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Thursday, June 8, 2017



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JAMES M. TOUR AND WILLIAM SIKKEMA OF RICE UNIVERSITY



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Longitudinal Unzipping of CNTs to Form Graphene Nanoribbons (GNRs)





Longitudinal Unzipping of CNTs to Form Graphene Oxide Nanoribbons (GONRs)





Alignment of Nanoribbons (no picture rotation)



SEM images of **(a-e)** monolayer ribbons, **(f,g)** GNRs with coexisting mono- and bilayer fragments, **(h,i)** bilayer ribbons, and **(k)** a multilayer stack of GNRs. All scalebars in (a-j) are 250 nm, except for (d) at 500 nm. All GNRs have a width of 180-320 nm, they can be up to several μ m long, as shown in (d) at 6.1 μ m and (e) at 3.2 μ m. All scale-bars are 250 nm except for (e) at 500 nm.



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Reduction of the Ribbons

Reductive splitting & in-situ modification





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Na-K MWCNTs to Form Graphene Nanoribbons



Split MWCNTs to Form Graphene Nanoribbon Stacks





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Carbon nanotubes are different than graphene nanoribbons. Is there a way to covalently functionalize a carbon nanotube to render it water soluble while maintaining its high conductivity?

A) No, because covalent functionalization would destroy its pi-conjugation

B) Yes, since its pi-conjugation would still largely remain after covalent functionalization

C) Yes, because one could functionalize the ends without affecting the central part of the nanotube

D) No, because any functionalization, covalent or non-covalent, would shield the nanotube from being accessible for use

J Neurosurg Pediatrics 11:575–583, 2013 ©AANS, 2013

Biocompatibility of pristine graphene for neuronal interface

Laboratory investigation

Deshdeepak Sahni, M.D.,^{1,2} Andrew Jea, M.D.,^{1,2} Javier A. Mata, M.D.,^{1,2} Daniela C. Marcano, M.S.,³ Ahilan Siyaganesan, B.A.,^{1,2} Jacob M. Berlin, Ph.D.,³ Claudio E. Tatsui, M.D.,¹ Zhengzong Sun, Ph.D.,³ Thomas G. Luerssen, M.D.,^{1,2} Shiyun Meng,⁴ Thomas A. Kent, M.D.,^{5,6} and James M. Tour, Ph.D.³

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Results. Statistically significant differences in the percentage of live or dead neurons were noted between graphene and PDL surfaces, as well as between the PDL-coated and bare surfaces, but there was little difference in cell viability between graphene-coated and bare surfaces. There were significantly lower LDH levels in the graphene-coated samples compared with the uncoated ones, indicating that graphene was not more cytotoxic than the bare control surface. According to phase contrast microscopy, neurons attached to the graphene-coated surface and were able to elaborate long, neuritic processes suggestive of normal neuronal metabolism and morphology.

Conclusions. Further use of graphene as a bioscaffold will require surface modification that enhances hydrophilicity to increase cellular attachment and growth. Graphene is a nanomaterial that is biocompatible with neurons and may have significant biomedical applications. (http://thejns.org/doi/abs/10.3171/2013.J.PEDS12374)



Neurons grow on graphene



Fig. 2. Phase contrast micrographs of live neuronal cell growth. Left: Neurons cultured on graphene at Day 7. Significant linear neurite outgrowth is noted, with normal morphology and evidence of synapse formation. Original magnification x10 hpf (Phase 1). Right: Magnified details of *red insets*. Original magnification x40 hpf (Phase 2).



Fig. 4. Neurons cultured at 14 (*upper*) and 20 (*lower*) days. A: Graphene surface. B: Poly-b-lysine-coated surface. C: Bare surface. Robust growth of neurons and neurites can be seen at 14 days on graphene, with decreasing density at 20 days, which is similar to the pattern on bare controls. Original magnification ×10 hpf (Phase 1).





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OPEN ACCESS Editor: James I. Aus University of

Original Article

Biocompatibility of reduced graphene oxide nanoscaffolds following acute spinal cord injury in rats

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Results: The graphene nanoscaffolds adhered well to the spinal cord tissue. There was no area of pseudocyst around the scaffolds suggestive of cytotoxicity. Instead, histological evaluation showed an ingrowth of connective tissue elements, blood vessels, neurofilaments, and Schwann cells around the graphene nanoscaffolds.

Conclusions: Graphene is a nanomaterial that is biocompatible with neurons and may have significant biomedical application. It may provide a scaffold for the ingrowth of regenerating axons after spinal cord injury.



