ADVANCED MATERIALS

Supporting Information

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Carbon Nanotube–Hydrogel Composites Facilitate Neuronal Differentiation While Maintaining Homeostasis of Network Activity

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Figure S10. Morphological classification of dendritic spines of neurons derived from NSCs cultured on PLL-coated glass substrates or c-PEG-20 samples.

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Experimental Section

Table S1. UV illumination time of CNT-PEG hydrogel composites with varying CNT content.

Notation	CNT concentration (g L^{-1})[a]	UV illumination time (s)[b]
c-PEG-0	0	10
c-PEG-5	5	60
c-PEG-10	10	120
c-PEG-20	20	240

[a)] The concentration here is referring to the CNTs in the ionic liquid. [b)] UV light: 365 nm, 250 mA (*i.e.*, 15.6 mW cm^{-2}).

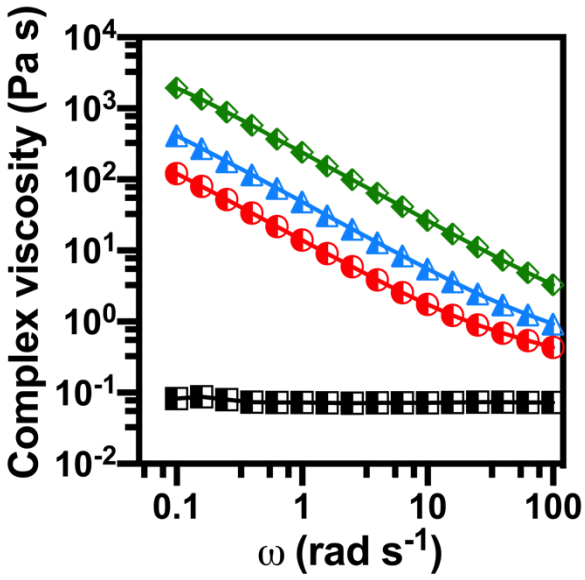

Figure S1. Frequency dependence of complex viscosity of the precursors of CNT-PEG hydrogel composites with varying CNT content (◧: c-PEG-0, ◐: c-PEG-5, ◭ c-PEG-10, ◆: c-PEG-20 samples).

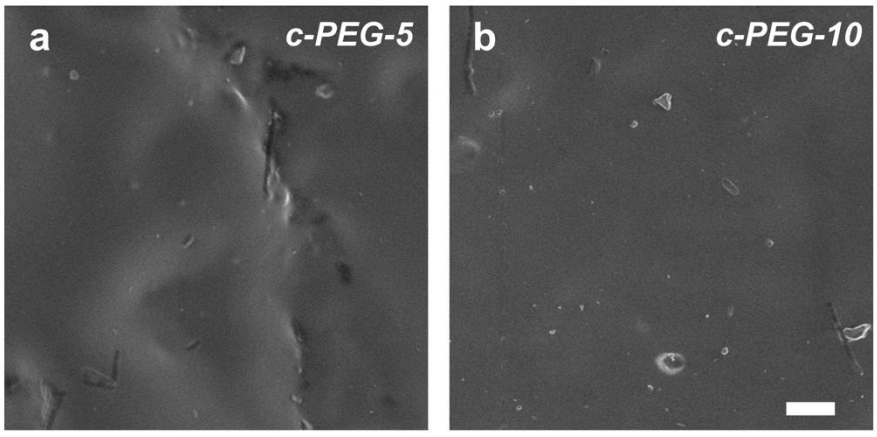

Figure S2. Scanning electron microscopy images of (a) c-PEG-5 and (b) c-PEG-10 samples after gently dehydration. Scale bar: 5 μm.

