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Supporting Section 1. Experimental Methods

A. Fabrication of substrates

The ECM protein in our experiments is laminin, which is essential for the adhesion, growth, and differentiation of hNSCs. Furthermore, ECM protein patterns and nanotopographical features have been shown to play a significant role in controlling the polarity of hNSCs and influence their neuronal differentiation. We thus created nanotopographical features within the ECM using monolayers of positively charged silica nanoparticles (300 nm) assembled using centrifugation at 2000 RPM for 1 min and then coated these monolayers with GO. Laminin was then adsorbed on these hybrid films to facilitate the attachment of hNSCs. We show that by using SiNP monolayers coated with GO, hNSCs not only align, but also show a remarkable increase in the expression of neuronal markers. We thus believe that incorporating GO along with nanotopographical features into the microenvironment of hNSCs provide significant advantages for the differentiation of hNSCs into neurons.

Cover glass (Number 1, 22 mm x 22 mm; VWR) was cut equally into smaller pieces (18 mm x 6 mm) and sonicated in Nanopure water (18.2 mOhm) for 10 mins and then cleaned in piranha solution (a 3:1 mixture of sulphuric acid and hydrogen peroxide) for 10 min (Caution: Piranha solution is extremely corrosive). The glass coverslips were then washed again in Nanopure water (18.2 Mohm) and dried under a stream of pure nitrogen. To generate films of nanotopographical features, 300 nm silicon oxide nanoparticles (SiNPs, Corpuscular Inc)) were utilized. The washed cover slips were centrifuged at 2000 RPM for 2 min in a 2 mL eppendorf tube containing 25 mg/mL of the positively charged (amine terminated) SiNP solution. The substrates were then washed with Nanopure water and dried under a stream of pure nitrogen. The

cover slips were then rinsed thoroughly with ethanol and dried under nitrogen. They were then baked at 100 °C in an oven for 10 min.

Graphene Oxide (GO), which is negatively charged, was assembled on glass cover slips through electrostatic interactions. Specifically, these glass cover slips were first functionalized with self-assembled monolayers of positively charged APTES. Each positively charged glass cover slip was then simply dipped once into a concentrated solution of nanometer sized GO (250 nm, 0.6 mg/mL) and dried using a stream of nitrogen. Furthermore, we generated substrates having nanotopographical features within the ECM using monolayers of positively charged silica nanoparticles (SiNP, 300 nm). The monolayers were fabricated using centrifugation as previously reported. The substrates having the nanoparticles were then dipped into a concentrated solution of GO (0.6 mg/mL) as before and dried under a stream of nitrogen. As controls, we used bare glass and generated substrates having only SiNPs (without GO) as shown in Figure 1. The presence of GO on the substrates was confirmed using Raman spectroscopy and scanning electron microscopy (SEM) (Supporting Figure S1).

B. Human neural stem cell (hNSC) culture and differentiation

Human neural stem cell line (ReNCell VM, Millipore) was purchased and routinely expanded according to the manufacture's protocol. The hNSCs were maintained in laminin (Sigma, 20 μg/ml) coated culture dishes precoated with poly-L-lysine (10 μg/ml) in ReNCell VM media (Millipore) supplemented with the antibiotics, geniticin (Life Technologies), in the presence of basic fibroblast growth factor (bFGF-2, 20 ng/ml, Millipore) and epidermal growth factor (EGF, 20 mg/ml, Peprotech). All of the cells were maintained at 37°C in a humidified atmosphere of 5% CO_2. For consistency, the experiments were carried out on cells between passages 2 and 5.

Neural differentiation was initiated by changing the medium to basal medium (without bFGF-2 and EGF) on the different substrates (SiNP, GO, SiNP-GO and control glass) coated with laminin. The cells were allowed to differentiate for 14 days with the basal medium in each being exchanged every other day.

C. Image Analysis

Image analysis on images taken from scanning electron microscopy was done to determine the axonal alignment and axonal length. Alignment is recorded by tracing axons through Adobe® Photoshop® software and measuring linear angle from connected cell bodies. Axonal length is done using a similar method by tracing axons and calculating the pixels spanned in Adobe® Photoshop®. Once the number of pixels has been obtained, it is converted to μm by referencing the amount of pixels the scale bar spanned. Since there needs to be a reference angle, SEM images are rotated so that the general directions of axons are similar among all conditions. The number of cells participated in image analysis is one hundred per condition. Data analysis was conducted after alignment angles were recorded for all four conditions. Due to the nature of sample loading of SEM, angles of axons are not a good statistical indicator of alignment. Instead, the angle of standard deviation is the better statistical representative of the effects of our platform. Figure. 2b depicts a compass plot plotted with MathWorks® Matlab® software illustrating axonal angles and lengths of four conditions.

