Electronic Supplementary Material (ESI) for Biomaterials Science.

Multiwall Carbon Nanotube Reinforced Biomimetic Bundled Gel Fibre

Young-Jin Kim,[a,b] Seiichiro Yamamoto,[a,c] Haruko Takahashi,[a] Naruo Sasaki,[c,d] and Yukiko T. Matsunaga[a,*]

[a] Center for International Research on Integrative Biomedical Systems (CIBiS), The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo, 153-8505, Japan

[b] Japan Society for the Promotion of Science (JSPS), 8 Ichibancho, Chiyoda-ku, Tokyo, 102-8472, Japan

[c]Department of Materials and Life Science, Seikei University, 3-3-1 Kichijoji Kitamachi, Musashino, Tokyo, 180-8633, Japan

[d]Department of Applied Physics, Faculty of Informatics and Technology, The University of Electro-Communications, 1-5-1 Chofugaoka, Chofu, Tokyo 182-8585, Japan

*Address correspondence to mat@iis.u-tokyo.ac.jp

Supporting Information

Contents

1. Experimental section

Materials. Hydroxypropyl cellulose (HPC, Mw: 100 kDa) and divinyl sulfone were purchased from Sigma-Aldrich (Tokyo, Japan) and Tokyo Chemical Industry (TCI, Tokyo, Japan), respectively. Sodium alginate (Na-Alg) was kindly provided by Kimica Corporation (Tokyo, Japan). Calcium chloride ($CaCl_2$) and sodium hydroxide (NaOH) were obtained from Kanto Chemicals (Tokyo, Japan). Trisodium citrate was purchased from Nacalai tesque, Inc. (Kyoto, Japan). Polydimethylsiloxane (PDMS, SILPOT 184) and its catalyst SILPOT were purchased from Dow Corning Toray (Tokyo, Japan). Multiwalled carbon nanotubes (MWCNTs, diameter ~10-20 nm, length ~5-15 μm, purity > 95%) were purchased from Wako Pure Chemical Industries (Osaka, Japan). All chemicals and solvents were used as purchased. Distilled water from a Milli-Q system (Merck Millipore, Darmstadt, Germany) was used in all experiments.

Oxidation of MWCNTs. To obtain oxidized MWCNTs, 100 mg of MWCNTs were refluxed in a mixed solution of H_2SO_4 (98%, Wako, Tokyo, Japan) and HNO_3 (68%, TCI, Tokyo, Japan) (3:1, 80 ml) at 70 °C. After 4 h of refluxing, MWCNTs were filtered using a membrane filter (pore size: 1.0 μm) and dried at 25 °C for 17 h. To increase the amount of carboxyl groups on the MWCNTs, the MWCNTs were sonicated twice in hydrochloric acid for 30 min and then filtrated using a membrane filter. Oxidized MWCNTs were finally obtained after drying at 25 °C for 17 h.[S1] These oxidized MWCNTs were used in further experiments after being sonicated for 10 min in MilliQ water. Zeta-potentials of MWCNTs before and after oxidation were determined using dynamic light scattering (DLS, n = 9, Nano ZS, Malvern Instruments Ltd., Malvern, WR, UK).

Fabrication of co-flow microfluidic device. Co-flow microfluidic device was fabricated using a slightly modified method from our previous report (Figure S1).[S2] Briefly, the co-flow microfluidic device is composed of two kinds of glass capillaries and a connector. First, a round glass capillary tube (outer diameter: 1 mm, inner diameter: 0.6 mm, G-1, Narishige, Tokyo, Japan) was pulled by a micropipette puller (PC-10, Narishige Co., Tokyo, Japan), and then its end-tip was cut and ground to an outer diameter of 300 μm. Next, this round glass capillary was inserted into a square glass tube (outer dimensions: 1.4 mm × 1.4 mm, inner dimensions: 1 mm × 1 mm, Vitrocom Inc., Mountain Lakes, NJ, USA) (inlet A and outlet). A PDMS connector (25 mm × 20 mm, 5 mm thick) with a channel (dimensions: 1.5 mm × 1.5 mm, length: 25 mm) was prepared by curing mixture of PDMS prepolymer and catalyst (10:1) with a polystyrene stick (dimensions: 1.5 mm × 1.5 mm) at 75 °C for 2 h. Another outer channel (inlet B) was prepared by punching a hole (1.5 mm in diameter) through the PDMS connector. Two glass tubes and connectors were assembled into one device, then finally, they were immobilized on a slide glass after O_2 plasma treatment for a minute using a plasma cleaner (Harrick Plasma, Ithaca, NY, USA).