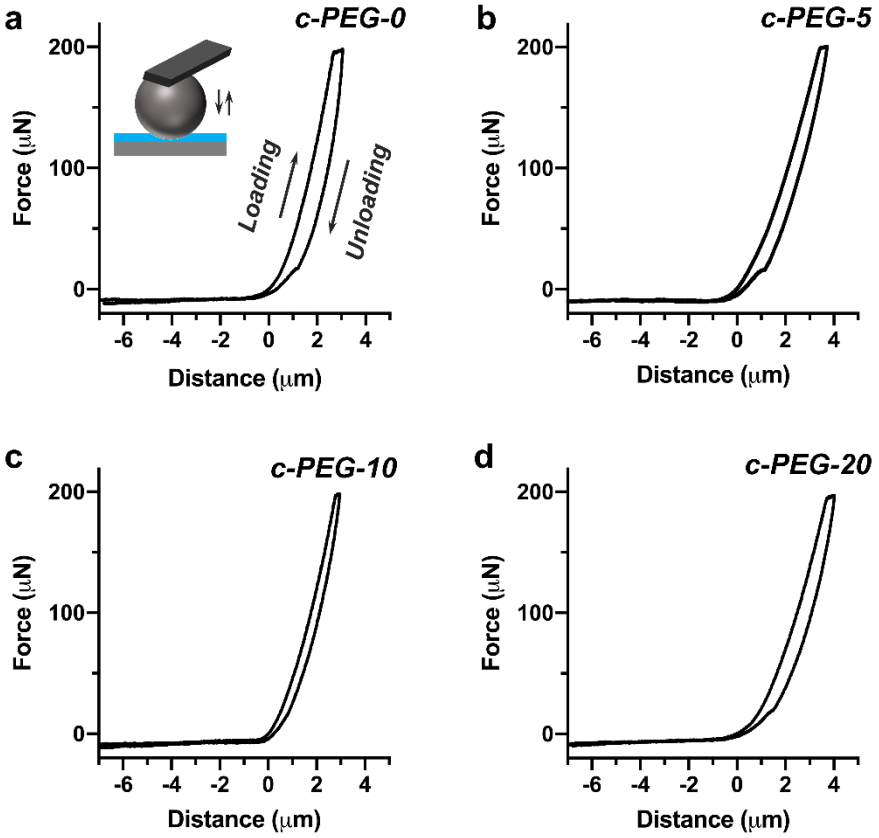

Figure S3. Indentation curves of CNT-PEG hydrogel composites with varying CNT content. (a) c-PEG-0, (b) c-PEG-5, (c) c-PEG-10, and (d) c-PEG-20 samples. The elastic modulus of the composites was obtained by fitting the loading curves with the Hertz model.

$$F = \frac{4}{3}\frac{E}{1-\mu^2}\sqrt{R\delta^3}$$

where E is the elastic modulus of the sample, μ is the Poisson's ratio of the sample, R is the radius of the probe, and δ is the indentation depth.

