Electronic supplementary information

for

Microfluidic Synthesis of Chemically and Physically Anisotropic Hydrogel Microfibers for Guided Cell Growth and Networking

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List of electronic supplemental information:

- **Figure S1**. Preparation of anisotropic hydrogel fibers with various diameters, obtained by using microdevices with 6 inlet channels.
- Figure S2. Preparation of solid-soft anisotropic fibers and fibers composed of three regions.
- **Figure S3**. Guided growth of HeLa cells in solid-soft-solid complex alginate fibers.
- Figure S4. Localization of FITC-labeled collagen in the anisotropic fibers on Day 1.

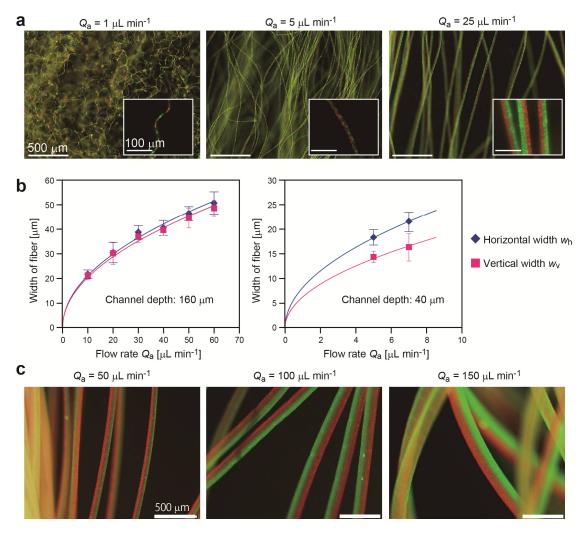


Figure S1. Preparation of anisotropic hydrogel fibers with various diameters, obtained by using microdevices with 6 inlet channels. (a) Fibers obtained by using a microchannel with the width and depth of 400 and 160 μm, respectively. Q_b and Q_c were 10 and 100 μL min⁻¹, respectively. (b) Relation between the alginate flow rate Q_a and the vertical/horizontal widths of the fiber, when microchannels with the depth of 160 or 40 μm were employed. The microchannel width was 400 μm. The ratios of the horizontal/vertical widths of the fibers were ~1.0 and ~1.2 for 160- and 40-μm deep microchannels, respectively. (c) Fibers obtained by using a broader microchannel with both the width and depth of 800 μm. Q_b and Q_c were 30 and 200 μL min⁻¹, respectively. The average diameters of the fibers were ~95, ~145, and ~200 μm, respectively.

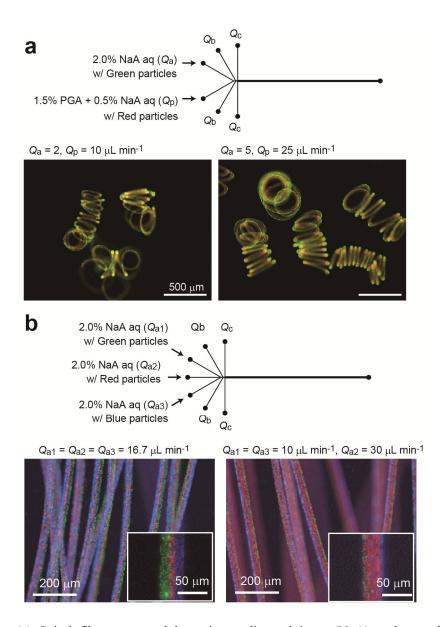


Figure S2. (a) Spiral fibers prepared by using sodium alginate (NaA) and propylene glycol alginate (PGA) using the 6-inlet microchannel with the width and depth of 400 and 80 μm, respectively. The obtained fibers were cut into 10 mm-long fragments. Q_c and Q_b were 100 and 10 μL min⁻¹, respectively, while Q_a and Q_p were changed as indicated. The average diameters ± SD of the curvature were 256 ± 52 μm (left) and 323 ± 49 μm (right), respectively. (b) Anisotropic fibers composed of three regions containing different-color particles. The width of each region could freely be controlled by changing the introduced flow rates.

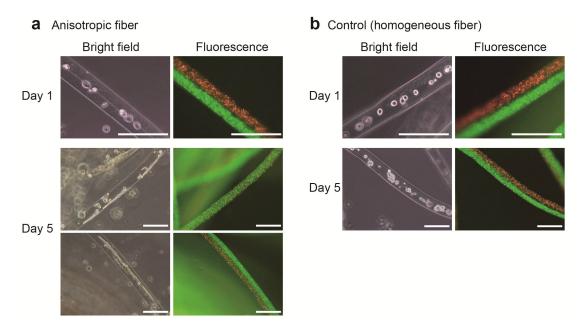


Figure S3. Guided growth of HeLa cells in the core of the solid-soft-solid anisotropic Ca-alginate fibers. The experimental conditions are the same as those in the experiments using NIH-3T3 cells described in the manuscript, except for the initial cell concentration $(2 \times 10^7 \text{ cells mL}^{-1})$, which is two times higher than that shown in Figure 4). (a) HeLa cells cultured in the solid-soft-solid anisotropic fibers for 1 or 5 days, and (b) HeLa cells in the homogeneous fiber, totally made from 2.0% NaA solutions. In (a), linear colonies were formed along the fiber direction on Day 5, while the colony shape was spherical in (b). Scale bar, 200 μm.

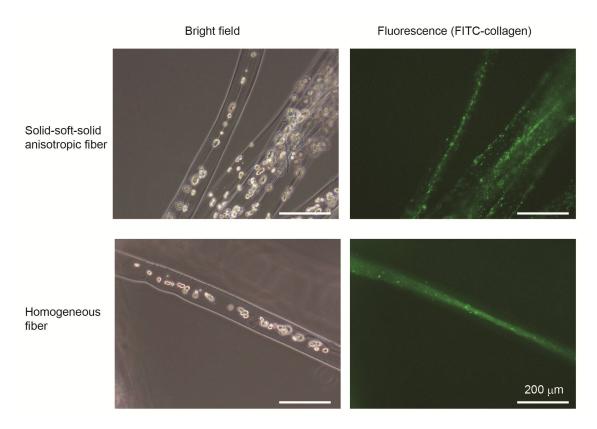


Figure S4. Localization of FITC-labeled collagen in the anisotropic fibers incorporating PC12 cells in the core on Day 1. The FITC-collagen molecules were localized in the core with a width of \sim 8 µm (solid-soft-solid fibers) or \sim 12 µm (homogeneous fibers). Scale bar, 200 µm.