Metre-long cell-laden microfibres exhibit tissue morphologies and functions

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3. **Supplementary references**
1. Materials and Methods

Chemicals. We obtained calcium chloride (CaCl$_2$), potassium chloride (KCl), sodium hydroxide (NaOH), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), dimethyl sulfoxide (DMSO), and glucose from Kanto Chemicals (Tokyo, Japan), sodium chloride (NaCl) from Wako Pure Chemical Industries (Osaka, Japan), and sucrose from Nacalai tesque (Kyoto, Japan). Two types of Dulbecco’s phosphate buffered saline solutions (DPBS(-), D1408 and DPBS(+), D8662) were purchased from Sigma-Aldrich Japan K K (Tokyo, Japan). All chemicals were used without further purification. Water was deionized to 18 M ohm cm with a Millipore purification system.

Animals. Wistar rats for cortical neural cells, ICR mice for NSCs, and BALB/c Slc-nu/nu mouse for implantation experiments were obtained from Sankyo Labo Service Corporation, Inc., Japan. Lewis rats (LEW/CrlCrlj) for isolation of pancreatic islet cells were obtained from Charles River, Yokohama, Japan. All of these animals were treated in accordance with the policies of the University of Tokyo Institutional Animal Care and Use Committee.

Cell culture conditions. Ten types of cells were used for evaluating cell fibre fabrication. All cells were maintained at 37°C, in water saturated 5% CO$_2$ environment. Their derivation and culture media are summarized in Table S1. We obtained Dulbecco’s modified eagle medium (DMEM, D5796) and low glucose DMEM (D5796). Table S1 | Cell culture conditions.

<table>
<thead>
<tr>
<th>Type of cell</th>
<th>Derivation</th>
<th>Culture medium (growth medium)</th>
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<tbody>
<tr>
<td>NIH/3T3</td>
<td>RIKEN Cell Bank (TKG0299)</td>
<td>DMEM + 10% FBS + 1% AB</td>
</tr>
<tr>
<td>Hep G2</td>
<td>JCRB Cell Bank (JCRB1054)</td>
<td>DMEM + 10% FBS + 1% AB</td>
</tr>
<tr>
<td>MS1</td>
<td>ATCC (CRL-2279)</td>
<td>DMEM + 5% FBS + 1% AB</td>
</tr>
<tr>
<td>HUVEC</td>
<td>Lonza Walkersville, Inc. (CC2519)</td>
<td>EGM®-2 BulletKit™</td>
</tr>
<tr>
<td>HeLa</td>
<td>ATCC (CCL-2.2)</td>
<td>Low glucose DMEM + 10% FBS + 1% AB</td>
</tr>
<tr>
<td>MIN6m9</td>
<td>Gift from Prof. Seino @ Kobe University</td>
<td>DMEM + 10% FBS + 1% AB</td>
</tr>
<tr>
<td>C2C12</td>
<td>ATCC (CRL-1772)</td>
<td>DMEM + 10% FBS + 1% AB</td>
</tr>
<tr>
<td>Cardiomyocyte</td>
<td>Primary Cell Co., Ltd. (CMC02)</td>
<td>Culture medium for cardiomyocyte (CMCM)</td>
</tr>
<tr>
<td>Neuronal stem cell</td>
<td>Primary culture from ICR mouse</td>
<td>Neurobasal-A + 2 mM L-glutamine + 1% AB</td>
</tr>
<tr>
<td>Cortical cell</td>
<td>Primary culture from Wistar rat</td>
<td>DMEM/F12 + 10% FBS + 1% AB + B27</td>
</tr>
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ATCC: America Type Culture Collection. JCRB: Japanese Collection of Research Bioresources.
glucose DMEM (D6046) from Sigma-Aldrich Japan K K, DMEM/F12 (11330, Gibco) and Neurobasal-A (10888, Gibco) from Invitrogen Japan K K, EGM®-2 BulletKit™ from Lonza Walkersville Inc., and Culture Medium for Cardiomyocyte (CMCM; the compositions are DMEM/F12 with 10% FBS, 10 units/ml penicillin and 10 µg/ml streptomycin) from Primary Cell Co., Ltd. Fetal bovine serum (FBS, S1650 or S1560) and penicillin-streptomycin solution (antibiotics, AB, P4333 or P4458) were purchased from Japan Bioserum Co Ltd. and Sigma-Aldrich Japan K K, respectively. We used basic fibroblast growth factor (bFGF, AF-100-18B, Peprotech), human epidermal growth factor (hEGF, AF-100-15, Peprotech) and B27 without vitamin A (12587-010, Invitrogen) for the supplement of neuronal stem cells (NSCs).

The cerebral cortices were dissected from brain of Wistar rats (embryonic days 17-19) and were dissociated with papain (3176, Worthington) as previously reported. Single cell suspensions of neuronal stem cells (NSCs) were prepared from striata of ICR mouse brain (embryonic days 14.5) by mechanical trituration as previously reported. The NSCs were used for experiments with one or two passages. Cardiomyocytes were purchased from Primary Cells Inc, Japan.

Double Coaxial Laminar Flow Microfluidic Device. The double coaxial laminar flow microfluidic device was composed of glass capillaries and connectors. The detailed design and images of the device are shown in Supplementary Fig. S1. A glass capillary tube (outer diameter: 1 mm; inner diameter: 0.6 mm, G-1, Narishige, Tokyo, Japan) was pulled to form a thin tip (~230 µm in diameter) with a puller (PC-10, Narishige) for the inner microchannels. Rectangular glass tubes (outer diameter: 1.4 mm; inner diameter: 1 mm, Vitrocom Inc., NJ, USA) were also used to guide the outer streams. These glass capillary tubes were assembled with connectors fabricated by stereolithography (Perfactory, envisionTEC, Marl, Germany). All inlets and outlets were connected to gas-tight syringes with Teflon tubes (inner diameter: 0.5 mm). The syringes were set to syringe pumps (KDS210, KD Scientific Inc., MA, USA) to precisely control the flow rates. We attached the microfluidic device on a vertical wall, and aligned the direction of the flow to the direction of gravitational force to avoid entangling the generated hydrogel microfibres in the device. This setup was built in a clean hood to prevent contamination.

Formation of cell-containing ECM protein/Ca-alginate core/shell hydrogel microfibre. For the
formation of the core-shell hydrogel microfibre, we prepared three solutions: (1) a pre-gel solution of a cell-containing ECM protein for the core stream, (2) a pre-gel solution of Na-alginate for the shell stream and (3) a CaCl$_2$ solution for the sheath stream.

We used three different ECM proteins, PCol, ACol and Fib, for the core solution. The composition of each pre-gel solution of the ECM protein is as follows:

(Note that SDS-PAGE analysis of each ECM protein is shown in Supplementary Fig. S2)

**PCol:** 2 mg/mL pepsin-solubilized type I collagen, neutralized in DMEM (AteloCell™, DME-02, Atelocollagen, KOKEN, Japan).

**ACol:** 3 mg/mL acid-solubilized type I collagen, neutralized in Hank’s buffered solution containing NaHCO$_3$ and HEPES. This type I collagen is composed of bovine type I collagen (AteloCell™, IAC-50, Native collagen, KOKEN, Japan) and pig type I collagen (Cellmatrix™ Type I-A, Nitta Gelatin Inc., Japan) at a molar ratio of 5:6.

**Fib:** 5 mg/mL fibrinogen. Fibrinogen derived from bovine plasma (F8630, Sigma-Aldrich Japan K K) was dissolved at 33.3 mg/mL in 20 mM HEPES buffered saline, and then was adjusted to 5 mg/mL by diluting with PBS (-).

Cells were suspended in the prepared pre-gel solution at 1.0×10$^8$ cells/mL except the case of cardiomyocytes that were suspended at 0.1×10$^8$ cells/mL. When we used NSCs for the fibre fabrication, we supplemented 100 ng/mL bFGF, 100 ng/mL hEGF and B27 to the NSC-containing pre-gel solution of an ECM protein. Note that in the case of ACol, the cell suspended pre-gel solution of ACol was kept at 4 ºC to prevent the gelation of the ACol at room temperature (RT: ~25 ºC). For the shell solution, we used 1.5% Na-alginate solution (80~120 cP, Wako Pure Chemical Industries); 3% w/w Na-alginate was sterilized with an autoclave and mixed with sterilized 2X saline (290 mM NaCl solution) at 1:1 ratio to obtain 1.5% Na-alginate solution in saline (145 mM NaCl solution). Note that in the case of fabricating Fib core in microfibres, the 3% w/w Na-alginate solution was mixed with 5 mg/mL fibrinogen solution at 1:1 ratio. The sheath solution contained 100 mM CaCl$_2$ and 3% w/w sucrose, and was sterilized with a 0.22 µm filter.

Before loading solutions into the microfluidic device, the device and all tubing were filled with 70% ethanol for 1 hour for sterilization. After that, the device was operated as follows. (1) Load core and shell solution in the device and introduce saline to the sheath channel instead of the 100 mM CaCl$_2$ solution to avoid clogging at the merge point of the shell and sheath streams. (2) Start syringe pumps to infuse the
core, shell and sheath solutions to generate double coaxial laminar flow in the device. The flow rates of each stream, core, shell and sheath, were $Q_{\text{core}} = 25 \, \mu\text{L/min}$, $Q_{\text{shell}} = 75 \, \mu\text{L/min}$ and $Q_{\text{sheath}} = 3.6 \, \text{mL/min}$, respectively. (3) Switch the saline stream to 100 mM CaCl$_2$ stream while keeping the flow rate at 3.6 mL/min. A core-shell hydrogel fibre is continuously generated and collected in a tube filled with saline. (4) After forming desired length of the fibres in the tube, switch the CaCl$_2$ stream to the saline stream again, and stop the pumps. The diameters of the core and shell can be adjusted by changing the flow rates of the streams (Supplementary Fig. S5). Note that in the case of ACol core hydrogel fibre, all the procedures were conducted in a cold room (4ºC) to avoid the gelation of the ACol in the microfluidic device.

**Gelation of the ECM protein in the core.** After collecting the core-shell hydrogel microfibre in the tube, the fibre was immediately moved to a culture dish filled with the culture medium corresponding to the encapsulated cells (Supplementary Table. S1). After that, in the cases of PCol or ACol pre-gel solution in the core, the formed microfibre was incubated at 37ºC for >15 min to set the encapsulated PCol or ACol pre-gel solution into gels. In the case of Fib pre-gel solution in the core, the fibre was immersed in 4 units/ml thrombin (T7513, Sigma-aldrich Japan K K) dissolved in 20 mM HEPES buffered saline with 2 mM CaCl$_2$ for 15 min at 37ºC to form fibrin gel in the core, and then moved in the appropriate culture medium. Subsequently, the cell-encapsulating core-shell hydrogel fibre was cultured at 37ºC in water saturated 5% CO$_2$ environment.

