deviation of 36, and the average aspect ratio is 85
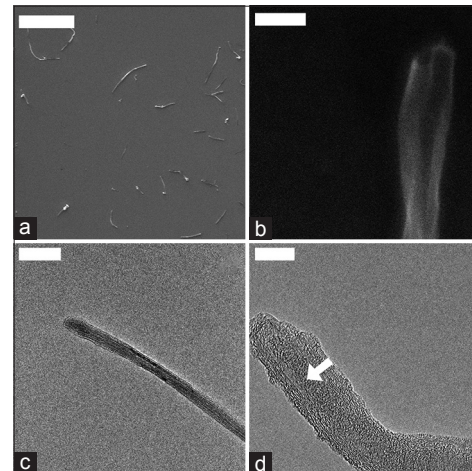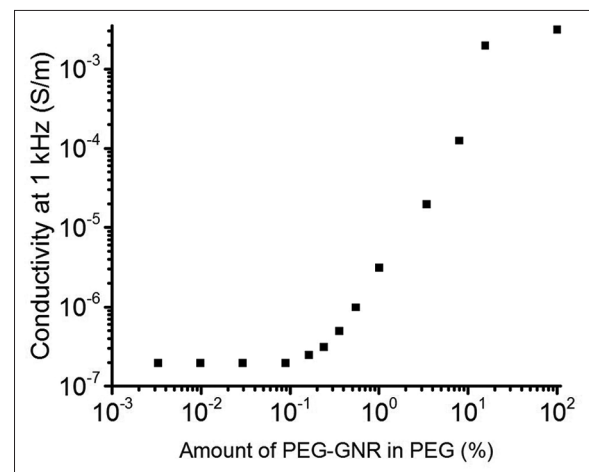

Figure 4: Experimentally measured conductivity of the PEG-GNR in PEG600 at varying concentrations

effect, these can these be administered systemically and topically in the PEG/PEG-GNR mixture.

Financial support and sponsorship

Conflicts of interest

There are no conflicts of interest.

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