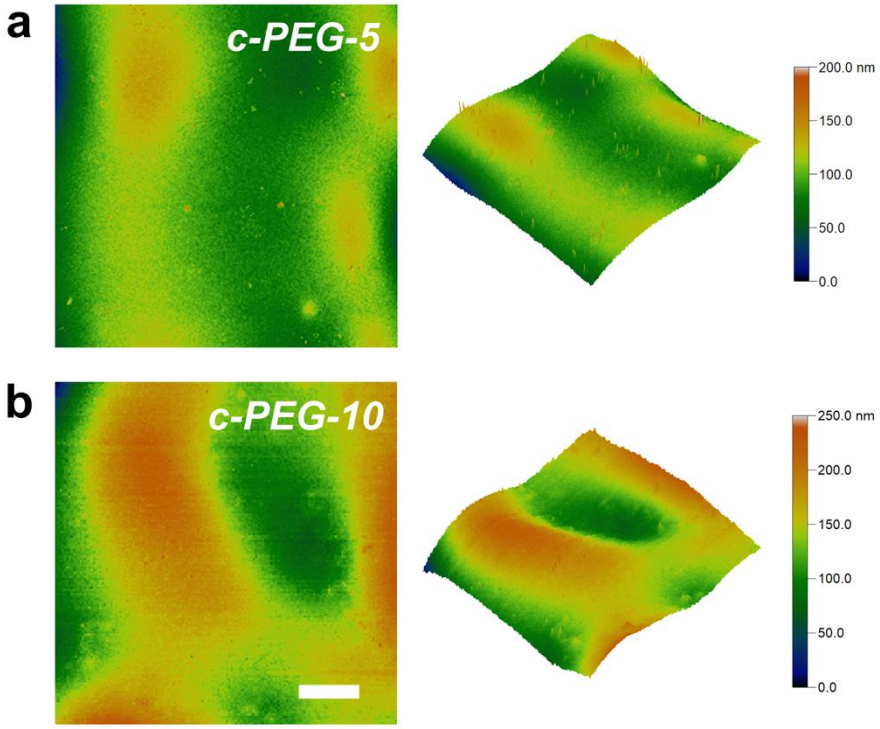

Figure S4. 2D and 3D topographies of (a) c-PEG-5 and (b) c-PEG-10 samples captured by atomic force microscopy in water. Scale bar: 5 μm.

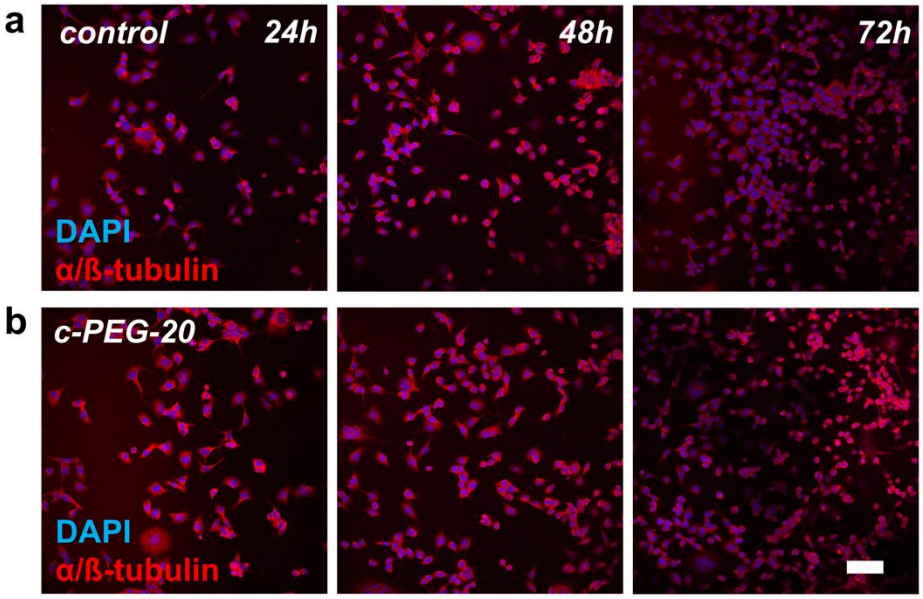

Figure S5. Confocal laser scanning microscopy images of PC12 cells cultured on PLL-coated (a) glass substrates and (b) c-PEG-20 samples for 24h, 48h, 72h. Cells were stained with Tubulin antibody (the nuclei were stained with dihydrochloride). Scale bar: 100 μm.

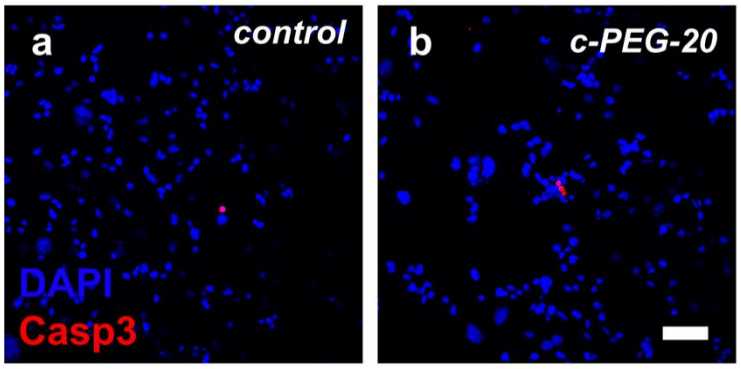

Figure S6. Confocal laser scanning microscopy images of PC12 cells cultured on PLL-coated (a) glass substrates and (b) c-PEG-20 samples for 48 h. The nuclei (blue) were stained with dihydrochloride, and the dead cells were marked with cleaved Caspase3 (red). Scale bar: 100 μm.

