ADVANCED HEALTHCARE MATERIALS

Supporting Information

for *Adv. Healthcare Mater.*, DOI: 10.1002/adhm.201701290

Impact of Graphene on the Efficacy of Neuron Culture Substrates

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Keywords: graphene; primary neuron culture; extracellular matrix; ion channel; cell survival

Table S1. *In vitro* viability of cell culture on graphene substrates.

Citation	Cell type	Substrates	Main results
[1] Veliev et al. 2016 Biomaterials	Primary hippocampal neurons (from embryonic E16.5 mice)	Poly-L-Lysine (PLL) coated glass, PLL coated graphene, bare graphene	Increased density of attached neurons on bare graphene; Decreased neurite number on bare graphene; Increased neurite outgrowth on coated graphene compared to bare graphene
[2] He et al. 2016 Biointerfaces	Primary hippocampal neurons (from postnatal P0 rats)	Graphene on glass, tissue culture polystyrene (TCPS) (both coated with poly-lysine)	Increased growth cone growth, neurite sprouting/outgrowth, & complexity of dendritic network on graphene; Higher frequency of spontaneous post synaptic currents (sPSC) on graphene; Neurons couldn't grow on graphene or TCPS without coating
[3] Lee et al. 2015 Biochem. & Biophys. Res. Comm.	Human neuroblastoma SH-SY5Y cells (neural differentiation with RA)	Glass, graphene on glass	Increased neurite outgrowth on graphene
[4] Fabbro et al. 2016 ACS Nano	Primary hippocampal neurons (from postnatal P2-P3 rats)	Graphene on glass, Control (glass or polyornithine-coated glass)	Normal morphology & cell density on all substrates; no effect on sPSC or induced PSC or synaptogenesis on all substrates
[5] Bendali et. al. 2013 Adv. Healthcare Mat.	Primary retinal ganglion cells (from adult rats)	Glass +/- Poly-D-Lysine (PDL)/laminin coating, graphene on sapphire +/- coating	Decreased cell survival, cell body area, & neurite outgrowth on graphene compared to glass (-coating); Decreased neurite outgrowth on graphene compared to glass (+coating); Decreased cell body area & neurite outgrowth on both substrates -coating compared to +coating
	Primary retinal ganglion cells (from postnatal P7 rats)	Glass +/- PDL/laminin coating, graphene on sapphire +/- coating, sapphire +/- coating	Decreased cell viability, neurite outgrowth, & total processes on all substrates -coating compared to +coating; Increased cell body area on all substrates -coating compared to +coating (cell aggregation)
[6] Sahni et al. 2013 J. Neurosurg. Ped.	Primary rat cortical neurons	Uncoated permanox dishes, PDL coated dishes, graphene	No deleterious effect of graphene on neuronal attachment, growth, or morphology; No evidence of cytotoxicity between substrates; Increased LDH activity on graphene compared to PDL coated; Decreased LDH activity on graphene compared to uncoated substrate
[7] Park et al. 2013 J. Microbio.	Human nerve SH-SY5Y cells	Glass +/- graphene coating, SiO_2/Si +/-	Normal percentage cell viability, cell survival, & morphology on all substrates

Biotech.		graphene coating	
[8] Li et al. 2011 Biomaterials	Primary hippocampal neurons (from postnatal P1 mouse)	TCPS, graphene on TCPS (both coated with PLL)	Normal neuron growth, morphology, density, metabolic activity, & membrane integrity on both substrates; Increased neurite outgrowth on graphene