D. Cell Viability Assay

The percentage of viable cells on the different substrates was determined after 3 weeks of differentiation using the MTS cell viability assay following standard protocols described by the manufacturer. All experiments were conducted in triplicate and averaged. The data is represented

as formazan absorbance at 490 nm, considering the differentiated hNSCs on SiNP-GO as 100% viable.

E. Immunocytochemistry

To investigate the extent of neuronal differentiation, at Day 14, the basal medium was removed and the cells fixed for 15 minutes in Formalin solution (Sigma) followed by two PBS washes. Cells were permeabilized with 0.1% Triton X-100 in PBS for 10 minutes and non- specific binding was blocked with 5% normal goat serum (NGS, Life Technologies) in PBS for 1 hour at room temperature. To study neuronal differentiation, antibodies against neuronal markers were used. Mouse primary antibodies were used against TuJ1 (1:500, Covance), NeuN (1:100, Millipore) and rabbit primary antibodies were used against Synapsin (1:100, Santa Cruz Biotechnology) and MAP2 (1:100, Cell Signaling). The fixed samples were incubated overnight at 4°C in solutions of primary antibodies in PBS containing 10% NGS. After washing three times with PBS, the samples were incubated for 1 h at room temperature in solution of anti-mouse secondary antibody labeled with Alexa-Fluor® 647 or Alexa-Fluor® 546 and anti-rabbit secondary antibody labeled with Alexa-Fluor® 546 or Alexa-Fluor® 488 (1:200, Life Technologies), Hoechst 33342(1:500, Life Technologies) in PBS containing 10% NGS to observe neuronal differentiation. After washing the samples thrice with PBS, the substrates were mounted on glass slides using ProLong® antifade (Life Technologies) to minimize photobleaching. The mounted samples were imaged using Nikon TE2000 Fluorescence Microscope.

F. PCR Analysis

Total RNA was extracted using Trizol Reagent (Life Technologies) and the mRNA expression level of TuJ1, MAP2, GAP43 and nestin were analyzed using Reverse Transcriptase PCR (RT-PCR) and quantitative PCR (qPCR). Specifically, cDNA was generated from 1 μg of total RNA using the Superscript III First-Strand Synthesis System (Life Technologies). Analysis of mRNA was then accomplished using primers specific to each of the target mRNAs. RT-PCR reactions were performed in a Mastercycler Ep gradient S (Eppendorf) and images were captured using a Gel Logic 112 (Carestream) imaging system. qPCR reactions were performed using SYBR Green PCR Master Mix (Applied Biosystems) in a StepOnePlus Real-Time PCR System (Applied Biosystems) and the resulting Ct values were normalized to Gapdh. Standard cycling conditions were used for all reactions with a melting temperature of 60°C. Primers are listed below:

Gene	F Primer	R Primer	Size (bp)
FAK	5'-CAATGCCTCCAAATTGTCCT-3'	5'-TCCATCCTCATCCGTTCTTC-3'	157
GAPDH	5'-ATGACTCTACCCACGGCAAG-3'	5'-GGAAGATGGTGATGGGTTTC-3'	87
Nestin	5'-GGAAGAGAACCTGGGAAAGG-3'	5'CTTGGTCCTTCTCCACCGTA-3'	122
GAP43	5'-AACCTGAGGCTGACCAAGAA-3'	5'-GGGACTTCAGAGTGGAGCTG-3'	118
MAP2	5'-GAGAATGGGATCAACGGAGA-3'	5'-CTGCTACAGCCTCAGCAGTG-3'	100
TUJ1	5'-ACTTTATCTTCGGTCAGAGTG-3'	5'-CTCACGACATCCAGGACTGA-3'	97

Supporting Section 2. Synthesis of chemically derived graphene, pristine graphene and MoS_2 nanoflakes

Chemically derived graphene (CDG) or graphene oxide was obtained by the exfoliation of graphite oxide made through the modified Hummers method. Details of this method can be found elsewhere [1]. Thin films of graphene oxide can be formed from this suspension either by dip coating, spin coating, drop-casting or vacuum filtration methods.

Chemical vapor deposition (CVD) of graphene was done on copper by modifying the conditions mentioned in [2-3] to obtain large area graphene. Copper foil (Alfa Aesar, item No.13382) was annealed in Argon atmosphere for 30 mins at 1000^{o} C and then methane was allowed into the furnace for two hours in the presence of argon at 1000^{o} C. Graphene thus formed on copper was transferred onto glass substrates after spin coating PMMA (poly(methyl methacrylate)) and dissolving copper using 1M iron chloride solution [3].

