with embedded Na_2HPO_4 particles (diameter of less than 25 μm) at a concentration of 30% (w/w) was poured onto the holes of the mold, and it was heat-cured at 85°C for 10 min. Thereafter, pure PDMS prepolymer without containing the phosphate particles was poured onto the entire region of the mold, and heat-cured at 85°C for an additional 30 min. The cured PDMS was peeled off from the mold and a PDMS plate having PP-PDMS microposts was obtained. The plate was bonded against a flat glass slide via O_2 plasma treatment, to obtain the microchannel having PP-PDMS microposts.

To produce the entirely PP-PDMS channels, we used a PDMS mold with a convex structure for the microchannel. PP-PDMS prepolymer was poured onto the channel region of the mold, and it was cured at 85 °C for 10 min. Thereafter, pure PDMS prepolymer was poured onto the entire mold and cured for an additional 30 min. The obtained PDMS plate was bonded against flat PP-PDMS plate to prepare a microchannel, of which the inner surface was entirely made of PP-PDMS.

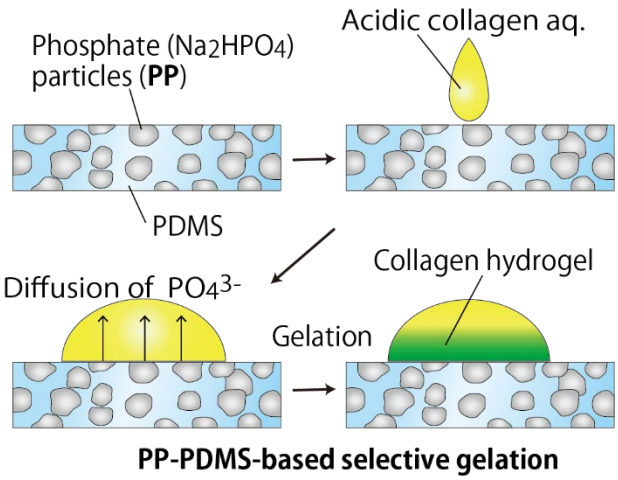

Figure 1. A schematic image showing the gelation process of an acidic collagen solution on the phosphate particle-embedding PDMS (PP-PDMS) substrate

2.2 Preparation of collagen hydrogels in microchannels

Figure 2 shows the preparation process of the hydrogel patterns in flat channels with PP-PDMS microposts. First, 0.5% acidic type I collagen solution was introduced into the channel. When the channel was completely filled with the collagen solution, the flow was stopped and it was incubated for several minutes. An aqueous solution of 10% dextran as the washing buffer was then introduced into the channel to remove the non-gelled collagen solution. For creating collagen hydrogel tubes in the entirely PP-PDMS channels, the collagen solution was kept flowing for several minutes, and then the non-gelled solution was removed similarly. To observe the formation behaviors of the collagen hydrogel layer in the channels, green fluorescent microparticles (Φ of 1–3 μm) were introduced into the collagen solution in advance.

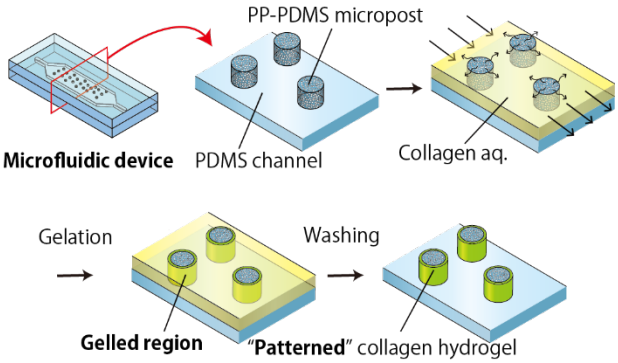

Figure 2. Fabrication process of the PP-PDMS micropost-incorporating microfluidic channels and the collagen microgels around the posts of the PP-PDMS

2.3 Cell culture experiments

HepG2 cells (human hepatoma cell line) were suspended in the collagen solution, and then it was introduced into the microchannel with PP-PDMS microposts at a flow rate of ~100 μL/min. The non-gelled collagen solution was washed away with 10% dextran solution. After forming the hydrogel patterns with encapsulated cells, the cell culture medium was introduced using a syringe pump at a feed speed of 10 μL/min. Perfusion culture was performed for up to 7 days at 37 °C in a humidified CO_2 incubator.