**Time-lapse observation of cell fibre during culture.** A cell fibre was embedded in 5 mg/mL type-I collagen hydrogel (IAC-50, KOKEN) to fix the position of the cell fibre. After adding culture medium, the cell fibre was observed with an inverted microscope (IX-71, Olympus) equipped with an incubation system (INUBUG2SF-ONICS, TOKAI HIT). The time-lapse images were obtained with a CCD camera (AxioCam MRm, Zeiss)

**Stiffness measurement of gelated ECM proteins with atomic force microscopy.** Three types of gelated ECM proteins, PCol, ACol and Fib, were prepared in a 35 mm dish and immersed in DMEM supplemented with 10% FBS for >24 h. The stiffness of each gelated ECM protein was measured in DMEM solution with an atomic force microscope (AFM, NanoWizard, JPK Instruments, Berlin) on the top of an inverted
microscope (IX71, Olympus, Tokyo). AFM cantilever tips were constructed by gluing glass beads (diameter: 48 µm, VitraBio, Steinach) onto the ends of tipless silicon cantilevers (TL-CONT, NanoSensors, Neuchatel) with a spring constant of 0.3 N/m. The glued glass bead at the tip of the cantilever was moved at 3.0 µm/s and pushed to the surface of gelated ECM proteins for measuring the stiffness. The measurement was conducted at 25 points (5-by-5 grid, 10 µm in gap) for each sample, and was repeated more than three times at each point. The data was analyzed and selected with built-in software (JPK Data Processing, JPK instruments, Berlin), and Young’s moduli were determined by fitting the Hertz model to the force curve.

**Removal of the alginate shell.** To remove the alginate shell of the cell fibre, 4 mg/mL alginate lyase (A1603, Sigma) in DPBS (+) was added at 1:100 ratio to the culture medium in which the cell fibre was dispersed. The fibre was then incubated for 15 min to enzymatically digest the alginate shell of the cell fibre.

**Cell count and viability assay for cell fibres.** A standardized cell fibre (170 cm in length) was used for the evaluation of the number of live/dead cells in the NIH/3T3 fibres. The cell fibre was collected in a centrifuge tube with 2 mL of the culture medium. Then, 20 µL of 4 mg/mL alginate lyase was added to the medium. The cell fibre was incubated at RT for 5 min to remove the alginate shell. After that, the cell fibre was centrifuged and all the supernatant was aspirated. The collected fibre was then treated with 0.5 mL of trypsin/EDTA solution for 5 min at 37ºC to dissolve the cell fibre into single cells, followed by adding 0.5 mL of culture medium to the cell suspension. The dispersed cells were suspended in 1:1 mixture of the cell suspension and 0.4% Trypan blue solution (T10282, Invitrogen), and the number of the cell was counted with a hemocytometer to obtain the numbers of live cells (not stained) and dead cells (stained with trypan blue). The number of samples (standardized cell fibres) for this assay is three for each type of a cell fibre.

**Immunocytochemistry for the HUVEC-ACol fibre.** A HUVEC-ACol fibre (Fig. 2 (c)) was buried with 0.5% native collagen (IAC-50, KOKEN) in a dish and incubated at 37ºC for 15 min. This process prevents the fibre from breaking down by liquid flows during the exchange of solution in the procedures of
immunocytochemistry. All the reactions to the cell fibre were done by diffusion through the collagen hydrogel. After the gelation of the collagen, the cell fibre was fixed for 1 h with 4% paraformaldehyde (Muto Pure chemicals Co., Ltd.) in DPBS (-), permeabilized with 0.1% Triton-X (A16046, Alfa Aesar) in DPBS(-), and blocked with 1% bovine serum albumin (BSA, A7906, Sigma-Aldrich) in DPBS (-) for 90 min to eliminate nonspecific bindings. Note that at this stage, the alginate shell of the fibre was completely dissolved by the ion exchange between the Ca-alginate and DPBS (-). The fibre was then reacted with Alexa488-conjugated phalloidin (1:200, A12379, Invitrogen) at 4°C for overnight. After rinsing twice with DPBS(-), treatment of Hoechst 33342 (1:1000, H3570, Invitrogen) was carried out for nucleus staining. After that, the HUVEC-ACol fibres were observed with a confocal laser scanning microscope (CLSM, LSM-780, Zeiss, or FV1000D, Olympus) (Fig. 2 (c-d) and Supplementary Fig. S12).

**Contraction measurement of cardiomyocyte-Fib fibre.** The contractile motion of the cardiomyocyte-Fib cell fibre was observed with an inverted fluorescent microscope (IX-71, Olympus) and recorded as a movie at 30 frames/sec with digital camera (EOS kiss X3, Canon, Japan). Each frame of the movie was converted to tagged image file (TIF) format with Premiere (Adobe, USA). The distance of the specific points of the cardiomyocyte-Fib fibre in the images was measured with ImageJ (NIH, USA).

**Immunocytochemistry for cortical cell-PCol fibres.** Cortical cell-PCol fibre was fixed, permeabilized, blocked as the same processes as those of HUVEC-ACol fibre written in *Immunocytochemistry for the HUVEC-ACol fibre section*, except the cortical cell-PCol fibres were not buried in native collagen hydrogel. Note that at this stage, the alginate shell of the fibre was completely dissolved by the ion exchange between the Ca-alginate and DPBS (-). The fibre was then reacted with mouse monoclonal antibodies to Tuj1 (1:500, T8578, Sigma-Aldrich) at 4°C for overnight. After rinsing twice with DPBS (-), the fibre was incubated with anti-mouse IgG-AlexaFluor488 (A11001, Invitrogen) for 2 hours at RT. Following a DPBS(-) rinse twice, the treatment of Hoechst 33342 (1:1000; H3570, Invitrogen) was carried out for nucleus staining. After that, the cortical cell-PCol fibre was observed with an inverted fluorescent microscope (Aixo Observer Z1, Zeiss).

**[Ca^{2+}]_{i} imaging of cortical cell-PCol fibres.** The observation of [Ca^{2+}]_{i} fluxes of the cortical cell-PCol
fibre (day 14) was performed as previously described. Briefly, 1 mM Fluo-4/AM (F14201, Invitrogen) dissolved in DMSO was diluted to a final concentration of 2.5 µM with basal salt solution (BSS) consisting of 130 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 5.5 mM glucose and 20 mM HEPES-NaOH (pH 7.4). The cortical cell-PCol fibre was incubated with the Fluo-4/AM solution at 37°C for 1 hour. After rinsing the fibre with BSS twice, the cortical cell-PCol fibre was observed with an inverted fluorescent microscope (Axio Observer Z1, Zeiss) to measure the relative concentration of Ca²⁺ quantified as \( \Delta F/F₀ = (F(t) - F₀) / F₀ \), where \( F₀ = F(0) \).

**Differentiation Induction of NSC-PCol fibre.** A NSC-PCol fibre was cultured in a growth medium (the composition is shown in Table S1) for 7 days until the space of the core is completely filled with the NSC cells. On day 4, both bFGF and hEGF were added to the medium at the concentration of 20 ng/mL. On day 7 of culture, we started differentiation induction (day 0 for differentiation induction); the NSC fibre was immersed with a growth medium without bFGF, hEGF and B27 for 15 min to rinse the fibre. The fibre was then cultured in a differentiation medium (B27 (+), 1% FBS (+), bFGF (-), and hEGF (-)) at 37°C in water saturated 5% CO₂ environment to induce the differentiation of the NSCs to glial cells and neurons. For the control, the NSC fibre was continuously cultured in the growth medium (differentiation-uninduced NSC-PCol fibres). At day 17 (10 days after starting the differentiation induction), both the differentiation-induced and the control NSC-PCol fibres were stained with immunocytochemistry for observation and analyzed by RT-PCR. For observing synapses in NSC-PCol fibres, NSC-PCol fibres were induced to differentiate for 77 days and stained with a synapse marker.

**Immunocytochemistry for NSC-PCol fibres.** NSC fibres before differentiation induction (day 0), Differentiation-induced NSC-PCol fibres (day 10 (+)) and differentiation-uninduced NSC-PCol fibres (day 10 (-), control) and NSC-PCol fibres induced were fixed, permeabilized, and blocked in the same processes as those of HUVEC-ACol fibre written in Immunocytochemistry for the HUVEC-ACol fibre and MSI-ACol fibre section, except the cortical cell-PCol fibres were not buried in native collagen hydrogel. We conducted double immunostaining to both differentiated and controlled NSC fibres as follows; (1) nestin
(neuronal stem cell marker) and glial fibrillary acidic protein (GFAP, glial cell marker), and (2) Tuj1 (neuron marker) and GFAP. For the nestin/GFAP staining, mouse monoclonal anti-nestin antibody (1:200, mab353, Millipore) and rabbit polyclonal anti-GFAP antibody (1:500, Z0334, DAKO) were reacted to the NSC fibre at 4°C overnight. For the Tuj1/GFAP staining, mouse monoclonal anti-Tuj1 antibody (1:500, T8578, Sigma-Aldrich) and rabbit polyclonal anti-GFAP antibody (1:500, Z0334, DAKO) were reacted to the NSC fibre at 4°C for overnight. For the NSC-PCol fibres with differentiation induction for 77 days, anti-Tuj1 antibody (1:500, T8578, Sigma-Aldrich) and anti-synapsin I antibody (1:500, AB1543, Millipore) were reacted to stain neurons and synapses. After rinsing twice with PBS (-), the fibre was incubated with anti-mouse IgG-AlexaFluor488 (A11001, Invitrogen) and anti-rabbit IgG-AlexaFluor568 (A11011, Invitrogen) for 2 hours at RT. Following a PBS (-) rinse twice, the treatment of DAPI (1 µg/mL in PBS (-), D1306, Invitrogen) was carried out for nucleus staining. After that, the NSC fibres were observed with an inverted fluorescent microscope (Aixo Observer Z1, Zeiss) or a CLMS (LSM-780, Zeiss).

Reverse transcription polymerase chain reaction (RT-PCR) of the NSC-PCol fibre. For RNA extraction, the shell of the NSC-PCol fibres were digested with 40 µg/mL alginate lyase for 5 min at 37 °C. Total RNA was isolated using a PureLink™ RNA Mini kit (Life technologies). Following the treatment with DNase I (Invitrogen), cDNA was produced using SuperScript III (Invitrogen). cDNA was amplified using Ex Taq DNA polymerase (Takara) under the following reaction conditions: 94 °C for 2 min and then * cycles at 92 °C for 15 s, ** °C for 15 s, and 72 °C for 40 s, followed by 72 °C for 7 min [asterisk (*) indicates GAPDH and Tuj1 primers at 30 cycles, GFAP primers at 25 cycles; double asterisk (**) indicates GAPDH primers at 57 °C, GFAP primers at 54 °C, and Tuj1 primers at 55.5 °C]. The following primer pairs were used for the PCR: GAPDH (5’-ACC ACA GTC CAT GCC ATC AC-3’ and 5’-TCC ACC ACC CTG TTG CTG TA-3’); GFAP (5’-TGG ATT TGG AGA GAA AGG TTG AAT-3’ and 5’-ATT TGC CGC TCT AGG GAC TC-3’); and Tuj1 (5’-GCC TTT GGA CAC CTA TTC AG-3’ and 5’-GTA GAG GGC TTC ATT GTC GA-3’).

