Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Heparin-mediated Electrostatic Immobilization of bFGF via Functional Polymer Films for Enhanced Self-renewal of Human Neural Stem Cells

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Table S1. The flow ratios for the deposition of a series of coGD polymer films

	Feed ratio (sccm)	Deposition rate
	GMA : DMAEMA : TBPO	(nm/min)
pG1D1	1 : 1 : 1	14.3
pG1D2	1 : 2 : 1	12.5
pG1D3	1 : 3 : 1	12.5
pG1D4	1 : 4 : 1	12.5

Table S2. Atomic ratio and DMAEMA fraction in a series of coGD polymer films

	Atomic Composition (at. %)			$\frac{[DMAEMA]}{[GMA]+[DMAEMA]}$
	O1s	C1s	N1s	
pG1D1	**22.65**	**73.72**	**3.17**	**0.368319**
pG1D2	**20.45**	**72.05**	**7**	**0.765027**
pG1D3	**18.33**	**73.33**	**8.07**	**0.917045**
pG1D4	**15.6**	**73.7**	**10.7**	**1.0**

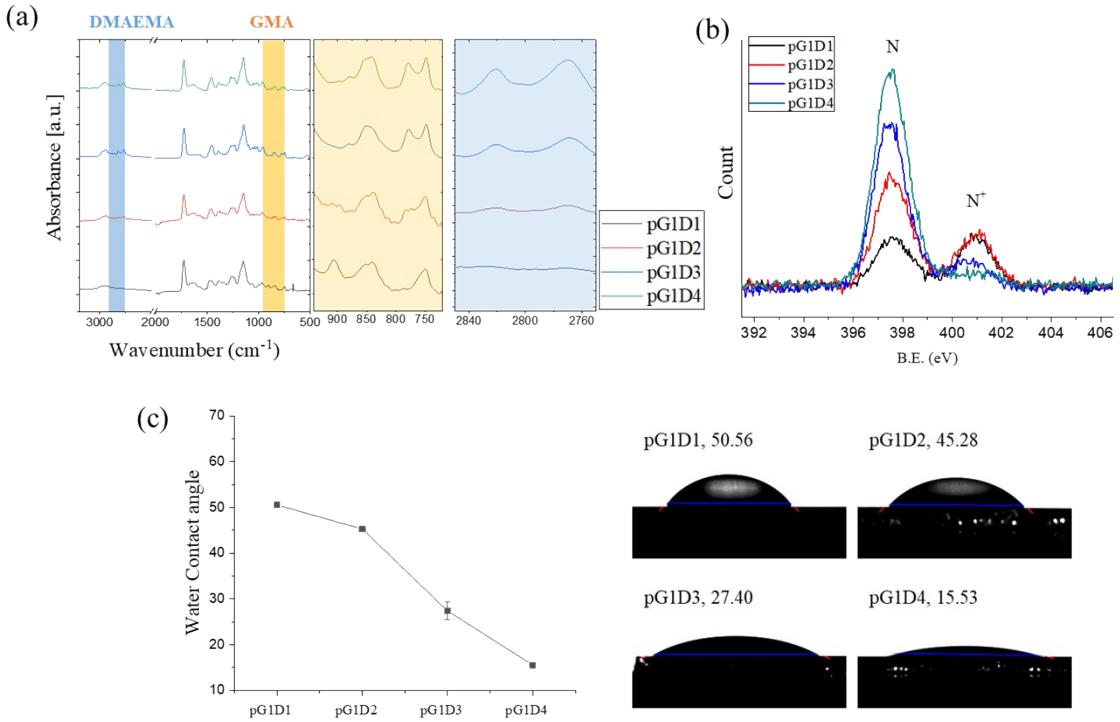

Fig. S1 (a) FT-IR spectra of a series of coGD polymers. Blue area at 2767 and 2819 cm^{-1} represents the characteristic peaks of DMAEMA, and yellow area at 759, 847, and 907 cm^{-1} represents the characteristic peaks of GMA. (b) XPS N1s scan of coGD polymers indicate amine and ammonium groups in coGD polymers. (c) Water contact angle analyses of coGD polymers.