[1] Veliev F, Briancon-Marjollet A, Bouchiat V, Delacour C. Impact of crystalline quality on neuronal affinity of pristine graphene. Biomaterials. 2016;86:33-41.
[2] He Z, Zhang S, Song Q, Li W, Liu D, Li H, et al. The structural development of primary cultured hippocampal neurons on a graphene substrate. Colloids Surf B Biointerfaces. 2016;146:442-51.
[3] Lee JS, Lipatov A, Ha L, Shekhirev M, Andalib MN, Sinitskii A, et al. Graphene substrate for inducing neurite outgrowth. Biochem Biophys Res Commun. 2015;460:267-73.
[4] Fabbro A, Scaini D, Leon V, Vazquez E, Cellot G, Privitera G, et al. Graphene-Based Interfaces Do Not Alter Target Nerve Cells. ACS Nano. 2016;10:615-23.
[5] Bendali A, Hess LH, Seifert M, Forster V, Stephan AF, Garrido JA, et al. Purified neurons can survive on peptide-free graphene layers. Adv Healthc Mater. 2013;2:929-33.
[6] Sahni D, Jea A, Mata JA, Marcano DC, Sivaganesan A, Berlin JM, et al. Biocompatibility of pristine graphene for neuronal interface. J Neurosurg Pediatr. 2013;11:575-83.
[7] Park H-B. Effect of Graphene on Growth of Neuroblastoma Cells. Journal of Microbiology and Biotechnology. 2013;23:274-7.
[8] Li N, Zhang X, Song Q, Su R, Zhang Q, Kong T, et al. The promotion of neurite sprouting and outgrowth of mouse hippocampal cells in culture by graphene substrates. Biomaterials. 2011;32:9374-82.

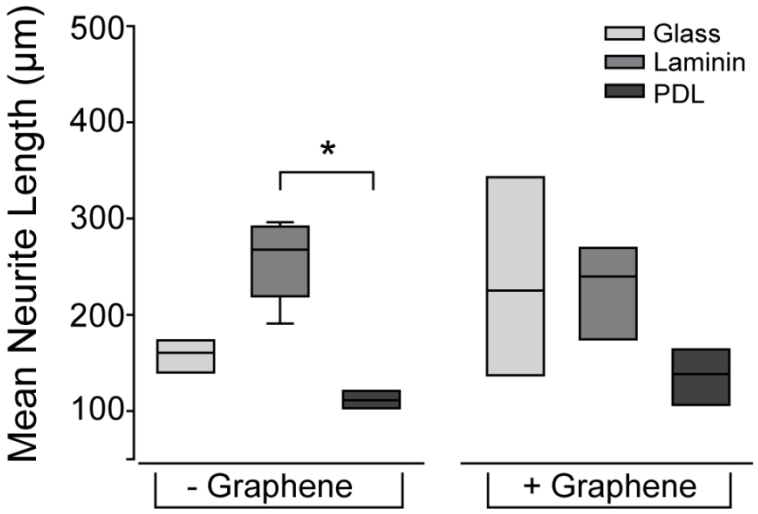

Figure S1: Box plot of the mean neurite length (µm) in each culture platform. Asterisks indicate $p<0.05$. Consistent with the results of Figure 4, average neurite length between graphene-integrated platforms and their respective substrate-only platforms did not differ ($p > 0.05$). Average neurite length was longer in the laminin platform than in the PDL platform ($p < 0.05$). These data confirm the results of the neurite intersections analysis that graphene overlay does not alter efficacy of glass, laminin or PDL substrates, and that laminin is the preferred substrate.