Microfluidic handling of cell fibres. In the demonstrations shown in Fig. 4 (a), we used HepG2-PCol fibre (day 7) in DMEM without phenol red (21063, Gibco, Invitrogen). Silicone tubes (inner diameter: 0.5 mm, LABORAN) were connected to syringes, and the fluid flow through the silicone tube was manually
controlled by hands. In the experiment shown in Supplementary Fig. S13, a Ca-alginate microfibre (diameter: 200 µm, fabricated with the double-coaxial microfluidic device without a core stream) with blue ink was introduced in a silicone tube (inner diameter: 1 mm) for easy observation. The microscopic images were taken by an inverted fluorescent microscope (IX-71, Olympus). The movie was taken by a stereoscopic microscope (SMZ1500, Nikon, Japan) equipped with a digital camera (EX-F1, CASIO, Japan).

**Assembly of cellular braid with cell fibres.** Three NIH/3T3-PCol cell fibres (day 2-4) were labeled with CellTracker Green, CellTracker Blue (C2110) and CellTracker Red (C34552), respectively. Note that DPBS (+) was used for the buffer solution instead of DPBS (-) to avoid the dissolution of the alginate shell by the exchange of ions. Silicone tubes (inner diameter: 0.5 mm) were fixed to a glass slide with glue. The glass slide was placed on the bottom of a bath and immersed with culture medium. The stained cell fibres were put in the culture medium and sucked into the silicone tubes one by one. The introduced length of the cell fibre was approximately ~20 cm to properly clamp the cell fibre during the knitting. The cell fibres were manually knitted in the medium with a sterilized glass tube (G-1, Narishige). The knitted braid was put on a glass slide, and observed with an inverted fluorescent microscope (Axio Observer Z1, Zeiss).

**Assembly of cellular fabric by the microfluidic weaving machine.** The setup of the microfluidic weaving machine is shown in Supplementary Fig. S17. The machine is composed of a set of arrayed silicone tubes (inner diameter; 0.5 mm, the number of the tubes; 8) fixed on a stage with glue, and two combs (upper comb and lower comb) where a silicone tube is fixed on each tooth of the combs (four silicon tubes were fixed to each comb). The combs (upper and lower) were fabricated with stereolithography (Perfactory, envisionTEC GmbH, Germany), and coated with a 5-µm-thick parylene film (PDS2010 LABCOATER, Special Coating Systems, USA) to avoid the sticking of cell fibres to the combs. The combs work as a heddle: The upper comb was attached to a manipulator so that the upper comb can move up and down; the lower comb was fixed to the bottom of the bath. The machine was built in the bath so that all the weaving procedures can be conducted in culture medium. All the suction and ejection of solution through the silicon tubes were independently controlled with syringe pumps (Micro4, World Precision Instruments, Inc., USA).
Cell fibres for the mechanical weaving were labeled with CellTracker Green, CellTracker Blue and CellTracker Red or CellTracker Orange (C34551, Invitrogen). Note that DPBS (+) was used for the buffer solution in the staining process instead of PBS (-) to avoid the dissolution of the alginate shell by the exchange of ions. The stained cell fibres were cut to 50-70 cm for the weaving, and stored in an incubator at 37°C until use. The operation of the mechanical weaving of the cell fibres are as follows: Eight warp cell fibres were bridged and clamped with the microfluidic handling method between the silicone tubes on the left stage and the combs. We used HepG2-PCol fibre and MS1-ACol fibre as a warp for the demonstration of the woven cellular fabric structure (Fig. 4 (c-d) and Supplementary Fig. S18 (e-f)) and the folded 3D structure of the cellular fabric (Fig. 4 (e) and Supplementary Fig. S19 (j-k)), respectively. As wefts, MIN6m9-PCol and HeLa-PCol fibres were used in Fig. 4 (c-d) and Supplementary Fig. S18 (e-f), and NIH/3T3-ACol and HepG2-PCol fibres were used in Fig. 4 (e) and Supplementary Fig. S19 (j-k). A weft cell fibre went right and left repeatedly by manual handling with a glass rod corresponding to the up-and-down motion of the upper comb. After completing the weaving, the woven structure was carefully pulled up from the culture medium, and fixed with 3% agarose (Low-melting agarose, Type IX-A, A2576, Sigma-Aldrich) in DPBS (+) or collagen hydrogel (AteroCell™, native collagen, IAC-50, KOKEN). The woven structure was observed with a fluorescent stereoscopic microscope (MVX10, Olympus) equipped with a digital camera (C7880, Hamamatsu Photonics K K, Japan).

**Folding woven cellular fabric.** The fabrication process of the folded woven tissue structures was shown in Supplementary Fig. S19. A woven cell fabric fixed with agarose or collagen hydrogel was placed on a polymer film (PM-996, PARAFILM M) so that we can handle the fabric with tweezers. A 1.5 mm-hole was punched at the center of the fabric. To make through holes in the structure, glass tubes (G-1, Narishige) were placed on the fabric. The woven fabric was then folded and fixed with an agarose or collagen hydrogel. After that, the glass tubes were carefully removed. Finally, the folded structure was trimmed with a razor blade into desired shape. The image of the folded structure was obtained with a fluorescent stereoscopic microscope (MVX10, Olympus) equipped with a digital camera (C7880, Hamamatsu Photonics K K).

**Helical tube structures.** At first, a glass tube (outer diameter: 1 mm, G-1, Narishige) was coated with
agarose (Type IX-A, A2576, Sigma-Aldrich) or collagen (IAC-50, KOKEN): The 3% agarose sol in DPBS(+) was coated on the surface of the glass tube by dipping, and gelated by cooling at 4ºC to produce 100-200 µm-thick agarose layer. In the case of collagen coating, molding approach was used: The glass tube was inserted in another glass tube (inner diameter: ~1.5 mm). Collagen sol was poured into the gap between the glass tubes and incubated at 37ºC for 10 min to set the collagen into gel. After that, the outer glass tube was removed to obtain a ~200 µm-thick collagen layer on the surface of the glass tube.

Next, cell fibres in a culture medium were picked up with the hydrogel coated glass tube to the air, and reeled them up to make a helical structure. To fabricate double-striped helical structure, two different cell fibres were picked up and reeled at the same time (Supplementary Fig. 20 (a)). To fabricate double-layered helical structure, two different cell fibres were picked up and reeled up one by one (Supplementary Fig. S20 (b)). HepG2-PCol fibre (stained with CellTracker Orange) and MIN6m9-PCol fibre (stained with CellTracker Green) were used in Supplementary Fig. S20 (e), and MS1-PCol fibre (stained with CellTracker Red) and NIH/3T3-ACol fibre (stained with CellTracker Green) were used in Supplementary Fig. S20 (g-h). After that, the helical structure was dipped in agarose or collagen sol and cooled at 4ºC or incubated at 37ºC to gelate the hydrogel to fix the helical structure. At last, the glass tube was carefully removed, and the helical tube structure was released into culture medium. The fabricated helical tube structure was observed with an inverted fluorescent microscope (IX-71, Olympus) and a fluorescent stereoscopic microscope (MVX10, Olympus).

**Co-culture of helical tube.** HepG2-PCol fibre and NIH/3T3-ACol fibre were prepared for the co-culture experiment. As written in *helical tube structures section*, a glass tube was coated with collagen, and double-striped helical tubes were then created with these two cell fibres. The helical tube structure was fixed with collagen and released from the glass tube to culture in DMEM supplemented with 10% FBS and 1% AB at 37ºC, 5% CO2 environment. For the control experiment, the same helical tube structures composed of only HepG2-PCol fibre were fabricated. Before starting the culture, 40 µg/ml alginate lyase (A1603, Sigma-Aldrich) was reacted to the helical tubes to remove the alginate shell of the reeled HepG2-PCol and NIH/3T3-ACol fibres. At 1 and 3 days after starting the culture, the medium was collected and stored in -20ºC. The amount of the albumin in the collected media was measured with...
albumin ELISA assay kit (EA3201-1, ASSAYPRO), and divided by the length of the HepG2-PCol fibre to estimate the normalized amount of secreted albumin per unit length of the cell fibre.

**Isolation of rat pancreatic islets**

Pancreatic islets were isolated from Lewis rats (LEW/CrlCrlj), 8- to 10- week old, weighing 250 to 300 g. The rats were anesthetized by Isoflurane (Isoflu®, Abbott) delivered at 4 mL/hour via Univentor 400 (Univentor Ltd., MALTA). They then underwent bile duct cannulation after clamping the distal common bile duct and pancreatic distention using 6 mL of ET-Kyoto solution (Otsuka Pharmaceutical, Tokyo) supplemented with Ulinastatin (Miracle®, Mochida Pharmaceutical, Tokyo), which contained 0.15 mg/mL of Liberase MTF-S (Roche Diagnostics, Mannheim, Germany). The pancreas was excised and digested in Liberase MTF-S (1 mg/mL) solution, without shaking, at 37 ºC for 20 min. The digested pancreas was washed by centrifugation (500 g for 2 min at 4 ºC) and then purified with a discontinuous density gradient (1.13 g/mL and 1.077 g/mL) in a modified ET-Kyoto/OptiPrep (Axis-Shield, Oslo, Norway) solution. Washes after purification were performed at 500 g for 2 min at 4 ºC. After isolation, islets were further purified using a hand-held pre-wet 70 µm nylon cell strainer (BD Biosciences, Bedford, MA) under a stereomicroscope. Obtained pancreatic islets were stored in Ulinastatin containing ET-Kyoto solution supplemented with 10% fetal bovine serum (Biowest, France) at 4 ºC and used within 48 hours.

**Formation of primary rat pancreatic islet cell fibre for implantation**

Primary rat pancreatic islets were dissociated with Accutase (AT104, Innovative Cell Technologies, Inc.) at 37 ºC for 15 min to obtain single cell suspension of pancreatic islet cells. As the core pre-gel solution for the cell fibre fabrication, the pancreatic islet cells were suspended at 3.0×10⁸ cells/mL in pre-gel solution of PCol. As the shell, we used pre-gel solution containing 1.5% Na-alginate and 2.5% type-IX agarose (Low-melting agarose, Type IX-A, A2576, Sigma-Aldrich) in saline to form the shell of alginate-agarose IPN hydrogel. Microfluidic formation of the core-shell hydrogel microfibre were the same as described in *Formation of cell-containing ECM protein/Ca-alginate core/shell hydrogel microfibre* section, except that the flow rate of the CaCl₂ solution was 10 mL/min. The fabricated primary islet cell-containing core-shell hydrogel microfibres were cultured at 37 ºC under water-saturated atmosphere in RPMI-1640 -based
medium (Funakoshi Co. Ltd., Tokyo), where the concentration of Ca(NO₃)₂·4H₂O, NaH₂PO₄·H₂O and NaHCO₃ were adjusted to 425.5 mg/L, 125.36 mg/L and 3700 mg/L, respectively, supplemented with 10% FBS and 1% AB. The fabricated primary islet fibres were cultured for 2-4 days before implantation.