Neurons and spinal tissue show good ingrowth into graphene gels

Surgical Neurology International 2016, 7:75



Figure 2: Representative photomicrographs showing spinal cord injury development after hemispinal cord transection at the T2 level (a) with reduced graphene oxide nanoscaffold performed immediately after transection and (b) without nanoscaffold implantation (control group). Notice the area devoid of tissue (*arrow*) at the lesion site in the control slide, suggesting possible pseudocyst formation. By contrast, cell proliferation (*asterisk*) is exuberant with implantation of the nanoscaffold, and no cavity is evident. Hematoxylin and eosin bar = 2 mm Surgical Neurology International 2016, 7:75



Figure 4: Representative phase contrast photomicrographs illustrate structural regeneration of spinal cord tissue using a nanoscaffold. The incompletely transacted spinal cord is bridged using a reduced graphene oxide scaffold for tissue ingrowth and cell infiltration. (a), Spinal cord adheres well to the nanoscaffold. (b), Loose connective tissue forms between the spinal cord tissue and the reduced graphene oxide scaffold. Bar = 150 µm



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Original Article

Spinal cord fusion with PEG-GNRs (TexasPEG): Neurophysiological recovery in 24 hours in rats

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Methods: Laminectomy and transection of cervical spinal cord (C5) was performed on Female Sprague-Dawley (SD) rats. After applying PEG-GNR on the severed part, electrophysiological recovery of the reconstructed cervical spinal cord was confirmed by somatosensory evoked potentials (SSEPs) at 24 h after surgery.

Results: While no SSEPs were detected in the control group, PEG-GNR treated group showed fast recovery of SSEPs at 24 h after the surgery.

Conclusion: In this preliminary dataset, for the first time, we report the effect of a novel form of PEG with the goal of rapid reconstruction of a sharply severed spinal cord.



Neuron regeneration



Neuron regeneration



Neuron regeneration



Neuron regeneration



Neuron regeneration



Neuron regeneration



Neurons growing on non-functionalized GNR in a 2D system



How neurons grow



Sharp filamentous actin protrusions from end of growth cone sense Electrical and physical environment and direct the tip of the neuron's process



Graphene nanoribbons positionally inform neuron regeneration



Graphene nanoribbons positionally inform neuron regeneration



Water solubility of GNRs is critical in a 3D system of neurons





Synthesis modified from: Kosynkin, D.; et. al. ACS Nano, **2011** Kosynkin, et. al. Tour, J. M. *Nature* **2009**, *458*, 872–876

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What do PEG-GNRs look like?



Scanning electron microscopy

Transmission electron microscopy



Sikkema, et. al. SNI (accepted)

TEM courtesy of Drew Metzger 38

How is the surgery performed?

- 1. Surgical window opened exposing spinal cord
- 2. Spinal cord supported with a gentle hook
- 3. Cord severed with a sharp blade
- 4. One drop of 1% PEG-GNR in PEG 600 applied to blunt ends of spinal cord
- 5. Surgical window closed and sutured



squamous occipital bone C3 vertebral spine cerebellum C1 C1 vertebral spine C2 nuchal ligament C6 C5 C4 C3 hindbrain C1 C3 basioccipital bone C7 C6 C5 C4 atlas axis 2.5 mm C5 C6 C2/C3 intervertebral disc C3 vertebral body

Harrison, et. Al. Neuroimage 68 (2013) 22-29



How are the nanoribbons aligned parallel with the spinal cord?

- Electrical fields
- Magnetic fields
- Shear forces
- Individually with a nano-manipulator
- Black Magic

Logs transported by river in 1903





They align similarly to the way logs align in a river with movement of the river

http://historycruise.blogspot.com/p/log-drives-on-river.html

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How do we measure recovery?

- 1. Before functional recovery is observable, electrical connectivity can be measured
- 2. Somatosensory evoked potentials (SSEP) show communication between extremities of the animal with its brain





Somatosensory evoked potential recovery

- 1. Showing recovery of SSEP signals after only 24 hours
- 2. Electrical connection prevents further degradation of distal neurons



PEG-GNR

Control





6/1/2017

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Laminectomy and complete transection of cervical spinal cord at C5. Then application of 1 wt% pegylated-graphene nanoribbons (PEG-GNR or "Texas PEG") in (polyethylene glycol)-600. Shown is the rat mobility at 7, 14 and 21 days post surgery.







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