Supporting Information

Effects of Hydrophilic Fullerene Nanoarchitectured Structure on the Behaviour of Neural Stem Cells

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Three supplementary tables, eight supplementary figures, and three supplementary videos

Supplementary Table

Table S1. The zeta potential of three different hydrophilic self-assembled fullerene structures in water.

Samples	Zeta potential (mV)
PFNR	-34.01
FNT	-41.29
eFNS	-41.21

Table S2. DLS results of three different hydrophilic self-assembled fullerene structures in PBS solutions after 3-day incubation at 37℃.

Samples	Number Mean (nm)	
	Fresh	After 3 days
PFNR	312.8 +/- 45.1	337.9 +/- 64.4
FNT	167.4 +/- 24.6	302.6 +/- 29.4
eFNS	46.6 +/- 6.1	88.1 +/- 12.2

Table S3. The primer sequences for each primer used in RT-PCR analyses.

Genes	Primer sequences
MAP2	Forward: TTGAAGGTTAAAATGCATCTGA Reverse: GGCATTTCAAGGAAAAACTCA
CNPase	Forward: ACCCTGAGCTGGCAAGAGTA Reverse: GGTAGGAGCATACATCCCAG
GAPDH	Forward: GGCTACAGCAACAGGGTGGT Reverse: CGAGTTGGGATAGGGCCTCT
Nestin	Forward: ACTGTGGAATCACCAGGAGG Reverse: ATTCCACCTCTCCCAGAGAC
β-tubulin	Forward: CAGGGCCAAGACAAGCAGCA Reverse: GGAGCCCTAATGAGCTGGTGA
GFAP	Forward: CTGAACCCTCTGAGCAAATG Reverse: GAATCAAACACAGAGCCTGC

Supplementary figures

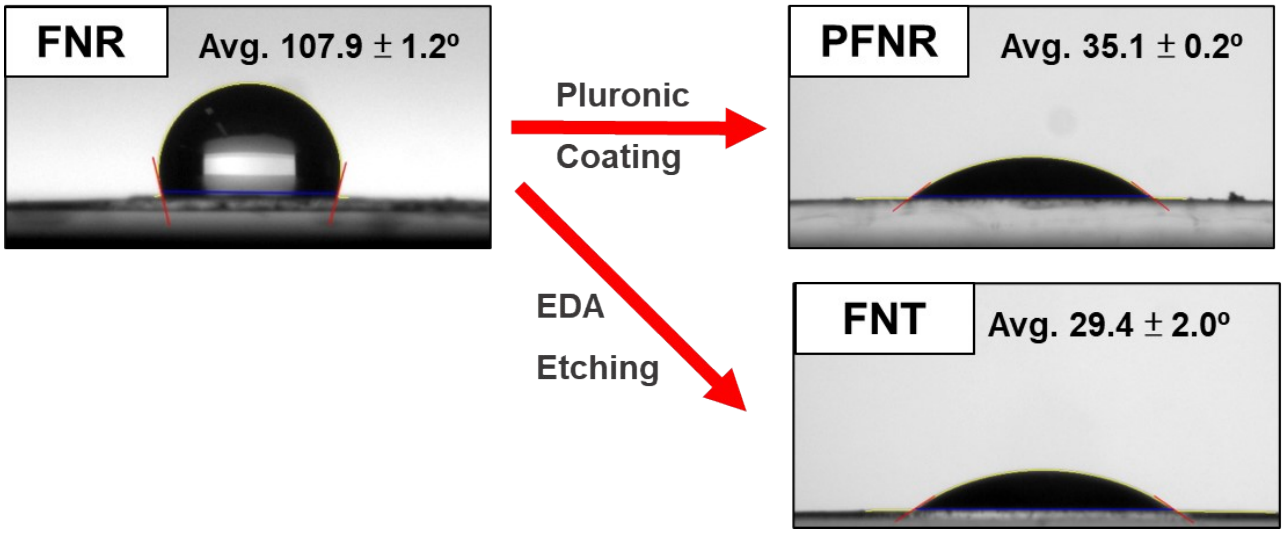

Figure S1. Contact angle measurement of FNR before and after hydrophilic surface modification by Pluronic P123 coating or EDA chemical etching.

