Electronic Supplementary Information (ESI) †

S1†: Top view of the microfluidic channels

S2†: Isolation and Culture of Neural Stem Cells (NSCs)

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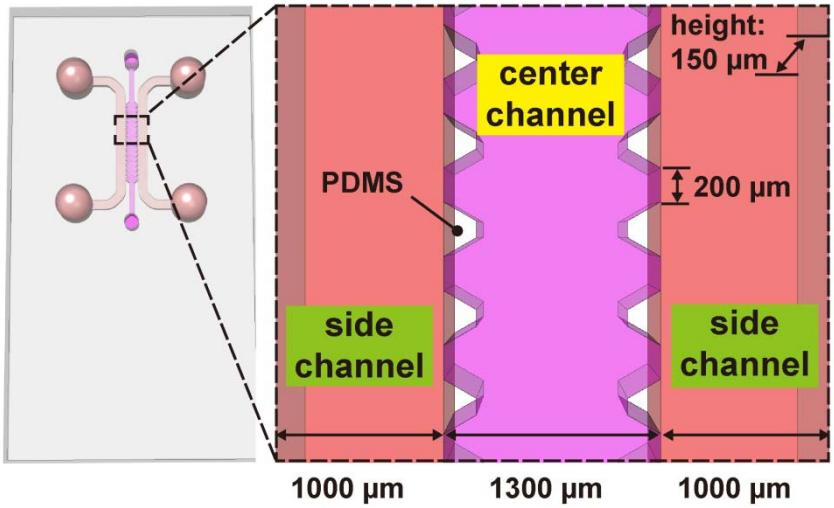
ESI S1†: Top view of the microfluidic channels



ESI S2†: Isolation and Culture of Neural Stem Cells (NSCs)

All procedures for primary isolation of mouse neural stem cells (NSCs) were carried out according to the guidelines for the care and use of laboratory animals of Yonsei University. NSCs were isolated as previously described.[1] Briefly, brains were explanted from E13 embryos of imprinting control region (ICR) mice (Samtako, Seoul, Korea). Cortices freed from meninges were mechanically dissociated in culture medium using a flame-polished Pasteur pipette. These dissociated NSCs were placed in cell culture dishes at a density of 2×10^5 cells/ml and cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 medium (DMEM/F12; Gibco BRL, MD, USA) supplemented with 20 ng/ml basic fibroblast growth factor (bFGF; Sigma, MO, USA), 20 ng/ml epidermal growth factor (EGF; Sigma), and N-2 supplement (Gibco BRL), which allows NSCs to grow as neurospheres. The culture medium was added to dishes every 48 hours.

ESI S3†: 2D NSC Culture

NSCs were plated on a 60 mm uncoated tissue culture dish at a seeding density of 2.0×10^5 cells/ml. To induce spontaneous differentiation, NSCs were maintained in DMEM/F12 medium in the absence of bFGF and EGF. After culture of 3 days, NSC differentiation was analyzed by immunocytochemical staining and qRT-PCR assay.

ESI S4†: NSC Culture in Extracellular Matrix (ECM) Hydrogels

Three types of extracellular matrix (ECM) solutions were prepared for NSC culture in 3D ECM hydrogels:

collagen, Matrigel, and a 1:1 mixture of collagen and Matrigel. Collagen solution (3.44 mg/ml, rat tail collagen type I; BD Bioscience, CA, USA) was mixed with 10X phosphate buffered saline (PBS; Thermo Scientific, MA, USA), 0.5 N NaOH, and distilled deionized water to adjust pH (7.4) and concentration (2.0 mg/ml) for gelation (collagen group). For the Matrigel group, growth factor-reduced Matrigel (GFR-Matrigel; BD Bioscience) was allowed to gel according to the manufacturer's instruction. For the mixture of collagen and Matrigel, a collagen solution (2.0 mg/ml, pH 7.4) was mixed with a GFR-Matrigel solution at a volume ratio of 1:1 (1:1 mixture of collagen and Matrigel). After preparation of hydrogel solutions, NSCs were suspended in each ECM hydrogel solution (5×10^6 cells/ml), and ECM hydrogels with NSCs were then allowed to gel in culture dishes for 3D macro-scale culture or micro-channels of microfluidic devices for 3D micro-scale culture. NSCs in 3D ECM hydrogels were cultured in DMEM/F12 without mitogenic factors (bFGF, EGF). The growth medium was exchanged daily.

ESI S5†: Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from each sample (n=4 per group) using the RNeasy Mini kit (Qiagen, Chatsworth, CA, USA) according to the manufacturer's instructions. The RNA concentration was determined by measuring absorbance at 260 nm using a spectrophotometer. A reverse transcription reaction was performed with 500 ng pure total RNA using the SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). qRT-PCR measurements of gene expression were performed using a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Universe Fast PCR Master Mix (Applied Biosystems) was used for the reaction. Gene expression in NSCs was quantified using TaqMan Gene Expression Assays (Applied Biosystems) for each target (Nestin, Mm00450205_m1 Nes; Tuj1, Mm00727586_s1; GFAP, Mm01253034_m1; Olig2, Mm01210556_m1; and GAPDH, Mm99999915_g1). mRNA levels of target genes were normalized to that of the endogenous reference (GAPDH), and relative differences in target gene expression were determined using the comparative Ct method.[2] The normalized expression of each marker in NSCs in 3D ECM hydrogels was expressed relative to that in the 3D macro Matrigel group.

ESI S6†: Immunocytochemistry

NSCs cultured in microfluidic devices were fixed by adding 4% (w/v) paraformaldehyde (in PBS) into each reservoir of the devices and incubating for 15 minutes. After blocking with 20% Block Ace (in PBS) (Dainihon-Seiyaku, Osaka, Japan) for 1 hour and washing the channels with PBS, solutions of mouse monoclonal anti-glial

fibrillary acidic protein (GFAP, 1:200; Millipore, CA, USA), mouse monoclonal anti-Tuj1 (1:100; Millipore), and mouse monoclonal anti-O4 (1:100; Millipore) primary antibodies were introduced into the devices and incubated for 1 hour at room temperature. After washing with PBS, Alexa Fluor 488-conjugated goat anti-mouse IgG secondary antibodies (1:500; Invitrogen, CA, USA) were added to the device and incubated for 1 hour. Cell nuclei were counter-stained with 4′,6-diamidino-2-phenylindole (DAPI; Sigma). Stained cells were observed under a fluorescence microscope (Axio Observer D1; Carl Zeiss, Germany).

ESI References

1. Y. H. Kim, J. I. Chung, H. G. Woo, Y. S. Jung, S. H. Lee, C. H. Moon, H. Suh-Kim and E. J. Baik, *Stem Cells*, 2010, **28**, 1816-1828.

2. S. W. Cho, M. Goldberg, S. M. Son, Q. Xu, F. Yang, Y. Mei, S. Bogatyrev, R. Langer and D. G. Anderson, *Adv. Funct. Mater.*, 2009, **19**, 3112-3118.