**Formation of MIN6m9-PCol fibre for implantation**

MIN6m9 cells were suspended in PCol pre-gel solution at 1.0×10⁸ cells/mL for the core solution. As the shell solution, we used pre-gel solution containing 1.5% Na-alginate and 2.5% type-IX agarose in saline. Formation of core-shell hydrogel fibre was the same as the procedure described in *Formation of primary rat pancreatic islet cell fibre for implantation* section. The fabricated MIN6m9-PCol fibre was cultured for 2-3 weeks before implantation.

**Measurement of glucose-responsive insulin secretion of pancreatic islet cell fibres.** Primary rat islet cell fibres or MIN6m9-PCol fibres were preincubated for 30 min at 37°C in HKRB solution (HEPES-added Krebs-Ringer bicarbonate buffer; NaCl, 134 mM; KCl, 4.7 mM; KH₂PO₄ (Wako Pure Chemical Industries, Japan), 1.2 mM; CaCl₂, 2.0 mM; MgSO₄ (Nacalai tesque), 1.2 mM; BSA, 0.5% and HEPES, 10 mM with NaOH (pH 7.4)) that contained 3.3 mM glucose. After that, the fibres were incubated for 60 min at 37°C in HKRB solution containing 3.3 mM glucose for the first low glucose stimulation, then for 60 min at 37°C in HKRB solution containing 22.2 mM glucose for high glucose stimulation, and finally for 60 min at 37°C in HKRB solution containing 3.3 mM glucose for the second low glucose stimulation. The amount of insulin released in the HKRB solution during the incubation was determined using ELISA (49170-51 for primary rat islet cell fibre and 49170-54 for MINm9-PCol fibre, MORINAGA) according to the manufacturer’s protocols.

**Bradford protein assay for evaluating the number of cells in MIN6mPCol fibres.** The numbers of cells in the MIN6m9-PCol fibres were determined using the Bradford protein assay using Coomassie Brilliant Blue (CBB) as follows: The MIN6m9-PCol fibres were treated with 0.004% alginate lyase in PBS(-) for 5 min at R.T. to dissolve the Ca-alginate in the shell. Consequently, they were rinsed in PBS(-) solution three times and treated with cell lysis solution including 10 mM Tris-HCl pH7.4 (Tris: 40326, Kanto Chemicals,
HCl: 081-05435, Wako), 0.1% Triton X-100 (A16046, Alfa Aeser), protease inhibitor cocktail and 0.5 mM EDTA (78410, Thermo), and mixed well for 30 sec on a vortex mixer. The supernatant of the solution was collected after centrifugation at 15000 rpm for 5 min at 4°C, and then examined with protein assay kit (500-0001JA, BioRad) to evaluate the amount of proteins that corresponds to the number of cells. The amount of secreted insulin per cell was calculated by dividing the amount of insulin secreted from a fibre by the number of cells in the fibre.

Implantation of pancreatic islet cell fibres to diabetic mice. 10–12-week-old male BALB/c Slc-nu/nu mice weighing 21–26 g were rendered diabetic by a single intraperitoneal injection of streptozotocin (54-0028, Wako Pure Chemical Industries, Osaka, Japan) at a dose of 200 mg/kg. Diabetes was defined as blood glucose concentration of more than 350 mg/dL (hyperglycemia) detected on two consecutive days after streptozotocin injection. Blood glucose concentrations of tail vein blood were monitored using glucose monitoring system (ACCU-CHEK Active; Roche Diagnostics, Japan) once a day throughout the experiment. The mice were anesthetized by Isoflurane (Forane®, Abbott) delivered at 1.5 mL/h via Univentor 400 (Univentor Ltd., MALTA). Primary rat islet cell fibres or MIN6m9-PCol fibres were implanted into the subrenal capsular space of the diabetic mice using the microfluidic handling technique (Movie S6; implantation of MIN6m9-PCol fibre) as follows. The prepared cell fibre was rinsed with serum-free culture medium once, and the fibre was sucked into a microcatheter (inner diameter: 0.4 mm, outer diameter: 0.6 mm). The subrenal capsular space was filled with 0.5% v/v hyaluronic acid in serum-free culture medium in advance for lubrication. Through the microcatheter, the cell fibre was injected into the subrenal capsular space. The tip of the microcatheter was precisely moved during the implantation procedure and the injected primary rat islet cell fibre or MIN6m9-PCol fibre was folded and placed in the subrenal capsular space.

Removal of the implanted pancreatic islet cell fibres from the treated mice. After anesthetizing the mice that received the primary rat islet cell fibres or MIN6m9-PCol fibres, the kidneys bearing the fibres were exposed. The implanted fibres were then removed from the subrenal capsular space either by sucking the implanted fibre with a microcatheter or by using tweezers.
2. Supplementary experimental results and figures

2-1 Double co-axial laminar flow microfluidic device

Figure S1 shows detailed information about the double coaxial laminar flow device we used in this work.

**Figure S1.** Double coaxial laminar flow microfluidic device. (a) Design of the device. The device was assembled with two square glass tubes, two pulled cylindrical glass tubes, and two connectors. As shown in the cross-sectional view at A-A', inner stream goes through the inside of the cylindrical tube, and the outer stream goes through the gap between the outside of the cylindrical tube and the inside of the square tube. (b) Image of the assembled device. (c) Microscopic image of the merge point of two streams. (d) Setup of the device with the tubing. Scale bars: 2 mm in (b), 1 mm in (c).
2-2 SDS-PAGE analysis of ECM proteins in the core

To examine the molecular weights and purity of the ECM proteins in the core (PCol, ACol and Fib), we performed sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

PCol is pepsin-solubilized bovine dermal type I atelocollagen, and ACol is acid-solubilized pig/bovine type I collagen (see Supplementary Materials and Methods for the detailed composition). Generally, a single type I collagen molecule has triple helix structure composed of two α1 chains and one α2 chain, and the molecular weights are known to be about 300 kDa for the type I collagen in a triple helix form, and approximately 100 kDa for the α1 and α2 chains. In SDS-PAGE analysis with the reduced PCol and ACol samples (Supplementary Fig. S2 (a)), we detected three major bands corresponding to two α chains at 100-150 kDa (α1 and α2 chain) and one β protein (double helix structures of two α chains) at approximately 250 kDa. The bovine plasma fibrinogen (Fib, see Supplementary Materials and Methods for the detailed composition) is a hexameric glycoprotein with its molecular weight of 330-340 kDa. Fibrinogen contains three pairs of different polypeptide subunits (α, β, γ) linked with each other through disulfide bonding and their molecular weights are approximately 63.5 kDa for α-chains, 56 kDa for β-chains and 47 kDa for γ-chains. Consistent with this, SDS-PAGE analysis of the reduced Fib sample revealed multiple major bands corresponding to α-, β-, and γ-chains and the N-glycosylated form of β-, and γ-chains, broadly ranging from 45-65 kDa (Supplementary Fig. S2 (a)). These results clearly indicate that the ECM proteins we used as the core of the fibres were highly purified.

Taken together, the ECM proteins, PCol, ACol and Fib, do not diffuse out through the Ca-alginate

Figure S2. SDS-PAGE analysis of the ECM proteins in the core. Denatured PCol, ACol and Fib samples were resolved on 10% acrylamide gel containing SDS.
shell because the molecular cut-off of the 1.5% Ca-alginate is approximately 60 kDa (diffusion coefficient of 60 kDa molecules in the Ca-alginate is less than 10% of that in water)\(^9\).

**Experimental procedures:**

**SDS-PAGE.** ACol, PCol and Fib were prepared as described in *Formation of cell-containing ECM protein/Ca-alginate core/shell hydrogel microfibre* section in Supplementary Materials and Methods. The concentration of these samples were adjusted to 1 mg/mL by adding PBS(-). The samples were then mixed with SDS sample buffer (50 mM Tris-HCl (pH6.8), 2% SDS, 50 mM dithiothreitol, and 10% glycerol) and heated at 95ºC for 5 min for denaturation. Two microgram of protein samples were loaded on each well and resolved on 10% acrylamide gel. The gel was then stained with Coomassie Brilliant Blue (021-02911, Wako), and de-stained with 5 % v/v methanol and 7.5% v/v acetic acid to visualize the protein bands.
Mechanical stiffness measurement of ECM proteins using AFM

We measured mechanical stiffness of PCol, ACol and Fib using AFM equipped with a bead-attached cantilever (Fig. S3).

Figure S3. Mechanical stiffness measurement of PCol, ACol and Fib using AFM. (a) Typical force-distance curves of PCol, ACol and Fib. (b) Young's modulus of PCol, ACol and Fib. The bars and the error bars indicate the averaged values and s.d., respectively, for \( n = 17-25 \).
Images of cell-encapsulating core-shell hydrogel microfibres

Metre-long and cell-containing core-shell hydrogel fibres were immersed in culture medium to form cell fibres (Fig. S4).

**Figure S4.** A metre-long cell-encapsulating core-shell hydrogel microfibre formed with the double-coaxial laminar flow microfluidic device. (a) Formed microfibres were suspended in culture medium in a 10-cm dish. (b,c) Microscopic images of the microfibre. (d) A metre-scale cell-encapsulating core-shell fibre was formed. The length of the fibre in (d) was more than 1.5 m. Scale bars: 500 µm in (b), 200 µm in (c).
Flow rate vs. diameter of the fibre

We examined the relationship between the flow rate and the diameter of the core in fabricated core-shell fibres (Fig. S5).

**Figure S5.** Relationship between the flow rate and the diameter of the generated fibre. (a) Images of the formed fibre at various flow ratios, $Q_{\text{core}}/(Q_{\text{core}} + Q_{\text{shell}})$. (b) Relationship between the diameter and flow rate. The sum of the flow rates of core and shell, $Q_{\text{core}} + Q_{\text{shell}}$, was fixed at 100 µL/min in the experiment. Each dot and error bar indicates the average and the standard deviation, respectively.
Results on the cell fibre formation for 10 types of cells in three different types of ECM proteins in the core

Sequential images of fabricated core-shell hydrogel microfibres are shown in Fig. S6 (1-3).