In addition, the entirely PP-PDMS channels were used for creating tubular collagen hydrogels, containing smooth muscle cells (SMCs), to produce blood vessel-like tissue models. SMCs were suspended in a collagen solution by the same procedure as described above, and the cell-containing collagen hydrogel tube was fabricated in the flow channel. Endothelial cells (ECs) were then introduced into the lumen. Perfusion culture was performed for up to 5 days in a humidified CO_2 incubator.

3 Results and Discussion

3.1 Observation of gelation on the PP-PDMS plate

First, we investigated if the collagen hydrogels were actually formed on the surface of the PP-PDMS because of the interaction with the embedded phosphate particles. Figure 3 shows the collagen hydrogel formed on the PP-PDMS plate. We confirmed that the gelation of the collagen solution took place on the PP-PDMS surface because the acidic collagen solution was neutralized by the embedded PP. We also confirmed that the thickness of the collagen hydrogel could be changed by adjusting the phosphate concentration and/or the incubation time period; the higher concentration of PP and the longer incubation time resulted in the formation of a thicker hydrogel layer. At high PP concentration conditions, the amount of PP present on the PDMS surface was increased, which was attributed to the increased hydrogel thickness. SEM observation revealed that the density of the microholes, which were formed by the dissolution of PP,

increased after the hydrogel formation with the increase in the PP concentration. In the following experiments we fixed the PP concentration in the PDMS prepolymer at 30% (w/w).

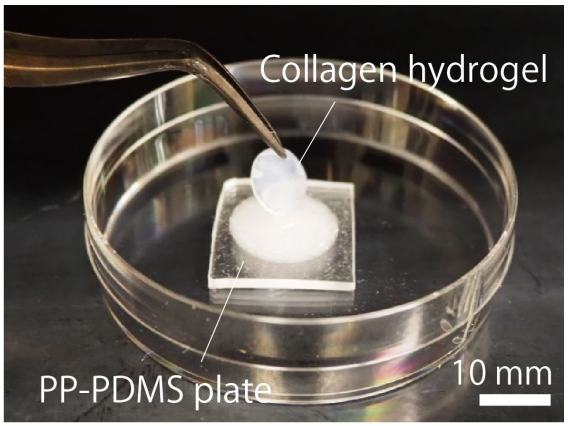

Figure 3. Photograph of the collagen hydrogel formed on a flat PP-PDMS plate.

3.2 Preparation of PP-PDMS microchannels

Next we prepared microchannel structures partially composed of PP-PDMS. Figure 4 shows several types of microchannels. We successfully prepared microchannels having PP-PDMS microposts with various shapes, and channels whose lumen was entirely composed of the PP-PDMS matrix. For the PP-PDMS microposts, phosphate particles with a size less than 25 µm were used so that these particles could be easily and uniformly incorporated into the microposts.