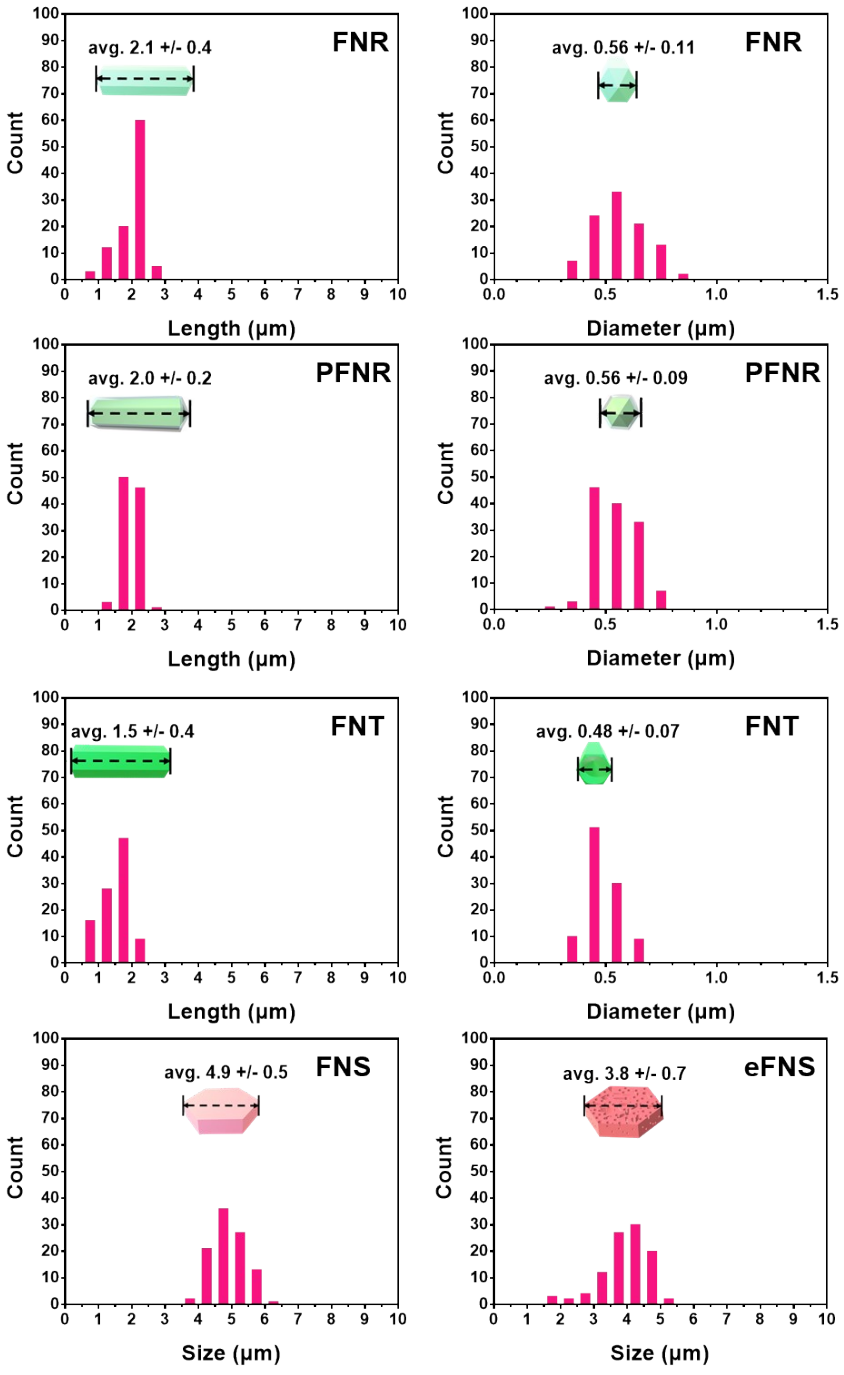

Figure S2. The size distribution of hydrophobic or hydrophilic self-assembled fullerenes obtained from the LLIP, Pluronic P123 coating, and EDA selective etching method.