Preparation and characterization of phase separated aqueous solution. Phase separated polymer blend aqueous solutions were prepared by dissolving 0.7 g of HPC and 0.1 g of Na-Alg in 10 ml of MilliQ water and stirring overnight; then the pH of the solution was adjusted to 13 by adding 5 M NaOH. To prepare polymer blend aqueous solutions containing oxidized MWCNTs, 1 and 5 mg of oxidized MWCNTs (0.1 and 0.5%) were added into the solutions, respectively. To obtain the microscopic images of the phase separated polymer, polymer solutions with different pH values (7 and 13) were dropped onto a small PDMS pit of 3-mm thickness and then covered with a coverslip. The polymer solutions were observed using a phase contrast microscope (AxioObserver D1, Carl Zeiss, Oberkochen, Germany) while increasing the temperature from 15 to 45 °C.

Fabrication and characterization of bundled gel fibres. A syringe filled with a MWCNT-containing polymer blend aqueous solution was connected to the inner capillary channel of the co-flow microfluidic device *via* a silicone tube, and then connected to a syringe pump A (inlet A)). Another syringe, containing 100 mM $CaCl_2$ solution as a cross-linker for Na-Alg, was connected to the outer capillary channel along a different silicone tube, and then connected to a syringe pump B (inlet B). The flow rates were fixed at 300 μl min^{-1} and 2,500 μl min^{-1} for syringe pump A and B, respectively. By injecting from both syringes, MWCNT-incorporated cross-linked HPC/Ca-Alg bundled gel fibres were generated and obtained from the outlet. The obtained fibres were immersed in divinyl sulfone solution (2.0 wt%, pH 13) to cross-link the HPC molecules for 12 h in agitated conditions. Ca-Alg molecules in the bundled gel fibres were removed by adding trisodium citrate (100 mM). Finally, MWCNT-incorporated HPC bundled gel fibres were obtained (all processes were performed at 28 °C). The morphologies of all bundled gel

fibres were observed using a phase contrast microscope and scanning electron microscope (SEM, SU8000, Hitachi, Tokyo, Japan).

For SEM, the samples were lyophilized, and then coated with Pt for 60 s using a magnetron-sputter coater (MSP-1S, Vacuum Device Inc., Ibaraki, Japan) before measuring. To measure the mechanical properties of the bundled gel fibres, all specimens were examined by a universal testing machine (n = 4, UTM, EZ-SX, Shimadzu, Kyoto, Japan). Each specimen was placed so that the chuck-to-chuck spacing was 20 mm, and pulled at a 10 mm min^{-1} load cell rate at 25 °C in a humid environment. Electrical properties of the bundled gel fibres were measured at 25 °C using a picoammeter (6487/J, Keithley, Tokyo, Japan) and two-point probe. All samples were completely lyophilized before the measurement to avoid the effects of moisture. The samples were cut into 20 mm long samples (n = 3). Resistance between the two probes was measured by applying a very small current (I = 0.001-10 nA, corresponding voltage V = 1-10 V) in a range where the current density-voltage (*I-V*) characteristics were linear. The electrical resistivity (ρ) and conductivity (σ) were calculated by standard methods from the obtained linear *I-V* curves as follow:

$$\rho = R \times \pi r^2 / S, \qquad \text{(eq. 1)}$$

$$\sigma = 1 / \rho, \qquad \text{(eq. 2)}$$

where R (Ω) is resistance, which is obtained from the *I-V* curves, S is the distance between two probes, r is the radius of the specimen, and ρ is the electrical resistivity, which is calculated using eq. 1.

Cell culture study. To investigate the feasibility of using bundled gel fibres for cell cultivation, the bundled gel fibres were coated with PLL-*g*-PEG-RGD (Susos AG, Dubendorf, Switzerland) to enhance cell attachment at the initial step after seeding. As a model cell, normal human dermal fibroblasts (NHDFs, Lonza, Basel, Switzerland) were used in this study. Harvested and suspended NHDFs in a cell culture medium, E-MEM (Eagle minimal essential medium), are supplemented with 10% fetal bovine serum (FBS, Japan Bio Serum, Fukuyama, Hiroshima, Japan), and penicillin-streptomycin (100 U ml^{-1} and 100 μg ml^{-1}), were seeded onto the MWCNT-incorporated bundled gel fibres (0.1 and 0.5% of MWCNTs) at 7,000 cell ml^{-1} of concentration, and then incubated in a 5% CO_2 atmosphere at 37 °C in an incubator. The culture medium was regularly changed every 2 days. After 3 and 6 days of cell cultivation, immunocytofluorescence staining for filamentous F-actin and nuclei was also performed. The cells were fixed in 4% para-formaldehyde for 10 min and then F-actin and nuclei were stained by Alexa488-conjugated phalloidin and Hoechst33342 (Molecular Probe, Invitrogen, Carlsbad, CA, USA), respectively. Stained cells were visualized by confocal laser scanning microscope (CLSM, LSM 700, Carl Zeiss, Oberkochen, Germany).

2. References

1. S. Zhang, L. Qin, H. Song, X. Chen, J. Zhou and Z. Ma, *RSC Adv.*, 2014, **4**, 54244-54248.
2. Y.-J. Kim, Y. Takahashi, N. Kato and Y. T. Matsunaga, *J. Mater. Chem. B*, 2015, **3**, 8154-8161.

