CHEMNANOMAT

Supporting Information

A Nanofiber-embedded Microfluidic Platform for Studying Neurobiology

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Figure S1. Methods of cell seeding to microfluidic devices. (a) Cell seeding without tilting the device caused the migration of NPCs during the differentiation process. (b) Cell seeding with tilting the device for 10 min prevented the initial migration of NPCs. Top panels: schematics of the device, bottom panels: phase contrast microscopy images of differentiated NPCs (neurons and glia cells) for 7 days in devices.



Figure S2. Width and height of microchannels were quantified with confocal microscopy. (a-c) Example images of microfluidic device with microgrooves filled with Alexa 488 conjugated dextran solution. z-stack images of channels were obtained with confocal microscopy. (d) An example of channel dimension calculation. The derivative of intensity profiles was used to quantify the channel width. The same method was used for the channel height calculation. The detailed quantification method is shown by Lee et al..^[1] The width and height of microgrooves are $12 \pm 0.3 \mu m$ and $5 \pm 0.6 \mu m$, respectively (n = 10). The height of a center chamber of nanofiber-embedded device is $5 \pm 0.6 \mu m$ (n = 5).



Figure S3. Quantification of fiber density. The original image (a) was converted into the grayscale image (b). The Otsu's thresholding method was used to generate the binary image (c) from the grayscale image. Complement of a binary image (d) was obtained from the binary image (c). To calculate the fiber density, the area of white pixels (fibers) in (d) was divided by the area of an entire image. All the image processing and fiber density calculation was done with a custom-made MATLAB code.



Figure S4. The effects of fiber density of aligned fibers on axonal growth. NPCs cultured in the microfluidic devices with different densities of aligned fibers. As the fiber density increases, more axons grew parallel to the orientation of aligned nanofibers. White dashed line: boundaries of central compartment of the device. Green: Beta-tubulin III staining. Blue: DAPI staining.

Video S1. Regeneration of axons along the microgroove after the laser-based axotomy.Video S2. Regeneration of an axon along the aligned fibers after the laser-based axotomy.

References

[1] D. Lee, M. M. Rahman, Y. Zhou, S. Ryu, *Langmuir* **2015**, *31*, 9684-9693.