Figure S6-1. Sequential images of fabricated core-shell hydrogel microfibres using NIH/3T3, HepG2, HeLa and MIN6m9.
Figure S6-2. Sequential images of fabricated core-shell hydrogel microfibres using HUVEC, MS1, C2C12 and Cardiomyocyte.
**Figure S6-3.** Sequential images of fabricated core-shell hydrogel microfibres using cortical cell and neuronal stem cell.
2-7 Removal of the Ca-alginate shell

Figure S7 shows that the removal of the Ca-alginate shells covering NIH/3T3-ACol fibres and MIN6m9-PCol fibres by adding an enzyme (alginate lyase) to culture medium.

**Figure S7.** Removal of the Ca-alginate shell after the cell fibre formation. The NIH/3T3-ACol fibre at day 7 and the MIN6m9-PCol fibre at day 14 were treated with alginate lyase. After the treatment, the cell fibres maintained their shape without the Ca-alginate shell. All scale bars: 200 μm.
Immunofluorescent analysis of cadherins in cell fibres

To examine cell-cell contacts in the NIH/3T3-ACol and MIN6m9-PCol fibres, we stained broad members of cadherins, which play important roles in cell-cell adhesion, with anti-pan cadherin antibody. Localization of cadherins were first tested using NIH/3T3 and MIN6m9 cells cultured on dishes; confocal laser scanning microscopic (CLSM) observation of these cells clearly showed that the signals of cadherins were intense at the border of the cells where cell-cell contact occurred (Supplementary Fig. S8). Similarly, strong signals of cadherins were observed at the border of the cells in day 7 NIH/3T3-ACol fibres (Supplementary Fig. S9 (a)) and day 14 MINm9-PCol fibres (Supplementary Fig. S9 (b)). These results clearly indicate that cadherin-based cell-cell contacts were formed over the whole NIH/3T3-ACol fibres and MIN6m9-PCol fibres.

**Experimental procedure:**

**Immunocytochemistry.** The NIH/3T3-ACol and MIN6m9-PCol fibres were fixed for 30 min with 4%

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**Figure S8.** Immunofluorescent analysis of cadherins in 2D cultured NIH/3T3 and MIN6m9 cells. NIH/3T3 cells (a) and MIN6m9 cells (b) were cultured on 2D culture dishes and immunostained with anti-pan cadherin antibody (cadherins, green). Cell nuclei were counterstained with DAPI (blue). The signal of the cadherins was intensely detected at the border of the cell-cell contact indicated by the arrows. Scale bars: 50 µm.
paraformaldehyde (Muto Pure chemicals Co., Ltd.) in DPBS(-), permeabilized with 0.5% Triton-X100 (A16046, Alfa Aesar) in Tris-buffered saline (TBS) for 5 min, and incubated with 5% Difco Skim Milk (232100, BD Difco) in TBS containing 0.2% Tween 20 (Sigma) for 1 h at 37 °C to block non-specific bindings. The cell fibres were then reacted with mouse anti-pan cadherin monoclonal antibody (1:200, ab6528, Abcam) for 1 h at 37 °C, followed by 1 h incubation with Alexa Fluor 488-conjugated goat anti-mouse IgG secondary antibody (Invitrogen) and DAPI (1µg/mL, D1396, Invitrogen) for nucleus staining. After that, the cell fibres were observed with a confocal laser scanning microscope (CLSM, LSM-780, Zeiss).

Figure S9. Immunofluorescent analysis of cadherins in NIH/3T3-ACol and MIN6m9-PCol fibres. NIH/3T3-ACol fibres at day 7 (a) and MIN6m9-PCol fibres at day 14 (b) were immunostained with anti-pan cadherin antibody (cadherins, green), and the nuclei were counterstained with DAPI (blue). The strong signal of the cadherins was observed at the border of adjacent cells. Scale bars: 50 µm.
Immunofluorescent analysis of type I collagen matrix in the core of cell fibres

We investigated how type I collagen matrix in the core of the cell fibres is changed or maintained during the culture (day 0 to 14). By utilizing species-specific type I collagen antibodies, we differentially visualized (i) type I collagen matrix initially provided as the ECM proteins in the core of the cell fibres (i.e. PCol, ACol and Fib) and (ii) cell-derived type I collagen matrix that was newly produced by the cells.

First, using NIH/3T3-ACol and MIN6m9-PCol fibres, we analysed the collagen matrix that was initially composed in the core of the cell fibres; ACol (a mixture of bovine and pig type I collagen) and PCol (bovine type I collagen) were specifically stained with anti bovine/pig type I collagen antibody. Although ACol in the NIH/3T3-ACol fibres were observed in the whole cell fibre, it gradually disappeared as the cell fibre formed from day 7-14 (Supplementary Fig. S10). On the other hand, PCol in the MIN6m9-PCol fibres disappeared at day 9 (Supplementary Fig. S11). (Note that immunofluorescence of the MIN6m9-PCol fibre at day 0 could not be carried out. The reason was that the PCol in the MIN6m9-PCol fibre was mechanically fragile and the cell-cell contact formation in the MIN6m9 cell fibres seemed to be

Figure S10. Immunofluorescent analysis of type I collagen matrix in NIH/3T3-ACol fibres. (a) ACol, a pig/bovine type I collagen, was stained with anti-pig/bovine type I collagen antibody (green) at day 0, 3, 7 and 14. (b) De novo synthesized mouse type I collagen by NIH/3T3 cells in the fibre was stained with anti-mouse type I collagen antibody (magenta), and nuclei were counterstained with DAPI (blue) at day 0, 3, 7 and 14. (c) Merged image of (a) and (b). Scale bars: 50 µm.
partial at the initial state. Thus, after the removal of the Ca-alginate shell, which was essential for immunostaining, the MIN6m9-PCol fibre at day 0 could not maintain its shape due to the multiple rinsing processes of the fluorescent immunostaining.

Next, we analysed \textit{de novo} synthesis of type I collagen in the fibres of mouse NIH/3T3 and MIN6m9 cells. Using anti-mouse type I collagen antibody that specifically reacts with mouse antigen, but not pig or bovine type I collagen of core gel, cell-producing type I collagen in the NIH/3T3-ACol fibres was immediately detectable even at day 0, and abundantly detected over the whole cell fibre area including extracellular space from day 3 onward. In contrast with NIH/3T3-ACol fibres, newly-produced type I collagen was hardly detected in MIN6m9-PCol fibres. This is indicative of low expression level of type I collagen in MIN6m9 cells, instead there was intense cell-cell contact formation (Supplementary Fig. S9).

Therefore, these results indicate that in our cell fibres type I collagen matrix initially provided in the core gradually disappeared, then cell-derived type I collagen matrix or cell-cell contact was newly formed and organized to maintain the fibre shape.

\textbf{Experimental procedure:}

\textbf{Immunocytochemistry.} The NIH/3T3-ACol and MIN6m9-PCol fibres were fixed for 30 min with 4% paraformaldehyde (Muto Pure chemicals Co., Ltd.) in DPBS(-), permeabilized with 0.2% Triton-X100

![Figure S11](image_url). Influence of cells on collagen in MIN6m9-PCol fibres. (a) ACol, a bovine type I collagen, was stained with anti-pig/bovine type I collagen antibody (green) at day 9, 14 and 21. (b) Mouse type I collagen produced by MIN6m9 cells in the fibre was stained with anti-mouse type I collagen antibody (magenta), and nuclei were stained with DAPI (blue) at day 9, 14 and 21. Scale bars: 50 µm.
(A16046, Alfa Aesar) in TBS for 5 min, and incubated with 5% Difco Skim Milk (232100, BD Difco) in TBS for 30 min to block non-specific bindings. The cell fibres were then reacted with mouse anti-bovine/pig type I collagen monoclonal antibody (1:1000, C 2456, Sigma-Aldrich) and rabbit anti-mouse type I collagen antibody (1:200, ab21286, Abcam) at 4°C overnight, followed by 1 h incubation with Alexa Fluor 488-conjugated goat anti-mouse IgG secondary antibody (Invitrogen), Alexa Fluor 568-conjugated goat anti-rabbit IgG secondary antibody (Invitrogen), and DAPI (1µg/mL, D1396, Invitrogen) for nuclei staining. After that, the cell fibres were observed with a confocal laser scanning microscope (CLSM, LSM-780, Zeiss).
Images of tube-like cellular structures in endothelial cell fibres

We examined the tube formation of two types of endothelial cells in cell fibres (Fig. S12).

Figure S12. Tube-like cellular structure in MS1-ACol and HUVEC-ACol fibres. (a) MS1 cells (mouse pancreatic islet endothelial cells) formed a tube-like structure in ACol core after two days of culture. The MS1-ACol fibre was embedded in collagen gel for immunofluorescent staining. (b) A sliced CLSM image of the MS1-ACol fibre. Green: actin, blue: nucleus. (c) Reconstructed cross-sectional image at the yellow line on (b). The green line indicates the position of the sliced plane in (b). (d) Image of a HUVEC-ACol fibre (day 2). (e) A sliced CLSM image of the HUVEC-ACol fibre (This is the same image as Fig. 2 (c). Green: actin, blue: nucleus. (f) Reconstructed cross-sectional images at the lines 1-4 in (e). Scale bars: 100 µm in (a), 50 µm in (b), (c) and (d), 20 µm in (e) and (f).
2-11 Microfluidic handling of cell fibres

As practical application including higher-order tissue construction and medical transplantation, cell fibres must be handled, arranged, assembled and implanted while retaining their shape and cellular functions. We thus developed a method for handling cell fibres using fluid flow and capillaries named “microfluidic handling” (Fig 4 (a) and Supplementary Fig. S13 (a)). Cell fibres floating in culture medium can be sucked into a capillary (inner diameter: 0.5 mm) without becoming entangled (Supplementary Fig. S13 (b-e)) and can also serve as a bridge between two capillaries in medium (Fig 4 (a)). Importantly, once a cell fibre enters the capillary to ~30 cm, the cell fibre is held by fluid resistance, $F_{\text{resistance}}$, that is larger than the breaking point of the cell fibres, ~1.8 µN (Supplementary Fig. S14), meaning that a cell fibre can be fluidically “clamped” without any mechanical attachment (the measurement of the mechanical strength of the cell fibres is described in 2-12 Mechanical strength measurement of cell fibres section and the detailed clamping principle is described in 2-13 Theoretical calculation of “clamp” in microfluidic handling section).

If the medium is pushed out, the “clamped” cell fibre is ejected from the capillary, corresponding to the “release” of the cell fibre. Consequently, this microfluidic handling provides a procedure for handling a living cell fibre as “a piece of string.”