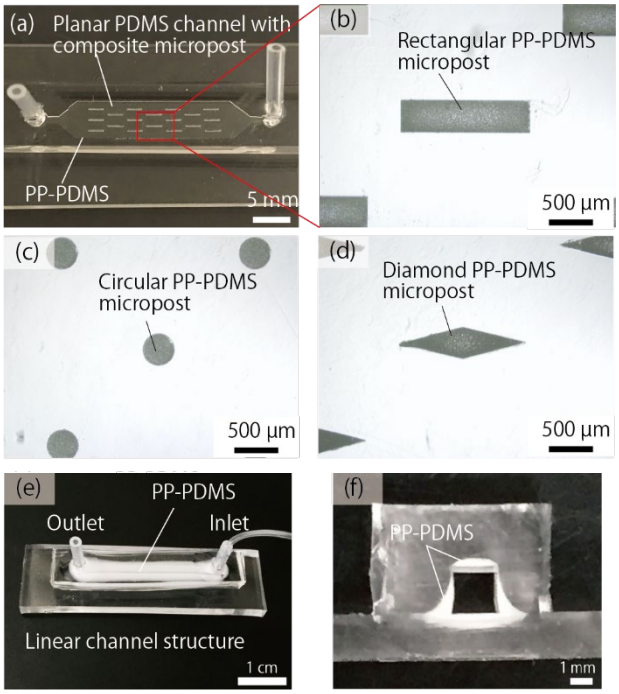

Figure 4. (a-d) Microchannels with PP-PDMS microposts with various shapes; (e, f) Microchannel entirely composed of PP-PDMS for creating tubular collagen hydrogels

3.3 Formation of collagen hydrogels in microchannels

We examined if micrometer-sized collagen structures were actually formed in the microchannels. Figure 5 shows the formation process of the collagen hydrogel around the PP-PDMS microposts. The hydrogels were started to form after ~1 min of introduction, when the flow of the collagen solution was stopped. The acidic collagen solution, introduced into the channel, was transformed into the hydrogel, selectively near the PP-PDMS region. After 2 min, a hydrogel layer with a thickness of ~500 µm was formed.

Next the entirely PP-PDMS channels were tested to form collagen hydrogel tubes on the lumen of the channels composed of PP-PDMS (Figure 6). After introducing a washing buffer, tubular collagen hydrogels were formed. The thickness of the hydrogel layer could be controlled by changing the input flow rate of the collagen solution and the gelation period (Figure 6 (c)). The higher flow rate resulted in the formation of the thinner hydrogel tube, because of the high shear force under the high flow-rate condition.

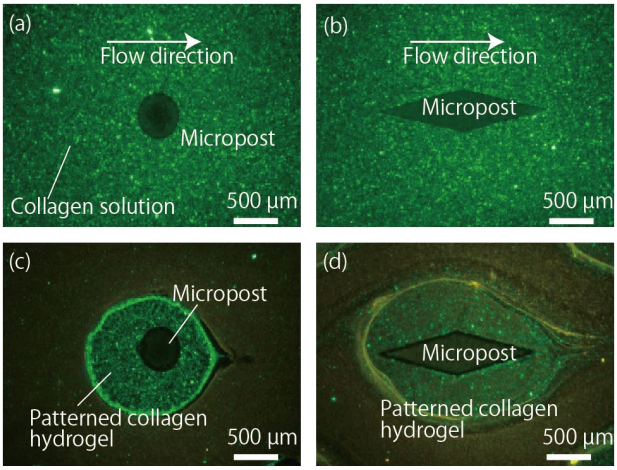

Figure 5. (a, b) Flow of the collagen solution with green fluorescent microparticles through the microchannel with the PP-PDMS microposts; (c, d) Formed collagen hydrogel structures around the circular and diamond microposts