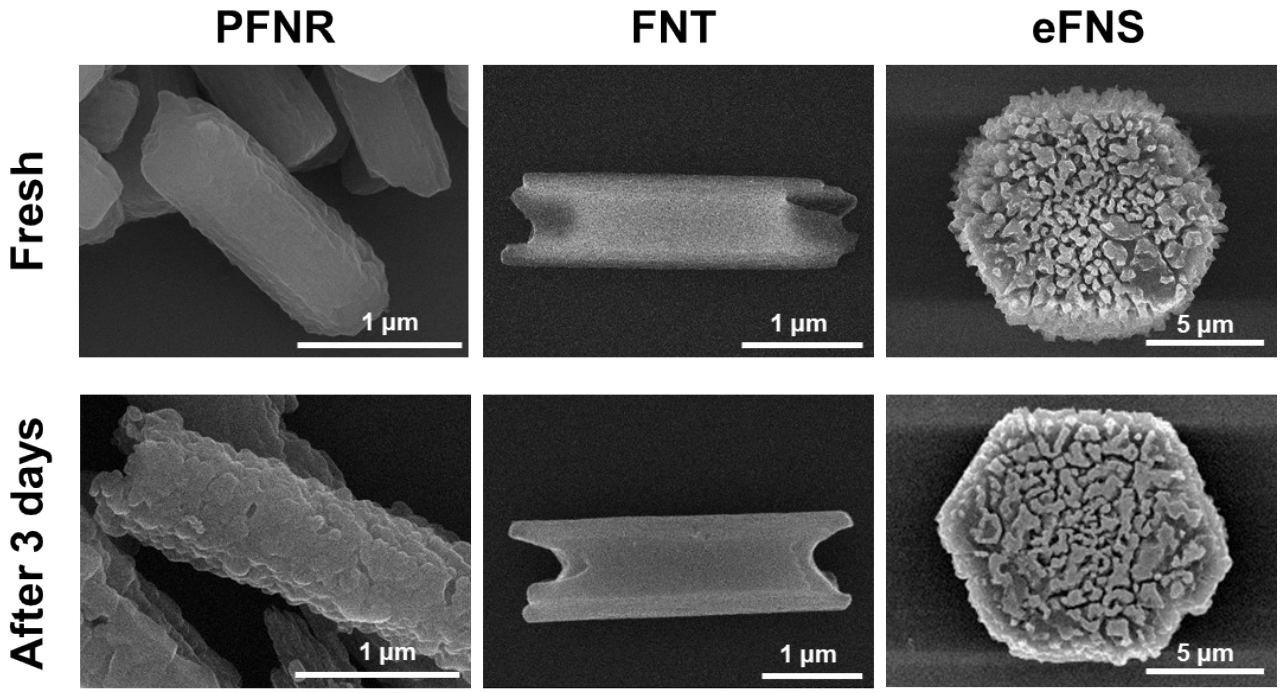

Figure S3. The morphology of hydrophilic fullerene self-assemblies in fresh aqueous solution and the changes after 3-day incubation at 37℃.