**Figure S13.** Microfluidic handling of cell fibres. (a) Conceptual illustration of the microfluidic handling. (b-c) A Ca-alginate microfibre (diameter: 200 µm), which was formed in the double coaxial laminar flow microfluidic device (Supplementary Fig. S1), was introduced into a silicon tube (inner diameter: 1 mm) by sucking the Ca-alginate fibre in a solution. The Ca-alginate microfibre was stored in the tube without breaking or entangling. (d-e) An introduced cell fibre in a silicone tube. The cell fibre was fluorescently labelled.
Mechanical strength measurement of cell fibres

Supplementary Figure S14 shows the setup for measuring mechanical strength of cell fibres, which is similar to the setup reported previously\textsuperscript{10,11}. A cell fibre (~5 cm in length) was fixed to two glass tubes with glue (Cyanoacrylate-based adhesive, Aronalpha, TOAGOUSEI, Japan). One of the glass tubes was a sensing glass tube (quarts, inner diameter: 0.1 mm, outer diameter: 0.12 mm, length: 70 mm, Quarakapillaren, Glas Müller, Germany) and the other was a manipulating glass tube (inner diameter: 0.6 mm, outer diameter: 1.0 mm, G-1, Narishige). These two glass tubes were attached in parallel to manipulators, and immersed in a culture medium in a bath so that we can stretch the fixed cell fibre in the medium. The fixed cell fibre was pulled by moving the manipulating glass tube that was controlled with XYZ precision stage (Sigma-Koki, Japan) (Supplementary Fig. S14 (a-b)).

The force applying to the cell fibre, $F$, was expressed as $F = k \Delta x$, where $k$ is the spring constant.

**Figure S14.** Mechanical strength measurement of cell fibres. (a) Principle of our measurement. The applied force to the fibre, $F$, was calculated as, $F = k \Delta x$. The measurement was conducted in a buffer solution (or culture medium). (b) Image of the experiment. A cell fibre was fixed to two glass tubes, sensing glass tube and moving glass tube, with glue. (c) Typical measurement data of stress-strain graph (picking up three typical data in the condition). A breaking point of each measurement (NIH/3T3-Fib fibre) is indicated with an arrow. (d) Comparison of the mechanical strength (breaking point) of cell fibres between with and without Ca-alginate shell. Results are shown as the mean ± s.e.m. for three independent fibres. Scale bar: 1mm.
of the sensing glass tube, and $\Delta x$ is the deformation of the sensing glass tube. The $\Delta x$ is obtained by the image of the microscope (VHX-100, KEYENCE, Japan). The spring constant of the sensing glass tube, $k$, is theoretically calculated to be 4.3 nN/$\mu$m. The change in the length of the cell fibre, $\Delta L$, was calculated by $\Delta L = l - \Delta x$, where the $l$ is the traveling distance of the manipulating glass tube.

We measured four different types of cell fibres: (1) HeLa-PCol fibre (day 7) with the alginate shell, (2) NIH/3T3-Fib fibre (day 7) with the alginate shell, (3) HeLa-PCol fibre (day 3) without the alginate shell, and (4) NIH/3T3-Fib fibre (day 5) without the alginate shell. Typical stress-strain curves were shown in Supplementary Fig. S14 (c). For each type of a cell fibre, the measurement was conducted for 3-5 times ($n = 3-5$). When we need to remove the alginate shell, alginate lyase was added to the culture medium in the bath (final concentration: 40 $\mu$g/mL) after fixing the cell fibre to the glass tubes.

The measurement result (Supplementary Fig. S14 (d)) shows that the mean of the breaking points for the HeLa (human epithelial carcinoma cell line)-PCol and the NIH/3T3-Fib fibres with alginate shells was ~1.8 $\mu$N, which was higher than that of the cell fibres without an alginate shell (<0.8 $\mu$N). This result indicates that the alginate shell dominates and increases the mechanical strength of the cell fibres.
2-13 Theoretical calculation of “clamp” in microfluidic handling

Principle of the “clamp” of a cell fibre using a thin capillary is based on fluidic resistance, $F_{\text{resistance}}$, occurring between the surface of the cell fibre and the inner wall of the silicone tube when the cell fibre is pulled out from the thin capillary at the velocity, $V$ (Supplementary Fig. S14). The behaviour of the fluid in the gap between the cell fibre and thin capillary is described by Navier-Stokes equation (cylindrical coordinate system, $z$-direction):

$$\rho \left( \frac{\partial v_z}{\partial t} + v_z \frac{\partial v_z}{\partial z} \right) = F_z - \frac{\partial p}{\partial z} + \mu \left( \frac{\partial^2 v_z}{\partial r^2} + \frac{1}{r} \frac{\partial v_z}{\partial r} + \frac{1}{r^2} \frac{\partial v_z}{\partial \theta} + \frac{\partial^2 v_z}{\partial z^2} \right),$$

(1)

where $\rho$ is the density of the fluid, $v_z$ is the velocity of the fluid in the $z$ direction, $F_z$ is the applied external force, $p$ is the pressure, and $\mu$ is the viscosity of the fluid. In the model shown in Supplementary Fig. S15, we hypothesized that the cell fibre moves to the $z$ direction along the centre of the thin capillary in static state, parallel flow, no external forces and no pressure differences. Thus, the equation (1) can be simplified to

$$\frac{d^2 v_z}{dr^2} + \frac{1}{r} \frac{dv_z}{dr} = 0.$$  

(2)

At the boundary conditions, $v_z(r_{\text{fibre}}) = V$ and $v_z(r_{\text{tube}}) = 0$, where $r_{\text{fibre}}$ and $r_{\text{tube}}$ are the radius of the cell fibre and the inner radius of the silicone tube, respectively, the equation (2) is solved as

$$v_z(r) = V \frac{\log r - \log r_{\text{tube}}}{\log r_{\text{fibre}} - \log r_{\text{tube}}}.  

(3)

Using equation (3), the fluidic resistance, $F_{\text{resistance}}$, is expressed as

$$F_{\text{resistance}} = 2\pi r_{\text{fibre}} l \tau = 2\pi r_{\text{fibre}} l \mu \left( \frac{dv_z}{dr} \right)_{r=r_{\text{fibre}}} = 2\pi r_{\text{fibre}} l \mu \left( \frac{r_{\text{fibre}}}{r_{\text{fibre}} (\log r_{\text{fibre}} - \log r_{\text{tube}})} \right) V,$$

(4)

where $l$ is the introduced length of the cell fibre, and $\tau$ is the shear stress on the surface of cell fibre.

The microfluidic “clamp” of a cell fibre works when the $F_{\text{resistance}}$ is larger than the applied

**Figure S15.** The model of the microfluidic “clamp” of a cell fibre using a thin capillary.
pull-out force, $F_{\text{pull}}$. The maximum value of the $F_{\text{pull}}$ must be lower than the breaking point of the cell fibres, $F_{\text{break}}$, otherwise the pull-out force will break the cell fibre. Thus, the relationship between the $F_{\text{pull}}$, $F_{\text{resistance}}$ and $F_{\text{break}}$ is written as follows:

- (The range of the $F_{\text{pull}}$) $0 < F_{\text{pull}} < F_{\text{break}}$, (5)
- (The clamp works) $F_{\text{pull}} < F_{\text{resistance}}$, (6)
- (The clamp does not work) $F_{\text{resistance}} < F_{\text{pull}}$. (7)

Consequently, when the $F_{\text{resistance}} > F_{\text{break}}$, the equation (6) is always satisfied, meaning that the cell fibre is “clamped”. The $F_{\text{resistance}}$ depends on the $V$ and $l$ (the equation (4)), indicating that the velocity of the fibre and the introduced length of the cell fibre determine whether the clamp works or not.

In the case of our experiment, we defined the typical value of the $V$ during the handling of cell fibres by estimating the speed for manual handling using a glass tube ($V = \sim 1\text{mm/s}$, data not shown). When the values of the $l$ are 10 cm and 30 cm, the calculated values of the $F_{\text{resistance}}$ are 1.4 $\mu$N and 4.2 $\mu$N, respectively, where $\mu$ is $8.9 \times 10^{-4}$ Pa (the value of water at room temperature), and $r_{\text{fibre}}$ and $r_{\text{tube}}$ are 100 $\mu$m and 250 $\mu$m, respectively. In our measurement, $F_{\text{break}}$ is measured to be $1.8 \pm 0.4$ $\mu$N (NIH/3T3 Fib fibre with the alginate shell, Supplementary Fig. S14 (d)). Thus, from the theoretical calculation, the clamp works at $l = 30$ cm (and does not work at $l = 10$ cm). This theoretical estimation reasonably matches the result of our experiments; we empirically introduced a cell fibre to 20-30 cm in a silicone tube for the successful clamp.
2-14  Braid assembled with cell fibres

As an example of higher-order cellular structure using cell fibres, we demonstrated three-stranded braid by using NIH/3T3-ACol fibres (Supplementary Fig. S16). The assembled braid was ~3 cm in length and its fabrication time was less than 15 min. In principle, because there are no limitations on the number or length of the cell fibres, not only simple braids but also more complex intertwining structures could be formed, such as wider ribbon-like bands, braided hollow cords or braided cylindrical cords.

Figure S16. Braid assembled with NIH/3T3-ACol fibres. (a) Image of three-stranded braid made of three NIH/3T3-ACol fibres. (b) Superimposed image of the braid. Each NIH/3T3 fibre was differentially stained with a different Cell Tracker. Scale bars: 5 mm in (a) and 100 μm in (b).
Design of microfluidic weaving machine

We conducted two different experiments for the demonstration of mechanical weaving of cell fibres using the setup described in Supplementary Fig. S17 (a-b). In the first experiment, we used alginate hydrogel fibres stained with blue inks for easy visualization (Supplementary Fig. S17 (c-d)). This blue-stained alginate fibres were made by the same method as our cell fibre, except that they do not have the core inside. In the second experiment, we used cell fibres as the material for the demonstration of the mechanical weaving to create centimetre-scale tissue constructs (Supplementary Fig. S17 (e-f)).
Images of woven cellular fabric

Two types of woven fabric were fabricated. Some were made of blue ink-stained Ca-alginate microfibres encapsulating fluorescent nanobeads (F-8815 (blue fluorescent, 1.0 mm), F8811 (yellow-green fluorescent, 0.2 mm) or F8810 (red fluorescent, 0.2 mm), Molecular Probes, Invitrogen, USA) (Supplementary Fig. S18 (a-c)) for easy observation, and others were made of cell fibres (Supplementary Fig. S18 (d-g)) for constructing higher-order macroscopic cellular constructs.

Figure S18. Woven cellular fabric. (a) A woven hydrogel fabric made of blue ink-stained Ca-alginate microfibers containing 1% fluorescent nanobeads. (b,c) Fluorescent images of the woven hydrogel fabric. (d) Centimetre-scale woven cellular fabric and (e) fluorescent image of the fabric (the same image as Fig. 4 (c)). (f,g) Fluorescent microscopic images of cellular fabric composed of HepG2-PCol, MIN6m9-PCol and HeLa-PCol fibres (f) (the same image as Fig. 3 (d)), and MS1-ACol, NIH/3T3-ACol and HepG2-ACol fibres (g). Scale bars: 1 mm in (a-c), (f) and (g), 5 mm in (e).
Folded 3D structures made of woven cellular fabric

Two types of folded 3D woven fabric were demonstrated by using the Ca-alginate hydrogel woven fabric (Supplementary Fig. S19 (b-h)) and the cellular fabric (Supplementary Fig. S19 (i-k)).