Bulk MoS_2 powder was purchased from Sigma-Aldrich (item number 69860) and exfoliated using lithium intercalation method to produce an aqueous suspension of exfoliated MoS_2 [4]. Thin films were made on glass substrates using vacuum filtration method and single layer was confirmed through optical microscopy and AFM images.

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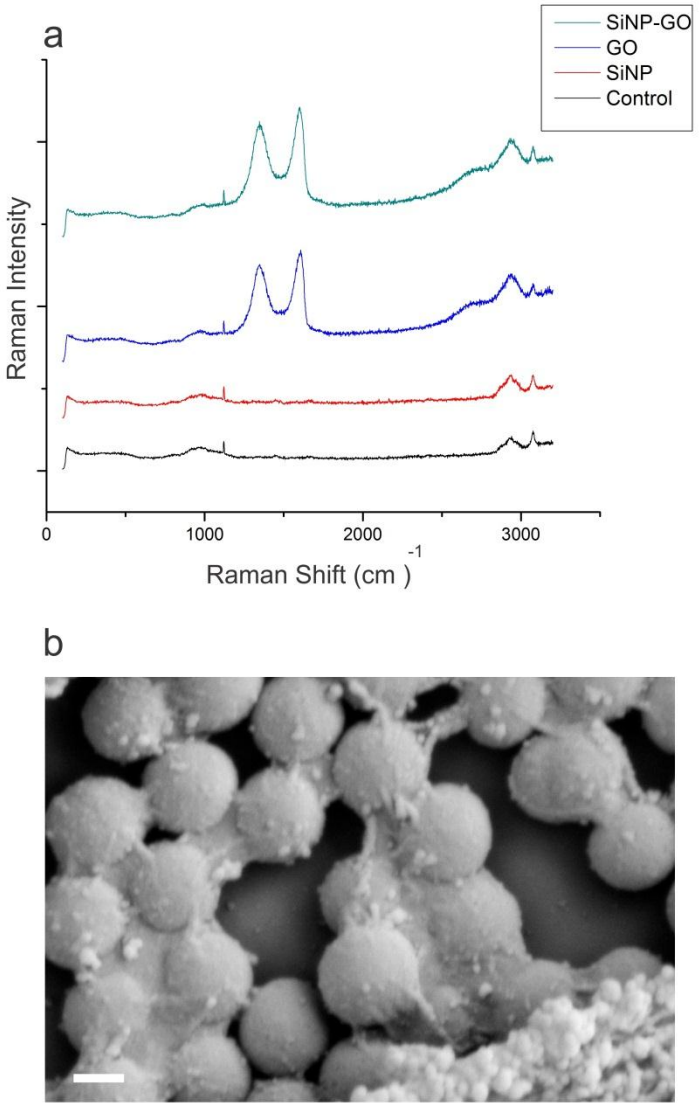
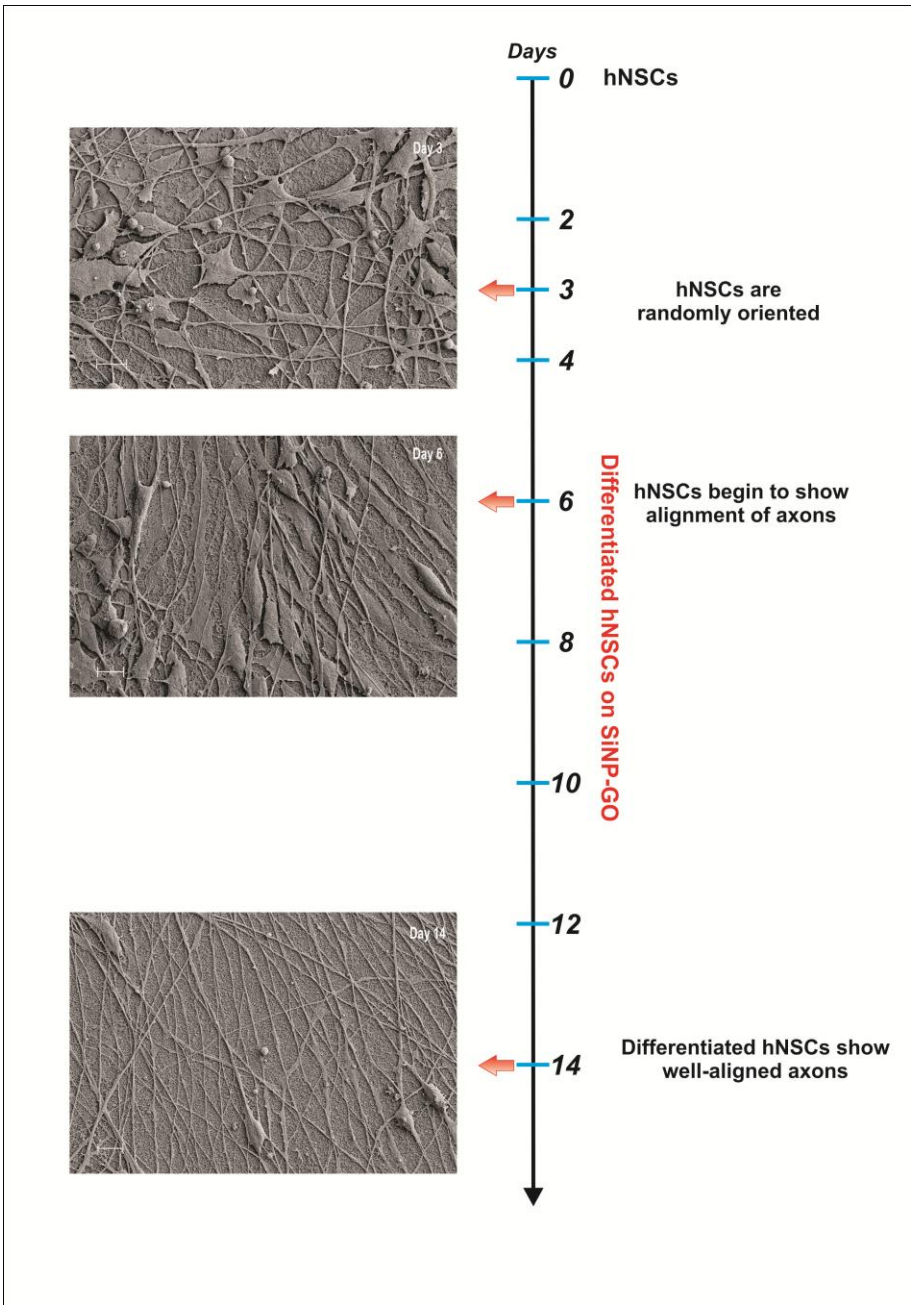
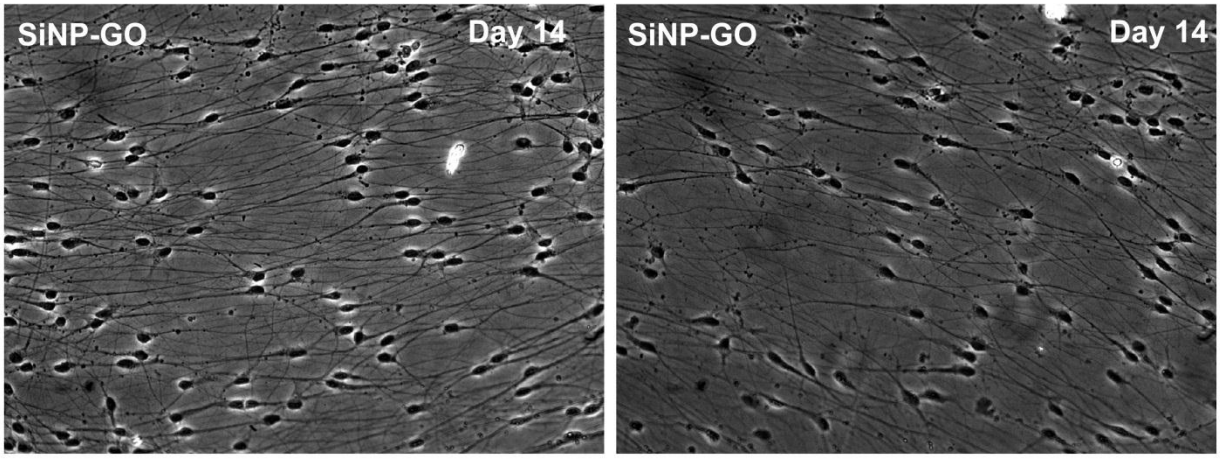