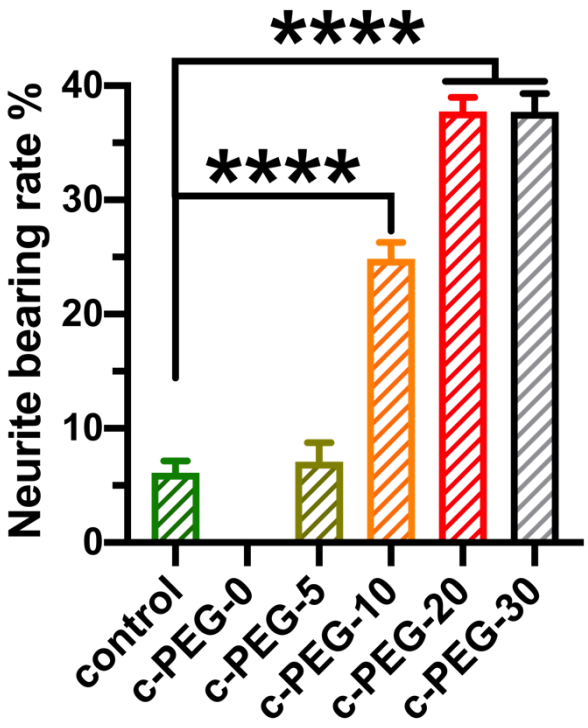

Figure S7. Neurite bearing rate of PC12 cells cultured on PLL-coated glass substrates and CNT-PEG hydrogel composites with varying CNT content. (n = 5 for PLL-coated glass plates, 3 for c-PEG -0, 3 for c-PEG-5, 8 for c-PEG-10, 16 for c-PEG-20 and 8 for c-PEG-30, one-way ANOVA). ****: $p < 0.0005$.