**Figure S19.** Folded 3D tissue constructs. (a) Fabrication process of the folded 3D structures using the woven hydrogel fabric or the cellular fabric. (b-f) Sequential images of the fabrication process. The woven Ca-alginate hydrogel fabric was put on a polymer sheet and fixed with agarose hydrogel. The hole at the centre was punched (b). The punched fabric was attached on a glass slide, and three glass tubes were then put on the fabric. The fabric was folded (c) and fixed with agarose hydrogel (d). All the polymer sheet and glass tubes were removed (e). The folded structure was trimmed with a razor blade to form a desired shape (f). (g,h) Top view (g) and front view (h) of the folded 3D structure observed by fluorescent microscopy. (i) The cellular fabric (Supplementary Fig. S18 (g)) fixed with agarose hydrogel on a polymer sheet. (j) Folded 3D tissue structure made of the cellular fabric. (k) Fluorescent image of the folded tissue structure. Scale bars: 1 cm in (b), (e), (f) and (i), 1 mm in (g), (h), (j) and (k).
Fabrication of reeled helical tubes

Double-striped and double-layered helical tube structures were fabricated by reeling two different cell fibres on glass rods (Fig. S20).

Figure S20. Reeled helical cellular tubes. (a) Fabrication process of the double-striped helical tube. (b) Fabrication process of the double-layered helical tubes. (c) An image of the reeled cell fibres with a glass tube. (d) A microscopic image of the double-striped helical tube made of HepG2-PCol and NIH/3T3-ACol fibres. (e) A microscopic image of the double-striped helical tube made of fluorescently labelled HepG2-PCol (red) and MIN6m9-PCol (green) fibres. (f) The double-striped helical tube released from the glass tube. (g) The inner layer (MS1-ACol fibre, stained with red fluorescence) and (h) outer layer (NIH/3T3-ACol fibre, stained with green fluorescence) of the double-layered helical tube. (i) The superimposed image of (g) and (h). (j) The double-layered helical tube construct released from the glass tube. Scale bars: 1 cm in (c), (f) and (j), 500 µm in (d), (e), (g), (h) and (i).
Co-culture of HepG2 and NIH/3T3 cell fibres in double-striped helical tube structures

We examined whether the assembled 3D structures maintained their cellular activity. As an indicator, we monitored the liver-specific function of HepG2 cells as triggered by NIH/3T3 cell\textsuperscript{12}. Double-striped helical tubes with HepG2-PCol and NIH/3T3-ACol fibres (Supplementary Fig. S19 (a)) were treated with alginate lyase to remove their alginate shells, resulting in a structure composed of cells and ECM proteins (Fig. 4 (i) and Supplementary Fig. S19 (b-c)). After 3 days of co-culture, the HepG2 and NIH/3T3 cells migrated and filled the gap between the cell fibres. Comparing the helical co-culture tube with a control helical mono-culture tube made of a single HepG2-AC fibre revealed that the HepG2 cells in the co-culture tube secreted approximately two times more albumin than those in the mono-culture tube at both day 1 and day 3 (Fig. 4 (j)). These results indicate that the cells are alive even after the physical manipulation, and that they can be activated via intercellular communication between heterogeneous cells in the assembled 3D construct.

Figure S21. A double-striped helical tube tissue construct for the co-culture of the HepG2-PCol and NIH/3T3-ACol fibres. (a) Reeled double-striped structure of the HepG2-PCol and NIH/3T3-ACol fibres on the glass tube. The entire structure was fixed with native collagen. (b) A microscopic image of the co-cultured helical tissue constructs after being released from the glass tube. (c) A magnified image of (b). Scale bars; 1 mm in (a) and (b), 500 \( \mu \text{m} \) in (c).
Effect of implanted MIN6m9-PCol fibres to control blood glucose concentration of diabetic mice

Using a microcatheter, MIN6m9-PCol fibres covered with alginate-agarose IPN hydrogel were implanted to the renal capsular space of diabetic mice to normalize the blood glucose concentration (Fig. S22). Nine days after the implantation, the implanted MIN6m9-PCol fibres were removed from the renal capsular space.

Figure S22. Implantation of pancreatic beta cell (MIN6m9) fibres into diabetic mice. (a) Optical image of MIN6m9-PCol fibres with an alginate-agarose IPN hydrogel shell at day 14. (b) Glucose-inducible insulin secretion by MIN6m9-PCol fibres on days 14 and 21 and by MIN6m9 cells cultured on a 2D plate on day 3. (c, d) Sequential images of the implantation of an 11.5 cm-long MIN6m9 fibre into the subrenal capsular space of a recipient mouse. The fibre was precisely folded in the capsular space. (e) Changes in the blood glucose concentration of mice implanted with 20 cm-long MIN6m9 fibres (solid lines; each symbol indicates an individual recipient) and two non-implanted diabetic mice (dashed lines). Nine days after implantation, the implanted fibres were removed from the subrenal capsular space. Scale bars: 100 µm in (a) and 2 mm in (c) and (d).
Histological analyses on host response to implanted hydrogel blocks and cell fibres

To examine host response to the implanted cell fibres, we conducted histological analyses using (i) alginate-agarose IPN hydrogel blocks that are made of the same material as the shell of cell fibres (Supplementary Fig. S23 (a-f)), and (ii) MIN6m9-PCol fibres (Supplementary Fig. S23 (g-j)) 3 hours after (day 0) and seven days after implantation (day 7).

Kidneys bearing implanted IPN hydrogel blocks were resected at day 0 (Supplementary Fig. S23 (a)) and day 7 (Supplementary Fig. S23 (b)) and sectioned for staining with haematoxylin and eosin (Supplementary Fig. S23 (c) and (d), respectively). These sections showed that host cells infiltrated between the hydrogel block and the renal capsule after the implantation (Supplementary Fig. S23 (d)). Fibroblast specific protein-1/S100A4 staining confirmed that the majority of the infiltrating cells were fibroblasts (Supplementary Fig. S23 (f)), indicating that fibrotic reaction occurred by the implantation as a host response. Similar to observations using the IPN hydrogel blocks, haematoxylin and eosin staining for the sections of kidneys and implanted MIN6m9-PCol fibres (Supplementary Fig. S23 (i-j)) showed that cells containing fibroblast as the major fraction infiltrated to the implanted MIN6m9 cell fibre (Supplementary Fig. S23 (j)). This result suggests that fibrotic response also occurred after the implantation of MIN6m9-PCol fibre as well as the IPN hydrogel block.

Experimental procedure:

Preparation of IPN hydrogel blocks. The pre-gel solution containing 1.5% Na-alginate (80–120 cP, Wako Pure Chemical Industries) and 2.5% agarose (Low-melting agarose, Type IX-A, A2576, Sigma-Aldrich) in saline was immersed in a solution of 100 mM CaCl₂ and 3% sucrose at 4°C for gelation of alginate-ararose IPN hydrogel. Then, the gelated IPN hydrogel was immersed in saline containing 2 mM CaCl₂ for overnight. The IPN hydrogel was cut into 1.5 mm × 1.5 mm × 0.3 mm using a scalpel.

Implantation of IPN hydrogel blocks. IPN hydrogel blocks were implanted to the subrenal capsular space of a kidney of mouse (BALB/c Slc- nu/nu mouse). The subrenal capsular space was filled with 0.5% v/v hyaluronic acid in serum-free culture medium in advance for lubrication. The IPN hydrogel block was inserted to the subrenal capsular space using tweezers.

Preparation and implantation of MIN6m9-PCol cell fibres. Preparation and implantation of MIN6m9-PCol cell fibres were described in Formation of MIN6m9-PCol fibre for implantation section and Implantation of pancreatic islet cell fibres to diabetic mice section.

Histological stain. Mouse kidneys with implanted IPN hydrogel blocks or MIN6m9-PCol fibres were treated by perfusion fixation using 4% paraformaldehyde (PFA), followed by immersion in 4% PFA overnight. The fixed kidneys were dehydrated and embedded in paraffin by conventional procedure. Ten-micrometre-thick paraffin sections were stained with haematoxylin/eosin or anti-S100A4 antibody
(AP16925PU-N, Acris Antibodies, Inc., Germany) and haematoxylin. The prepared sections were observed with an upright microscope (BX51WI, Olympus).

Figure S23. Histological analyses on host response to implanted IPN hydrogel blocks and MIN6m9-PCol fibres. (a-b) Kidneys bearing implanted IPN hydrogel blocks (arrows) (a) 3 hours after implantation (day 0) and (b) 7 days after implantation (day 7). (c-d) Sections of kidneys and implanted IPN hydrogel blocks (arrows) stained with haematoxylin and eosin at (c) day 0 and (d) day 7. (e-f) Sections of kidneys and implanted IPN hydrogel blocks stained with S100A4 (fibroblast marker) and haematoxylin (brown: S100A4 positive, blue: nuclei). (e) and (f) are the higher magnification of the areas framed in (c) and (d), respectively. (g-h) Kidneys bearing implanted MIN6m9-PCol fibres at (g) day 0 and (h) day 7. (i-j) Sections of kidneys bearing implanted MIN6m9-PCol fibres stained with haematoxylin and eosin at (i) day 0 and (j) day 7. Scale bars: 200 µm in (c-d) and (i-j) and 100 µm in (e-f).
FT-IR and laser Raman scattering analyses on implanted IPN hydrogel blocks

Using FT-IR and laser Raman scattering spectroscopies, we examined how the shell of the cell fibres were affected by implantation to the subrenal capsular space of mice. We used alginate-agarose IPN hydrogel blocks as described in Histological analyses on kidneys with implanted hydrogel blocks or cell fibres section. As reference hydrogel blocks, we prepared 1.5% alginate hydrogel block and 2.5% agarose hydrogel block. The details of the preparation are described in Experimental procedure of this section.

Figure S24. Analyses of hydrogel blocks using FT-IR spectroscopy and laser Raman scattering spectroscopy. (a) FT-IR spectra of a 1.5% Ca-alginate hydrogel block (blue), a 2.5% agarose hydrogel block (green), an alginate-agarose IPN hydrogel block (1.5% Ca-alginate and 2.5% agarose) before implantation (red) and an alginate-agarose IPN hydrogel block after implantation for 7 days (black). (b) Laser Raman scattering spectra of 1.5% Ca-alginate hydrogel block (blue), a 2.5% agarose hydrogel block containing Ca²⁺ ions (green; upper), a 2.5% agarose hydrogel block without Ca²⁺ ions (green; lower) and an implanted alginate-agarose IPN hydrogel block (1.5% Ca-alginate and 2.5% agarose) after implantation for 7 days (black).
FT-IR spectra (Supplementary Fig. S24 (a)) showed that the major peak at 1599 cm\(^{-1}\) shown on the spectra of Ca-alginate and IPN hydrogel blocks disappeared on the implanted IPN hydrogel block, and split peaks were observed on the spectrum of the implanted IPN hydrogel block at 1532 cm\(^{-1}\) and 1644 cm\(^{-1}\). The peak spectrum of carboxylate in Ca-alginate would appear around 1600 cm\(^{-1}\) \(^ {13,14}\). Thus, the peak shift of carboxyl group at 1599 cm\(^{-1}\) peak on the implanted IPN hydrogel indicated that the cross-link between alginate molecules and Ca\(^{2+}\) ions in the IPN hydrogel would decrease after the implantation.