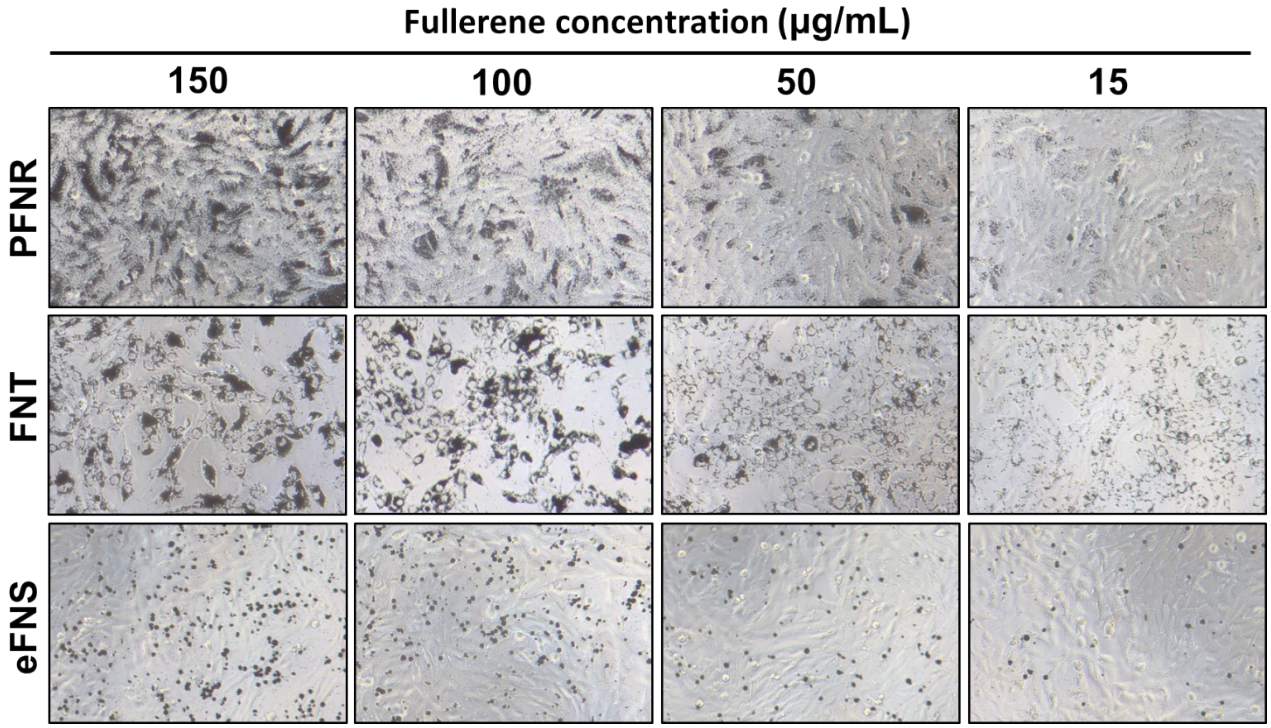

Figure S4. The morphology of NSCs cultured on tissue culture plates after adding different types of fullerene assemblies for 48 h.