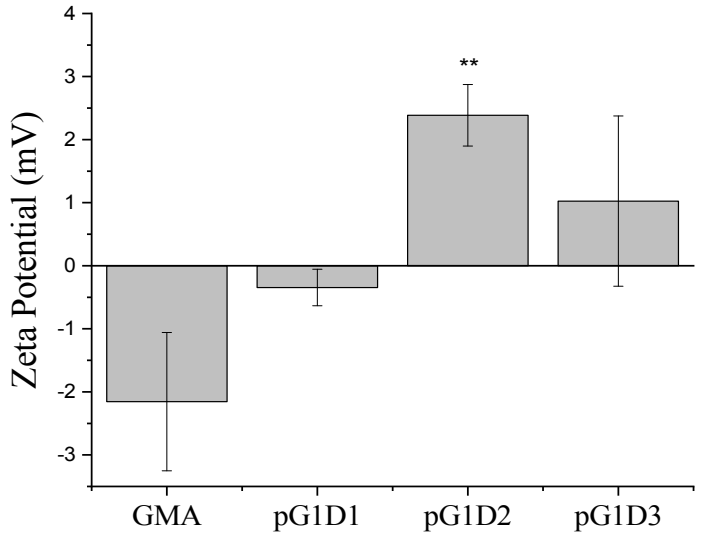

Fig. S2 Zeta potential of GMA and coGD surfaces (**$p < 0.01$).

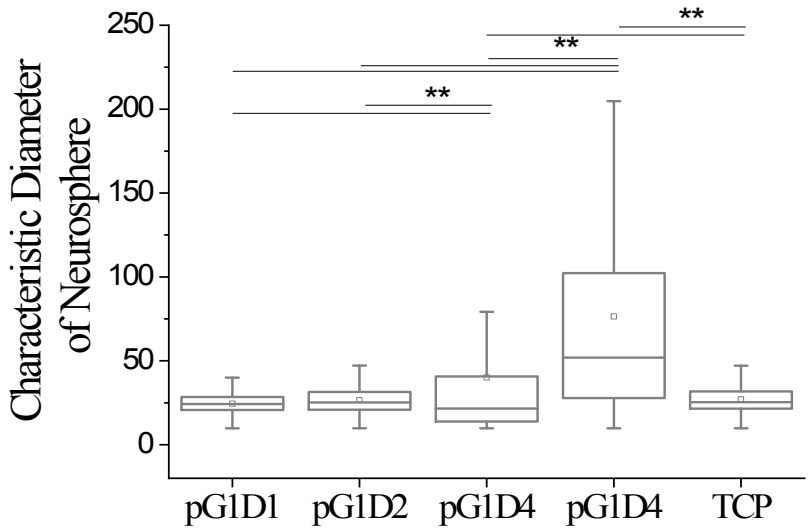

Fig. S3 Characteristic diameter of hNSC neurospheres after 2 days of culture on a series of coGD polymers (**$p < 0.01$).

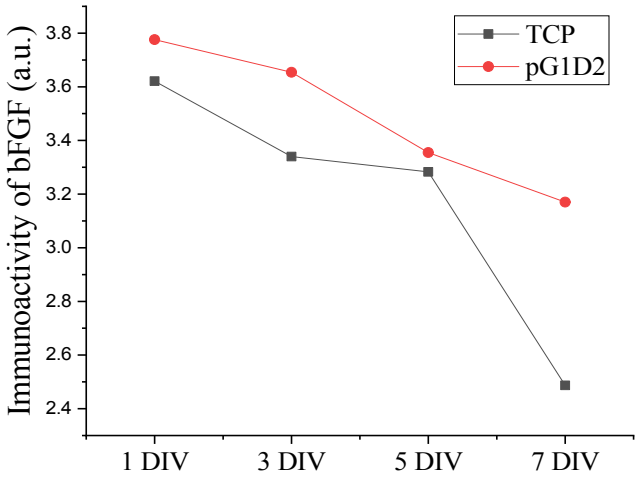

Fig. S4 Long-term stability of bFGF on TCP or pG1D2 surface.