Laser Raman scattering spectra of the hydrogel blocks gave similar results with FT-IR spectra analysis (Supplementary Fig. S24 (b)). The Raman spectrum of the control calcium-alginate IPN hydrogel contained peaks assigned to carboxyl groups carboxyl groups in Ca-alginate (1633 cm\(^{-1}\))\(^ {14,15}\). The peak of the carboxyl group band disappeared in agarose gels without calcium. However, the several peaks around 1600 cm\(^{-1}\) on the implanted IPN hydrogel block showed the existence of peptide bonds, indicating adsorption of proteins to the implanted IPN hydrogel blocks. Taken together, the results of FT-IR and Raman scattering spectroscopic analyses suggest that the interaction of alginate molecules and calcium ions decreased in the implanted IPN hydrogels, whereas the adsorption of proteins over the IPN hydrogel blocks increased.

Furthermore, the laser Raman scattering analysis also indicated the stability of nanostructures created by the network of agarose molecules, by following the approach of Ratajska-Gadomska and Gadomski\(^ {16}\) (Supplementary Fig. S25). Using the IPN hydrogel blocks before and after the implantation,
we fitted these spectra to five Gaussian peaks around 3050 cm\(^{-1}\), 3200 cm\(^{-1}\), 3400 cm\(^{-1}\), 3500 cm\(^{-1}\) and 3600 cm\(^{-1}\). The relative ratio of the area of 3600 cm\(^{-1}\) peak to the whole spectra is inversely proportional to agarose concentration\(^{16}\). In both IPN hydrogel blocks, the averaged values of relative ratio of the 3600 cm\(^{-1}\) peaks were almost the same (0.141 for the IPN hydrogel block before implantation (\(n=3\)), 0.146 for the IPN hydrogel block after implantation (\(n=3\), \(p>0.05\) (U-test)). The result indicates that the nanostructures of agarose molecules in the IPN hydrogel are preserved after the implantation.

Thus, all the results indicate that the alginate-agarose IPN hydrogel changes after the implantation as follows:

(i) The binding between carboxyl-groups of the alginates and Ca\(^{2+}\) ions changed after the implantation. This result indicates that a portion of the Ca-alginate network might dissociate after the implantation.

(ii) The structure of agarose in the IPN hydrogel remained after the implantation. This result indicates that the shell of the implanted cell fibre is stable to encapsulate the cells after the implantation.

(iii) The detection of peptide binding suggests that proteins and/or cells adhered to the IPN hydrogel after the implantation.

**Experimental procedure:**

**Preparation of hydrogel blocks.** The preparation processes of the alginate-agarose IPN hydrogel blocks before and after implantation were described in \(2-21\) *Histological analyses on kidneys with implanted hydrogel blocks or cell fibres* section. In addition to the IPN hydrogel blocks, we prepared three types of hydrogel blocks, (i) 1.5% Ca-alginate hydrogel block, (ii) 2.5% agarose hydrogel block, and (iii) 2.5% agarose hydrogel blocks with Ca\(^{2+}\) ions. For 1.5% Ca-alginate block, the pre-gel solution containing 1.5% Na-alginate (80-120 cP, Wako Pure Chemical Industries) was immersed in a solution of 100 mM CaCl\(_2\) and 3% sucrose and then immersed in saline containing 2 mM CaCl\(_2\) overnight. For the agarose blocks, 2.5% agarose (Low-melting agarose, Type IX-A, A2576, Sigma-Aldrich) in saline was cooled at 4°C for the gelation, and then immersed in saline and saline containing 2 mM CaCl\(_2\) to obtain 2.5% agarose hydrogel blocks and 2.5% agarose hydrogel blocks with Ca\(^{2+}\) ions, respectively.

**Analysis by Fourier transform infrared (FT-IR) spectroscopy.** The hydrogel blocks were analysed by FT-IR spectrometer (FT/IR-4100, JASCO) with KBr pellet method. Briefly, each hydrogel block was
embedded into potassium bromide (KBr) plates and tableted with hand presser after being lyophilized for 1 day.

**Laser Raman spectroscopy measurements.** Laser Raman spectroscopy (NRS-5100, JASCO) was used to obtain the Raman Scattering spectra of hydrogel blocks. Green laser (532 nm) was used as the Strokes beam. Spectral data were processed by Igor Pro 6.0 (WaveMetrics).
Mechanical stiffness measurement using AFM on implanted IPN hydrogel blocks

To evaluate the change in the implanted IPN hydrogel blocks before (day 0) and after (day 7) the implantation, we measured Young’s modulus of these IPN hydrogel blocks using AFM equipped with bead-attached cantilevers. The IPN hydrogel blocks were prepared as described in 2-21 Histological analyses on kidneys with implanted hydrogel blocks or cell fibres section. Young’s moduli of the IPN hydrogel blocks before and after the implantation were 12.8 ± 8.39 kPa and 82.6 ± 44.3 kPa (mean ± s.d.), respectively. The increase of the mechanical stiffness of the IPN hydrogel block after the implantation may be caused by the fibrotic reaction covering the hydrogel block.

**Experimental procedure:**

**Mechanical stiffness measurement using AFM.** The mechanical stiffness of the IPN hydrogel blocks was measured in DMEM solution with an atomic force microscope (AFM, NanoWizard 3, JPK Instruments, Berlin) on the top of an inverted microscope (IX71, Olympus, Tokyo) with quantitative imaging (QI) mode. The AFM cantilever tips were constructed by gluing glass beads (diameter: 25 µm, VitraBio, Steinach) onto the ends of tipless silicon cantilevers (TL-CONT, NanoSensors, Neuchatel) with a spring constant of 0.3 N/m. 4096 force curves were obtained in 64 µm x 64 µm area on the samples.
2-24 Relationship between the length of implanted MIN6m9-PCol fibres and the blood glucose concentration of diabetic mice

We implanted various lengths (5, 10, 15, 20 and 25 cm) of the MIN6m9-PCol fibres to diabetic mice to examine the relationship between the length of the implanted fibres (corresponding to the number of the MIN6m9 cells in the fibres; 0.8, 1.6, 2.4, 3.2 and $4.0 \times 10^5$ cells) and the degree of lowering of the blood glucose concentration in the diabetic mice. Supplementary Fig. S27 (a) and (b) show the implanted MIN6m9 fibres of 5cm and 15 cm, respectively. By increasing the length of the implanted fibres, the degree of lowering of blood glucose concentration in the diabetic mice became larger, when evaluated 2 days after the implantation (Supplementary Fig. S27 (c)). After the implanted fibres were removed, the blood glucose concentration in all treated mice rose and reached the level of hyperglycaemia. These results indicate that the MIN6m9 fibres maintained their function even after the implantation procedures and the amount of the secreted insulin is directly proportional to the length of the fibres, namely the cell number of MIN6m9 cells in the fibres.

Figure S27. Implantation of MIN6m9-PCol fibres to diabetic mice. (a-b) Images of implanted MIN6m9-PCol fibre to a subrenal capsular space of diabetic mouse. The length of the fibre was 5 cm and 15 cm in (a) and (b), respectively. (c) Blood glucose concentration of recipient mice before implantation of MIN6m9-PCol fibres (left bars), 2 days after the implantation (centre bars), and after removal of the fibres (right bars). The length of the implanted MIN6m9 fibres varied from 5 cm (~0.08 x 10^6 cells) to 25 cm (~0.40 x 10^6 cells). The results are shown as the mean ± s.d.; six mice were used as recipients in each group. (d) Blood glucose concentration of recipient mice before (left) and 2 days after (centre) implantation of dispersed MIN6m9 cells. The results are shown as the mean ± s.d.; three mice were used as recipients in each group.
To examine whether the MIN6m9 cells in cell fibres reconstitute their intrinsic function as in tissue, we compared the effectiveness in lowering of the blood glucose concentration between the MIN6m9 cells in cell fibres (Supplementary Fig. S27 (c)) and dispersed MIN6m9 cells (Supplementary Fig. S27 (d)). Even when we implanted $1.0 \times 10^6$ dispersed MIN6m9 cells, the degree of the lowering of the blood glucose concentration was smaller than that of the 25-cm long MIN6m9 cell fibre that had less than half of the number of MIN6m9 cells (i.e. $\sim 0.4 \times 10^6$ cells) within it. These results suggest that the cells in the cell fibres are more effective in treating diabetes mellitus than dispersed cells, and this superiority could be ascribed to a more suitable microenvironment for cells in cell fibre than that for dispersed cells after implantation. The cells in cell fibres connect with each other (Supplementary Fig. S9) and are ready to function at the implantation, while the dispersed cells need to adhere to get ready to function after transplantation. During that post-transplantation period, some dispersed cells might die.
Implantation of dispersed primary islet cells to diabetic mice

Dispersed primary islet cells (0.6 x 10^6 cells) were introduced in a microcatheter and injected to subrenal capsular space of diabetic mice (Fig. S28).

Figure S28. Implantation of dispersed primary rat islet cells to kidneys of diabetic mice. (a) The dispersed primary islet cells (0.6 x 10^6 cells) were implanted to the subrenal capsular space using a microcatheter. (b) The implanted primary islet cells.
2-26 Removal of implanted pancreatic islet cell fibres

We removed implanted primary islet cell fibres and MIN6m9-PCol fibres from the subrenal capsular space 15 days and 9 days after implantation, respectively. Supplementary Figure S29 shows the representative images of the kidneys at different stages: immediately after transplantation (Supplementary Fig. S29 (a)), 9 days after transplantation (Supplementary Fig. S29 (b)) and immediately after removal of the fibres (Supplementary Fig. S29 (c)).

**Figure S29.** Representative photographic images of the removal of implanted pancreatic islet cell fibres. (a) Images of a 20-cm long primary islet cell fibre (left) and a 10-cm long MIN6m9-PCol fibre (right) immediately after the implantation. (b) Images of an implanted primary islet cell fibre (left) and a MIN6m9-PCol fibre (right) 15 days and 9 days after implantation, respectively. (c) Images of a kidney just after removal of a primary islet cell fibre and a MIN6m9 fibre.
3. Supplementary references