3. Supporting figures

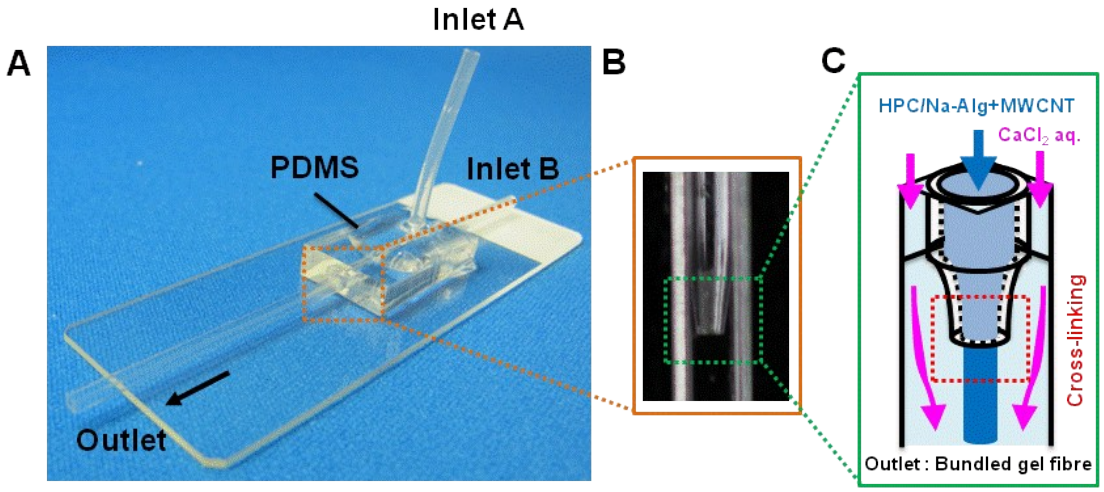

Figure S1. Microfluidic device with two inlets and one outlet for preparation of MWCNT-incorporated bundled gel fibres (A). Microscopic photograph and illustration of assembled round and square glass capillaries (B and C). The phase-separated polymer blend solution and cross-linker ($CaCl_2$ aq.) flow through inlet A and B, respectively. Na-Alg molecules in phase separated polymer blend solutions are immediately cross-linked in the area outlined by the dotted square *via* $CaCl_2$ aq., which flows in inlet B.

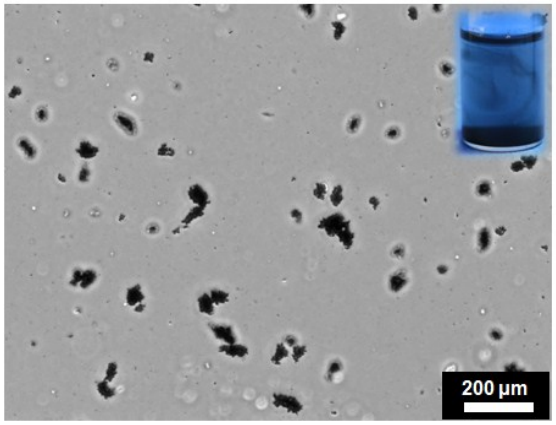

Figure S2. A microscopic image of a neat-MWCNTs/HPC/Na-Alg blend aqueous solution at pH 7 and 28 °C. A insert image indicated aggregated and precipitated MWCNTs in the solution.

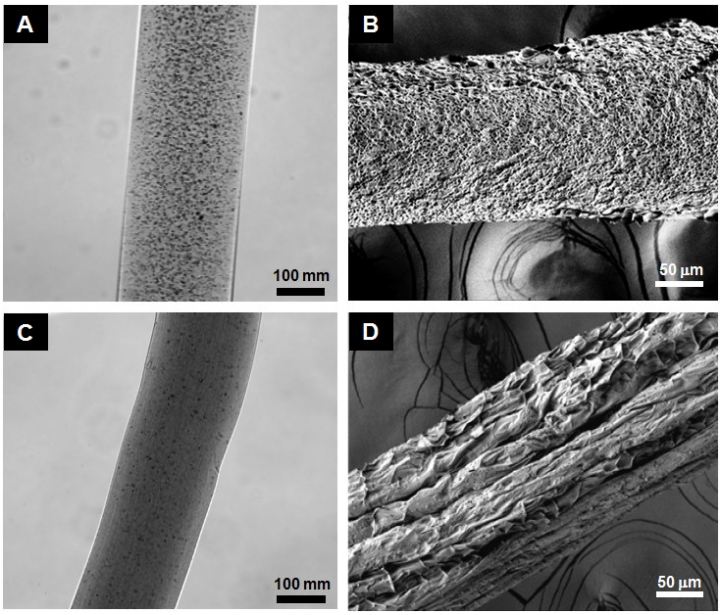

Figure S3. Morphological characteristics for non-bundled and bundled gel fibres. (A) Microscope image and (B) SEM image of MWCNT-incorporated non-bundled gel fibre. (C) Microscope image and (D) SEM image of MWCNT-incorporated bundled gel fibre.