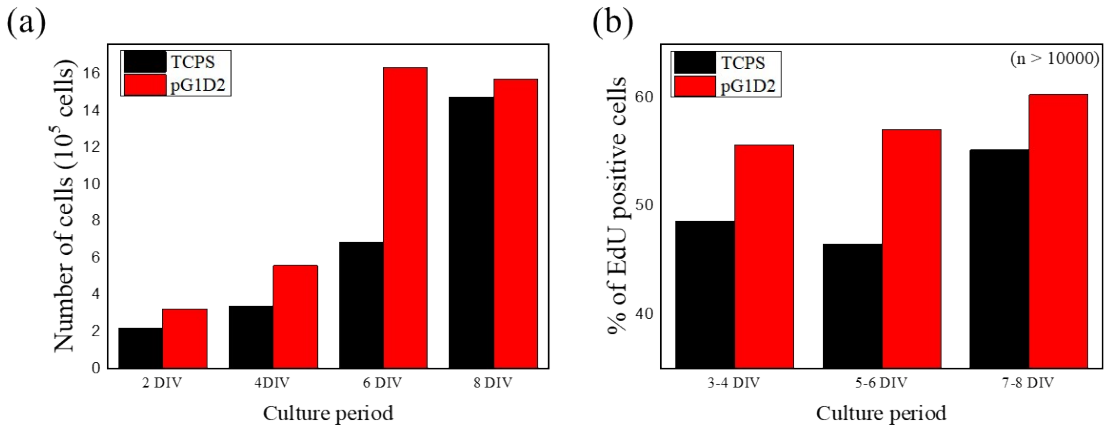

Fig. S5 Proliferative property of hNSC cultured on TCP and pG1D2 surface. (a) Cell numbers of hNSCs at each culture periods. (b) Quantification of EdU-positive cells on TCP and coGD surface.