Supporting Figure S1. Raman Spectroscopy and SEM confirming the presence of graphene oxide. (a) Raman Spectroscopy imaging shows the presence of the characteristic D and G bands on graphene oxide. The bare glass substrate and the substrate having on SiNP do not show the characteristic bands. (b) SEM image of SiNP-GO coated with laminin clearly shows the presence of GO on the SiNPs. Scale bar: 200 nm

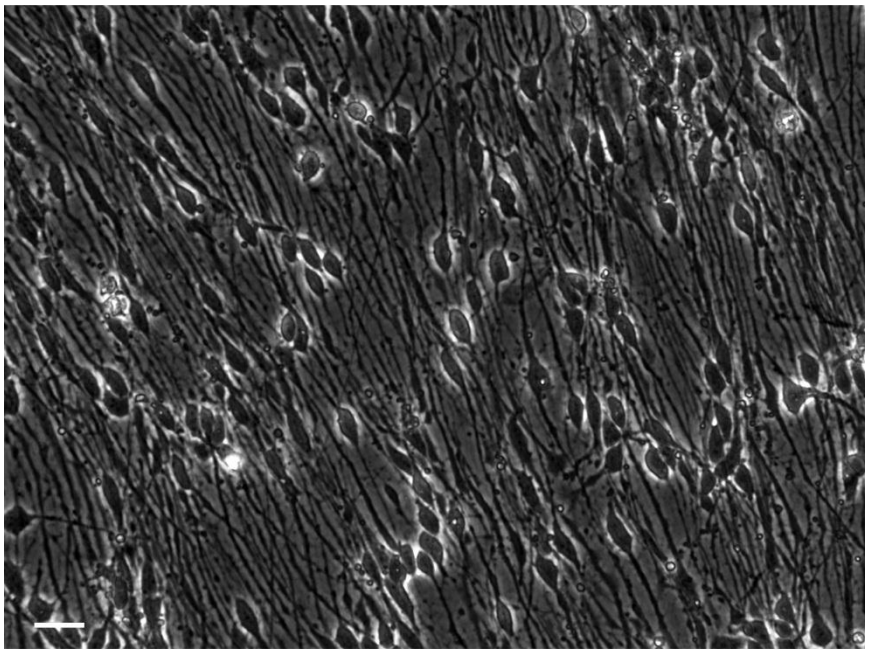


Supporting Figure S2. SEM images showing axonal alignment of differentiated hNSCs on SiNP-GO substrates. The hNSCs were randomly oriented when seeded on SiNP-GO substrates, and the emerging axons did not show any alignment until Day 6. On Day 6, the axons were still

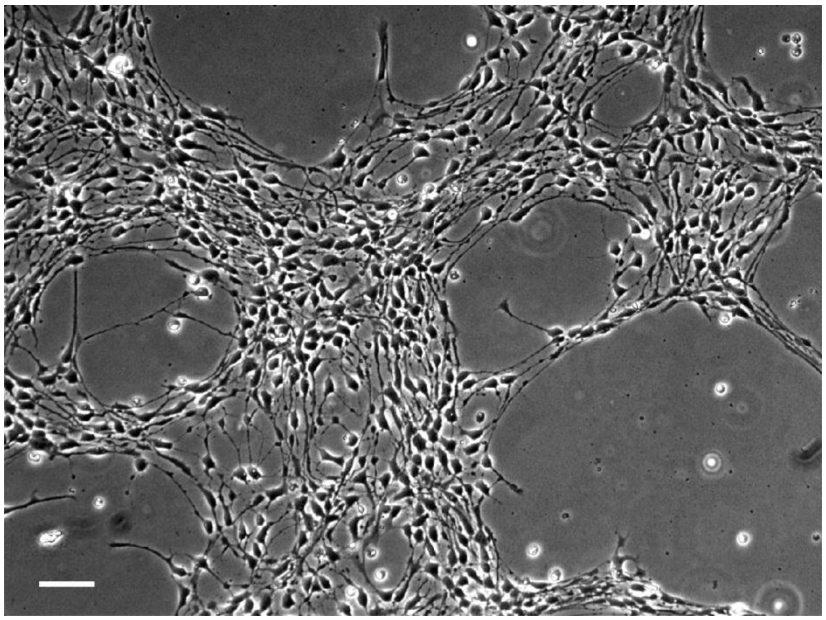
growing but begin to align. On Day 14, the differentiated hNSCs show very well-aligned axons. Scale bar: 10 μm.



Supporting Figure S3. Axonal alignment and cell density. Phase contrast images showing axonal alignment of differentiated hNSCs cultured on SiNP-GO at different initial seeding densities. The axonal alignment was observed even when the initial seeding density was reduced by 50%.



Supporting Figure S4. Axonal alignment on pristine graphene. Phase contrast image showing axonal alignment of differentiated hNSCs on pristine graphene. Scale bar: 10 μm



Supporting Figure S5. hNSCs differentiated on molybdenum disulfide (MoS_2). Phase contrast image showing hNSCs differentiated on molybdenum disulfiude. Scale bars: 20 μm