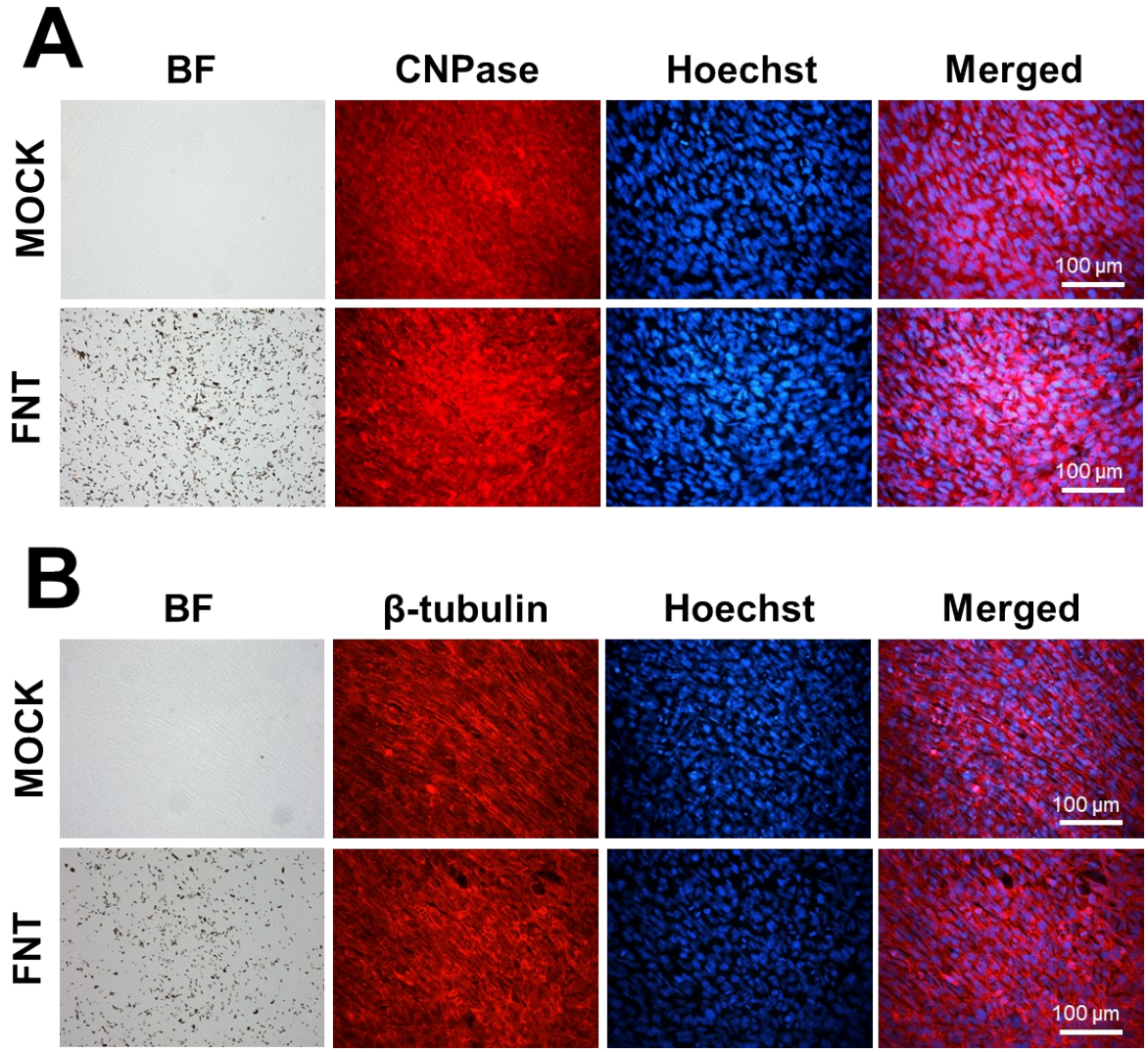

Figure S5. The expression of CNPase (A) and β-tubulin (B) of NSCs in the presence of FNT (15 μg/mL) was analyzed by immunofluorescence staining at 7 days. The expression levels of CNPase and β-tubulin were slightly upregulated in the presence of FNT. In the experiment, the NSCs were treated with FNT for 7 days and fixed with 4% paraformaldehyde solution for 15 min. The samples were blocked with 2% bovine serum albumin for 1 h and stained with β-tubulin (BioLegend, 802001) and CNPase (BioLegend, 836404) overnight at 4 °C. The samples were washed with PBS and incubated with a secondary anti-mouse or anti-rabbit antibody (Alexa Fluor® 594) for 1 h at 25 °C. The images were taken by the fluorescence microscope. BF: bright field; blue: Hoechst; red: neural-related markers. Scale bar = 100 μm.

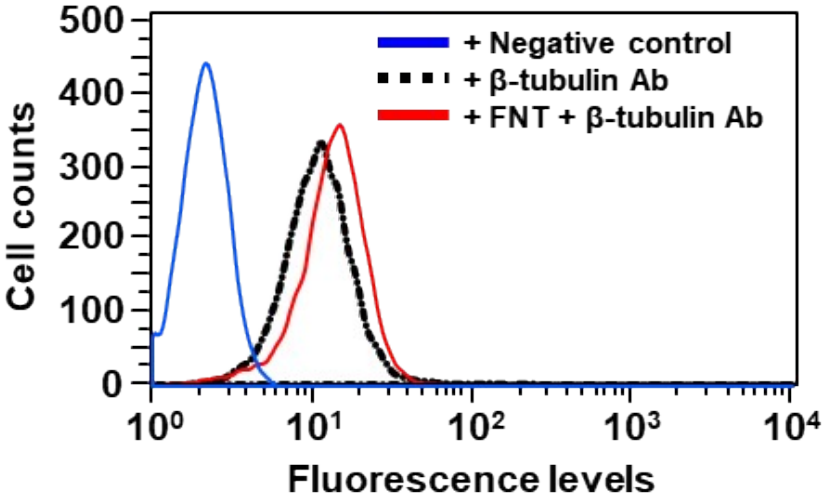

Figure S6. The protein level of β-tubulin in NSCs was semi-quantified by flow cytometry after the treatment of FNT (15 μg/mL) for 7 days. The expression of β-tubulin was slightly increased in the presence of FNT. In the experiment, the NSCs were stained with β-tubulin antibody (BioLegend, 802001) and subjected to flow cytometric (Guava, Millipore) analyses to determine the relative fluorescence levels. The blue line represents the negative control in flow cytometric analyses. The black dot line represents the pristine NSCs stained with β-tubulin antibodies. The red line represents the NSCs previously treated with FNT (15 μg/mL) and stained with β-tubulin antibody.