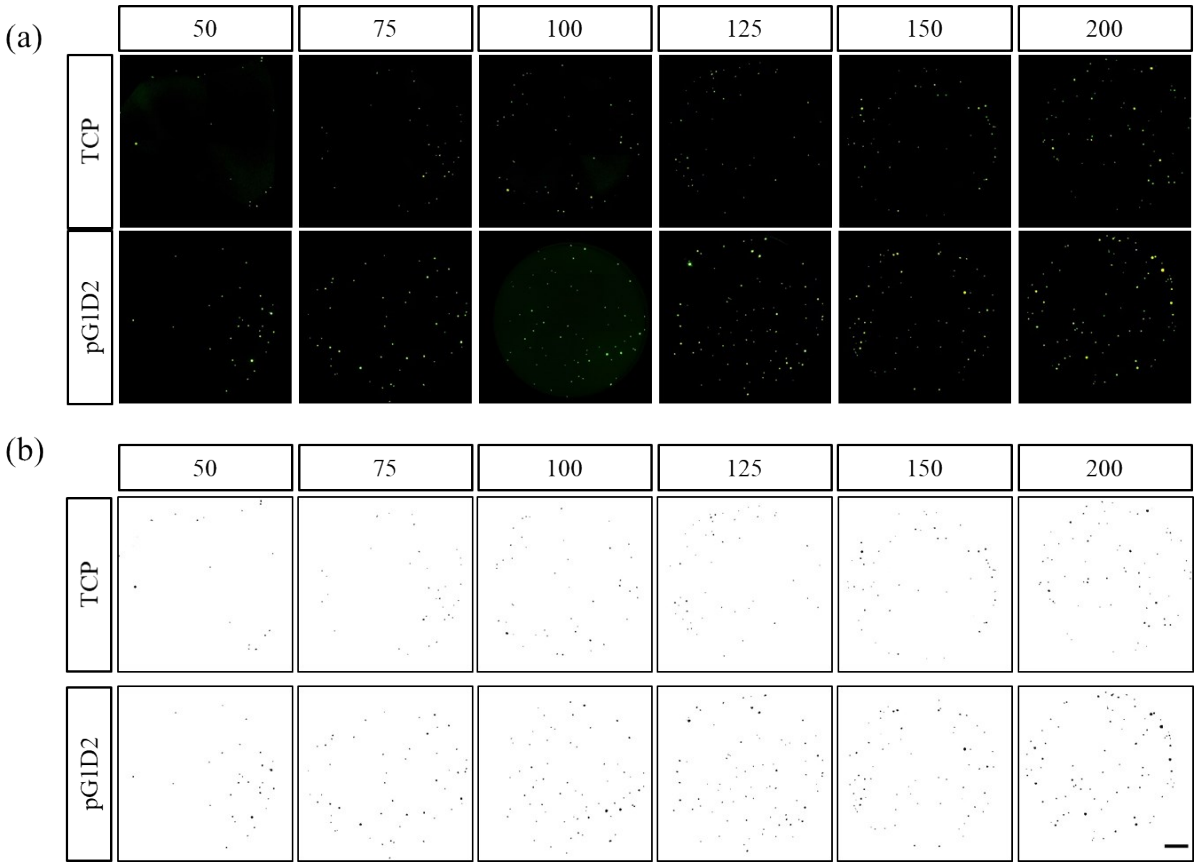

Fig. S6 (a) Representative images of neurosphere forming ability of hNSCs on G1D2 and TCP surface. (b) Green-filtered binary image of representative images (Scale bar: 1 mm).

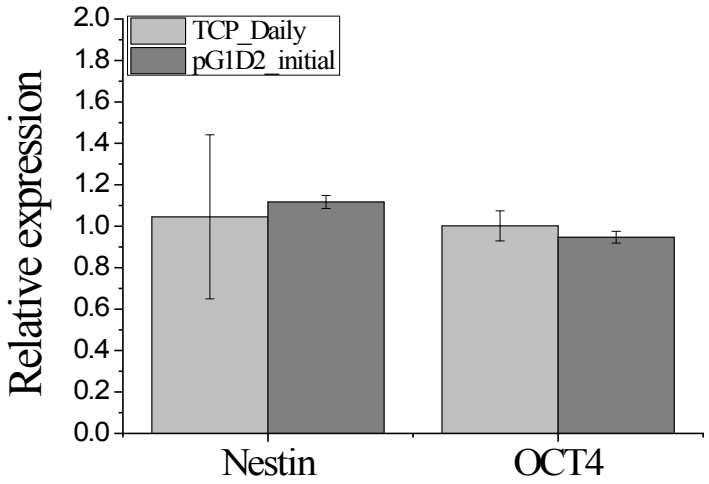

Fig. S7 Relative mRNA expression of self-renewal genes in hNSCs cultured on pG1D2 without media change after initial supplement for 6 DIV, compare to hNSCs cultured on TCP with daily supplement.

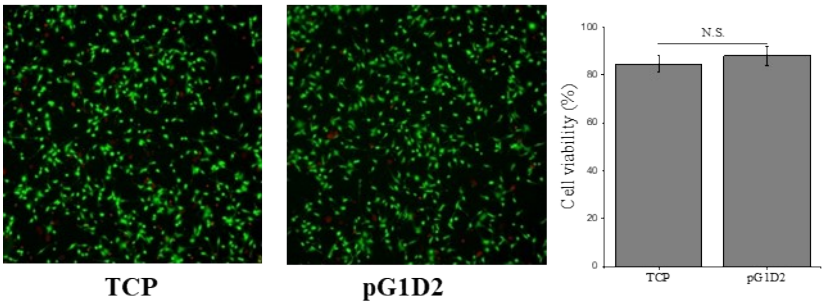

Fig. S8 Live-dead assay of hNSC on TCP and pG1D2 surface

Detailed Experimental Section

Immunofluorescence: The cells were rinsed with Dulbecco's phosphate buffer saline (DPBS, Welgene) and fixed with the formalin solution (Sigma-Aldrich, neutral buffered, 10%) for 20 min