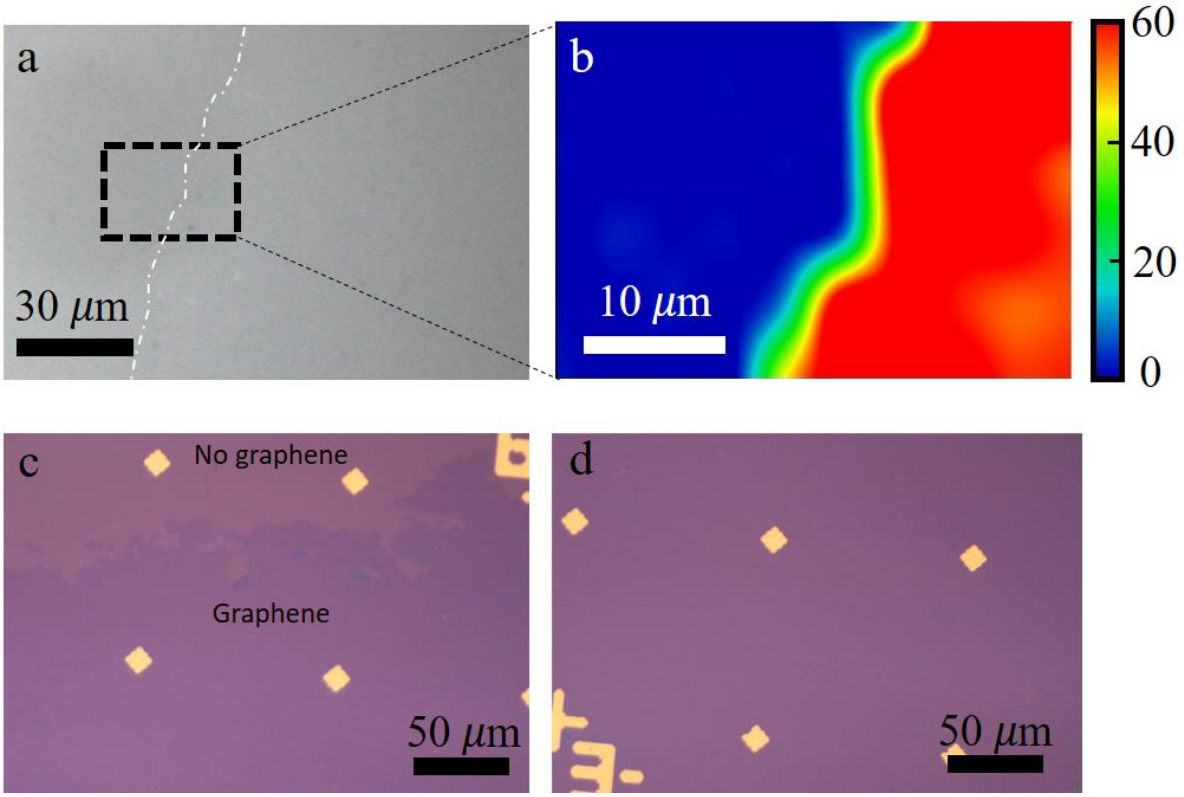

Figure S2: Optical images of graphene on top of (a) a laminin coated coverslip and (c, d) a SiO_2/Si wafer. (b) Raman mapping shows the 2D-peak intensity distribution in the black dashed line circled region in (a).

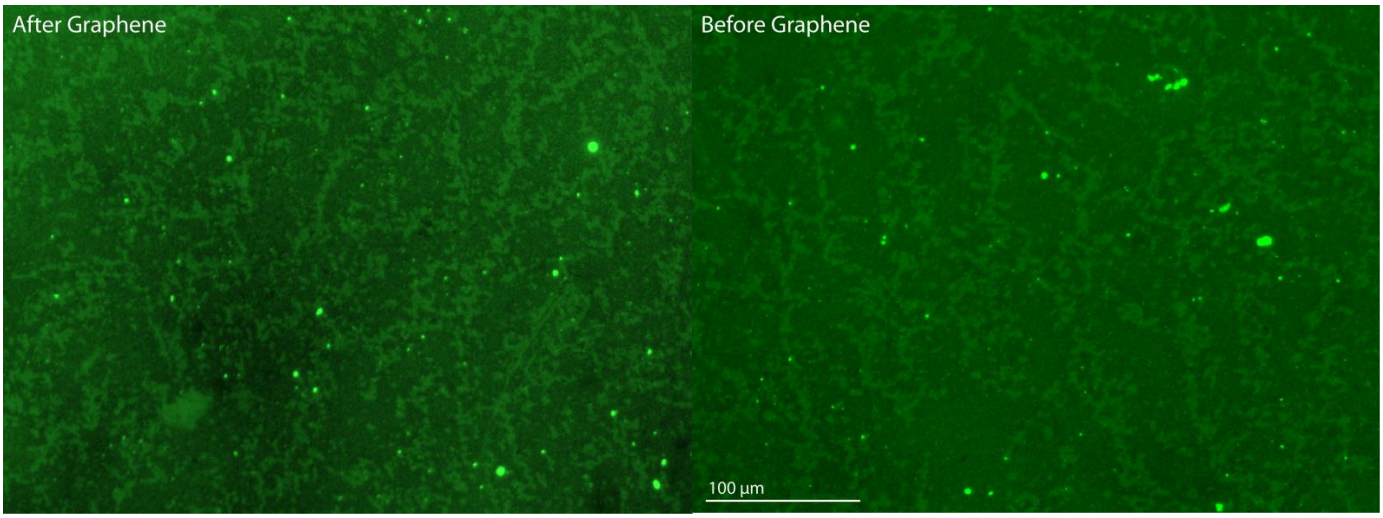

Figure S3: Fluorescence images of a laminin substrate labeled with anti-laminin antibodies (green) before and after graphene coating.