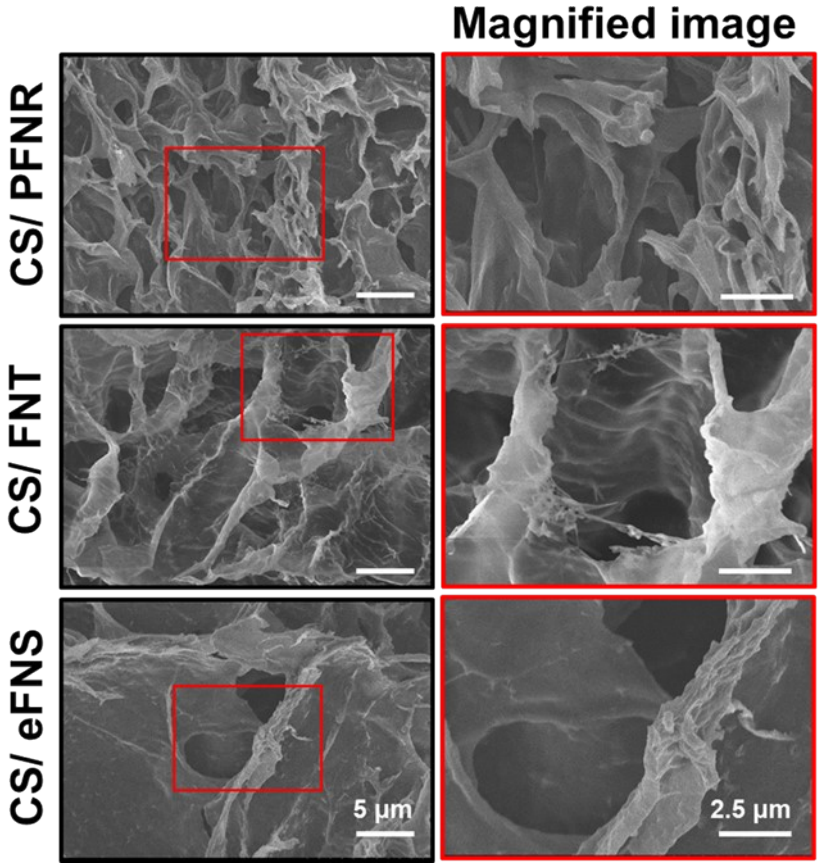

Figure S7. SEM images of freeze-dried composite hydrogels with the same concentration (150 μg/mL) of fullerenes.

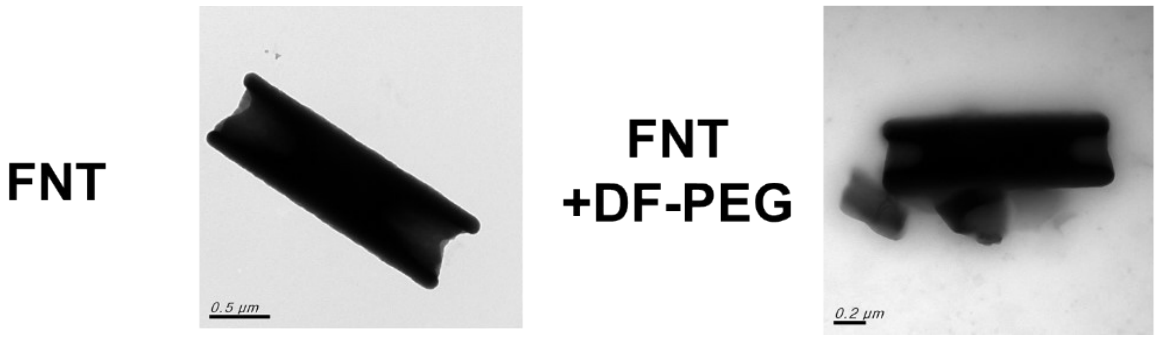

Figure S8. Interactions between fullerene nanomaterials and the crosslinker. FNT powder was added to a 2% DF-PEG solution and reacted for 30 min, and then the supernatant was removed by centrifugation. After washing the precipitate with DI water, the precipitate was dropped on a copper grid and dried. TEM images show that DF-PEG was attached around FNT, which was possibly attributed to the chemical bonding between amino groups on FNT and aldehyde groups on DF-PEG.

Supplemental videos:

Video S1. Time-lapse images recorded for NSCs after the treatment of PFNR for 24 hrs.

Video S2. Time-lapse images recorded for NSCs after the treatment of FNT for 24 hrs.

Video S3. Time-lapse images recorded for NSCs after the treatment of eFNS for 24 hrs.