at RT. The fixed cells were then permeabilized with the 0.1% (v/v in DPBS) Triton X-100 for 10 min and subsequently blocked with the 0.1 % bovine serum albumin (BSA) solution for 45 min at RT. Then the cells were incubated with the primary antibodies at 4 °C overnight. The primary antibodies were mouse monoclonal anti-neuronal class III b-tubulin (Tuj1, Millipore, 1:1000); mouse monoclonal anti-glial fibrillary acidic protein (GFAP, Millipore, 1:1000); and nestin antibody (Millipore, 1:1000). After the incubation with the primary antibody, the cells were rinsed with DPBS three times, then incubated with the corresponding secondary antibodies, Alexa Fluor-488 goat anti-mouse IgG (Thermo Fisher, 1:500) or Alexa Fluor-594 donkey anti-rabbit IgG (Thermo Fisher, 1:500) for 45 min at RT. The cell nuclei were stained with 40,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich, 1:1000). Fluorescent image was obtained by fluorescene microscope (Nikon). Captured images were further analyzed using ImageJ software.

Quantitative RT-PCR analysis: mRNA was extracted using mRNA extraction kit (QIAshredder, Rneasy Mini kit, QIAGEN) from 3.0×10^5 cells. Extracted mRNA was reverse transcribed using Ace qPCR RT Master Mix (Toyobo). Synthesized complementary DNA was used for quantitatve RT-PCR with SYBR (Toyobo) for each target genes, which were Nestin (forward: GAAACAGCCATAGAGGGCAAA, Reverse: TGGTTTTCCAGAGTCTTCAGTGA), SOX2 (forward: GGGAAATGGGAGGGGTGCAAAAGAGG, Reverse: TTGCGTGAGTGTGGATGGGATTGGTG), FGFR1 (forward: AACCTGACCACAGAATTGGAGGCT, Reverse: ATGCTGCCGTACTCATTCTCCACA), FGFR2 (forward: GGTCGTTTCATCTGCCTGGT, Reverse: CCTTCCCGTTTTTCAGCCAC), FGFR3 (forward: CGTGGGGCGAGCGGCAGAAGTC, Reverse: TCCGCTCGGGCCGTGTCCAGTAAG), GAPDH (forward:

TCGGAGTCAACGGATTTGGT, Reverse: TTGCCATGGGTGGAATCATA). Expression relative of each target gene to the housekeeping gene (GAPDH) was quantified using the delta delta C_T method (n = 3).

Western blot: hNSCs were harvested from each substrate and lysed by RIPA lysis and extraction buffer (thermo) containing protease inhibitor for 10 min in ice. Then the lysate was centrifuged with 13000 rpm in 4 °C. The supernatant was isolated carefully, then quantified with Bradford assay (Biorad). Lysate protein (20 μg) was mixed with LDS buffer (Thermo, 4x), then denatured in 95 °C for 10 min. The denatured protein lysate was loaded on 10% Bis-Tris Plus Gels (Thermo), then electrophoresed under 160 V for 30 min. Separated proteins were transferred to the polyvinylidene fluoride (PVDF) membrane using iBlot2 transfer stacks (Thermo). The PVDF membrane was blocked with BSA solution (2 % w/v, tris buffered saline) overnight in 4 °C. Then the membrane was incubated with primary antibody of p44/42 MAPK (1:1000, Cell Signaling technology), phosphor-p44/42 MAPK (1:2000, Cell Signaling technology), and GAPDH (1:2000, Abcam) overnight in 4 °C. Then the PVDF membrane was treated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:2000, ab6721, Abcam) for 30 min at RT. The chemical fluorescence signals were detected using a ChemiDoc MP system (Bio-Rad).