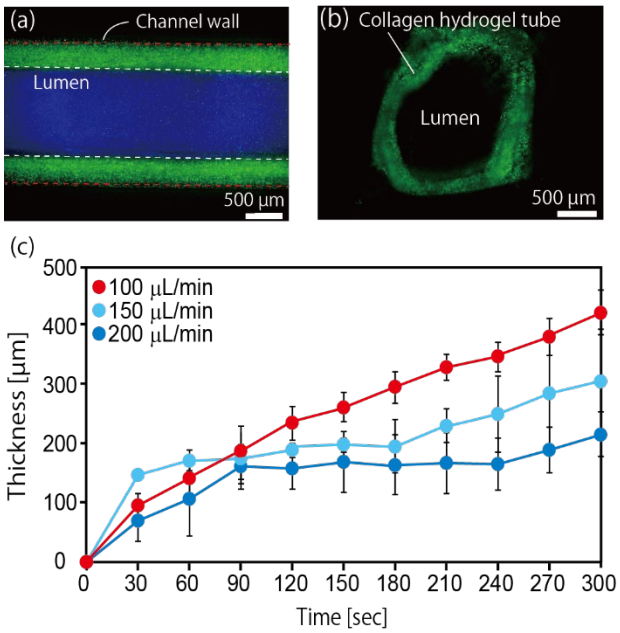

Figure 6. (a) Fluorescent micrograph showing the formed hydrogel layer (green) and the flow of the washing buffer (blue) in the entirely PP-PDMS microchannel; (b) Cross section of the formed hydrogel microtube; (c) Relation between the hydrogel thickness and the incubation time period, when the flow rate was changes as indicated

3.4 Cell encapsulation and cultivation in the hydrogel

Finally we attempted to encapsulate living mammalian cells in the hydrogel matrix to perform perfusion culture of the cells. Cells were suspended in the 0.5% acidic type-I collagen solution and introduced into the microchannel, to prepare cell-containing collagen hydrogel structures. In the channels with the PP-PDMS microposts, HepG2 cells were encapsulated and perfusion culture was performed for 7 days. During the perfusion culture, we confirmed that most of the cells were viable and proliferative (Figure 7 (a-d)).

For the tubular hydrogels, SMCs were introduced into the hydrogel matrix, whereas the ECs were seeded on the lumen, to form a multilayered vasculature tissue model. Figure 7 (e, f) shows the results of the Live/Dead assay of the cells at Days 3 and 5. We successfully cultured cells in the collagen hydrogel tubes while maintaining high cell viability. The presented approaches would be therefore useful for perfusion cell culture systems that can realize proper cell-matrix interactions.

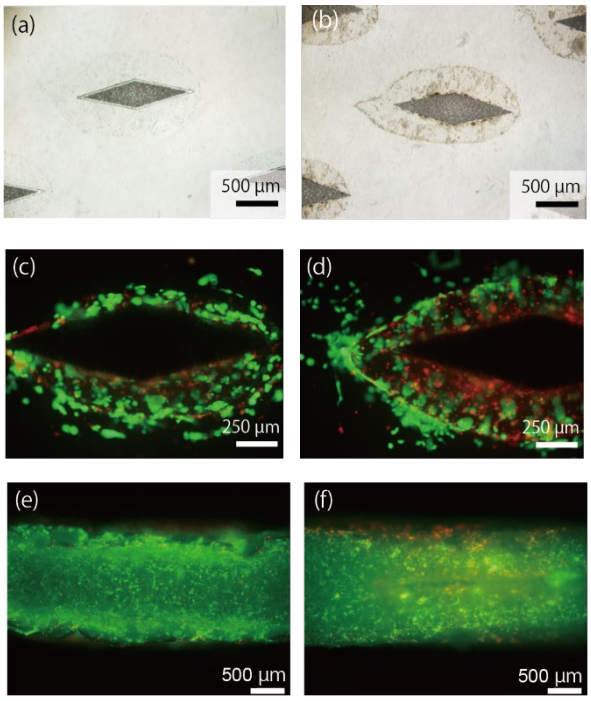

Figure 7. (a, b) Bright field micrographs showing the HepG2 cell-encapsulating collagen hydrogels at (a) Day 0 and (b) Day 7; (c, d) Live/Dead cell assay for the Hep G2 cells at (c) Day 3 and (d) Day 7; (e, f) Live/Dead cell assay for the cells in the collagen tubes at (e) Day 3 and (f) Day 5

4. Conclusions

We have proposed a new approach to precisely fabricating collagen microstructures in microfluidic devices, without necessitating complicated operations or devices. We successfully applied the formed collagen microstructures to the perfusion culture of mammalian cells, which can mimic the circulation environment in our body. The presented strategy would be useful as a variety of perfusion cell culture platforms, including organs-on-chip devices for drug development and basic biological studies.

Acknowledgements

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