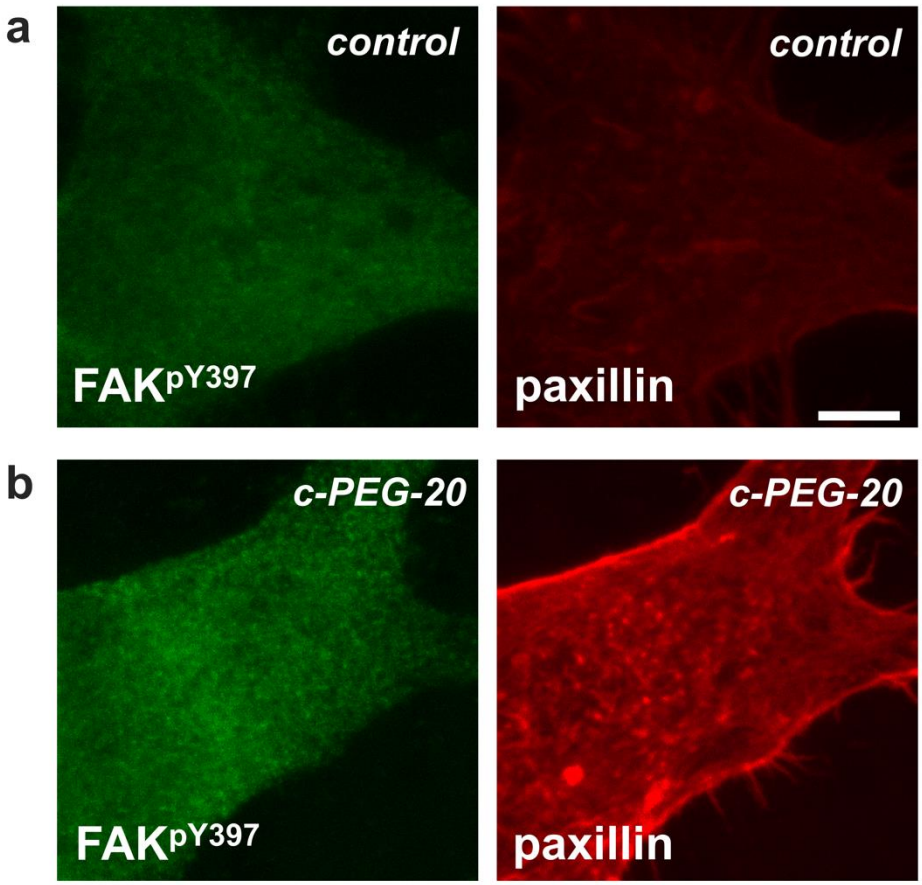

Figure S8. Confocal laser scanning microscopy images of PC12 cells cultured on PLL-coated (a) glass substrates and (b) c-PEG-20 samples for 72 h. Cells were stained with anti-Phospho-FAK (Tyr397) antibody and anti-Paxillin antibody. Scale bar: 5 μm.

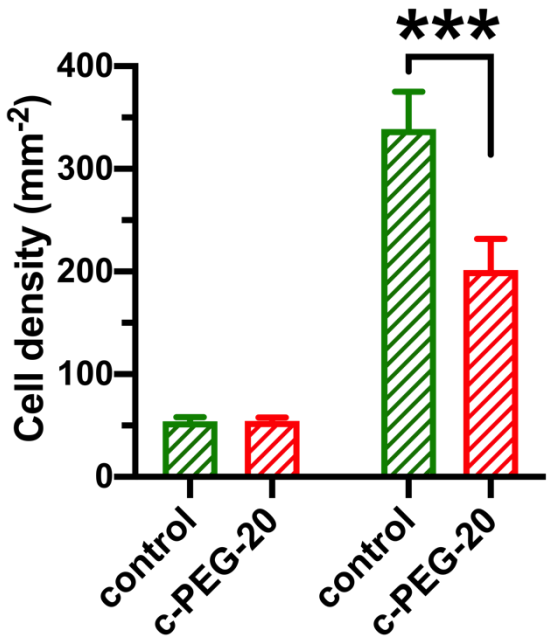

Figure S9. Cell density of neuron (left) and astrocyte (right) from NSC differentiated on PLL-coated glass substrates (control) and c-PEG-20 samples (n = 8 control and 7 c-PEG-20) ***: $p < 0.0001$, two-sided t-test.

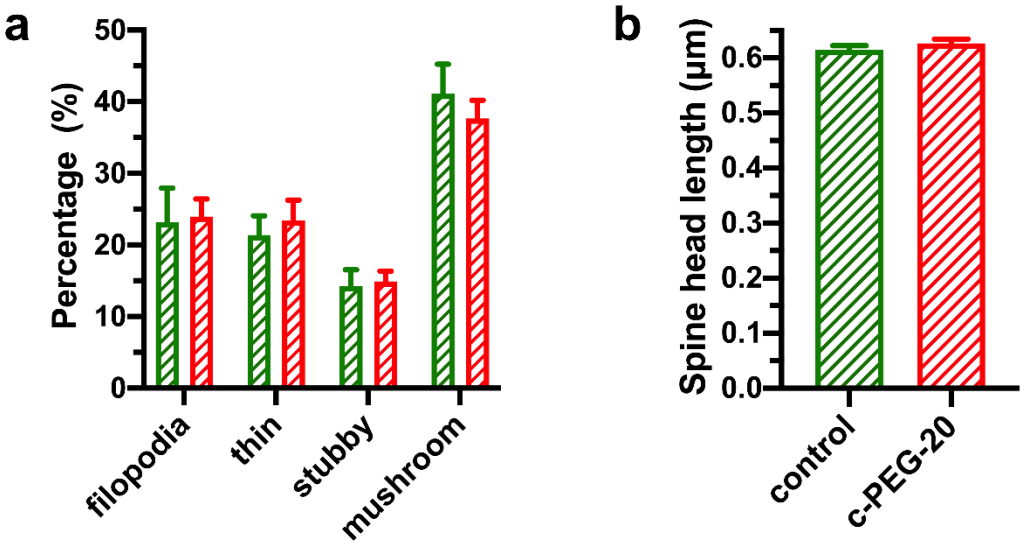

Figure S10. Results comparing the morphological classification of dendritic spines by shape (a) and the head length of dendritic spines (b) of neurons derived from NSCs cultured on PLL-coated glass substrates (green) or on c-PEG-20 samples (red) (for a: n = 614 total spine numbers in percent from 11 fields of view for NSC-derived neurons cultured on PLL-coated glass plates and total 534 spine numbers in percent from 14 fields of view for NSC-derived neurons cultured on c-PEG-20 substrates, for b: n = 737 spines from neurons cultured on PLL-coated glass slides and 875 spines from neurons cultured on c-PEG-20 substrates. two-sided t-test).

Experimental Section

DNA Transfection. Lipofectamine® 2000 (Invitrogen) was used for DNA transfection. The complexes for transfection were prepared as follows: 4 µL of Lipofectamine® 2000 was diluted in 100 µL of Opti-MEM I Reduced Serum Medium and incubated for 5 min at room temperature. In parallel, 2.0 µg of DNA was diluted in 100 µL of Opti-MEM I Reduced Serum Medium and mixed gently. After 5 min incubation, the diluted DNA and diluted Lipofectamine® Transfection Reagent was mixed gently and incubated for 20 min at room temperature. The samples were placed in 6-well plates. 200 µL of complexes was added to each well and mixed gently by rocking the plate back and forth. The medium was replaced with fresh growth medium after 6 h. The cells were used 48 h post-transfection.

Western Blotting (WB). PC12 cells were plated on PLL-coated glass slides and c-PEG-20 samples in DMEM medium containing 10% FBS. 24h later, the medium was replaced with 2.5% FBS culture medium and cells were cultured for another 48 h. Then the medium was removed, and cells were washed gently by ice cold PBS. Cells were lysed on ice by adding 100 µL RIPA buffer (50 mM Tris-HCl (pH 7.5). 150 mM NaCl, 1 mM EDTA, 1% Triton X-100 and 0.1 % SDS) supplemented with protease inhibitor (cOmplete™ Protease Inhibitor Cocktail, Roche) and phosphatase inhibitor (PhosSTOP™, Roche). WB was performed according to standard procedures. Briefly, samples were separated by 10% SDS-PAGE and blotted on a nitrocellulose membrane (0.45 µm, BIO-RAD, USA). Then the membrane was blocked in 10% non-fat dry milk in PBS at room temperature for 1 h and was further incubated with primary antibodies in 1% non-fat BSA at 4 °C for overnight. The following primary antibodies were used: FAK Antibody (Cell signaling, #3285, 1:4000), Phospho-FAK (Tyr397) Antibody (Cell signaling, #3283, 1:4000) and ß-actin (MP Biomedicals, clone B4，1:5000). After three washes, membranes were incubated with a corresponding secondary

fluorescent antibody (IRDye® 800CW Goat anti-Rabbit IgG Secondary Antibody or IRDye® 800CW Goat anti-Mouse IgG Secondary Antibody) at 1:10000 dilution. Immunofluorescent bands were detected using Fusion FX Spectra machine. Quantification of immunosignals was adjusted to ß-actin as loading control and was performed using ImageJ.

Immunostaining. Cells for imaging and analysis were fixed in 4% paraformaldehyde at 4 °C for 30 min followed by washed with PBS for three times and were blocked with 10% normal goat serum and 0.2% Triton-X 100 for 1 h at room temperature. Then, cells were incubated with primary antibodies at 4 °C overnight followed by washing with PBS, and further stained with Alexa-labeled secondary antibodies (Invitrogen, 1:1000 dilutions) for 1 h at room temperature and finally with DAPI (Invitrogen, 1:20000 dilutions) to visualize cell nuclei.