MICROFLUIDIC SYNTHESIS OF MICROMETER-SIZE COLLAGEN HYDROGEL PARTICLES FOR CELL MANIPULATION APPLICATIONS

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ABSTRACT

In this paper, we present a microfluidic system to produce micrometer-size collagen hydrogel particles. Aqueous droplets containing collagen molecules were generated in the continuous phase of a polar organic solvent at a microchannel junction, and then, collagen molecules were concentrated by the dehydration of the droplets. The collagen molecules in the shrunk droplets were chemically cross-linked, forming collagen hydrogel particles with sizes of 10~20 μm. As a biological application of the obtained particles, heterogeneous spheroids made of cells and magnetic beads-incorporated collagen particles were formed by incubating them in non-adhesive hydrogel microwells, and the formed cell-particle complexes were manipulated and recovered by applying a magnetic field. The presented collagen particles would be useful as functional materials for cell manipulation and tissue engineering applications.

KEYWORDS

Collagen, Hydrogel microbeads, Spheroid, Polar organic solvent

INTRODUCTION

Collagen is a group of bioactive proteins forming hydrogel, and plays as one of the most essential ECMs [1]. Collagen hydrogels provide suitable 3D environments for functional cell culture platforms. Researchers have demonstrated cell culture applications utilizing collagen hydrogels, including tubular hydrogels for endothelial tubulogenesis [2] and spherical beads for 3D tissue construction [3]. Microfluidic technologies have been used to produce monodisperse hydrogel beads with diameters of > 50 μm. However, production of small collagen hydrogel particles with sizes similar to mammal cell sizes has not been reported yet, mainly because of its difficulty in forming small-size droplet of the sol solution, as well as the relatively-low gelation speed and insufficient physical stiffness. We have developed a microfluidic technique to produce micrometer-size hydrogel particles (alginate and chitosan) by using non-equilibrium droplets [4], with sizes of 10 μm or less. In this paper, we applied this technique to producing collagen hydrogel particles. In addition, collagen hydrogel particles incorporating magnetic beads were prepared for cell manipulation applications. Cells attached on the collagen particles and heterogeneous cell spheroid composed of cells and collagen particles were formed by culturing cells with the collagen particles. We demonstrated the manipulation of these cell-collagen particle complexes by applying magnetic fields.

EXPERIMENT

The procedure of preparing collagen hydrogel particles is shown in Fig. 1. Droplets of a diluted collagen solution are formed in a polar organic solvent. The formed W/O droplets are shrunk because of the dissolution of water into the continuous phase. The shrunk droplets are then chemically cross-linked by adding a cross-linking reagent in the downstream of the microchannel. In the experiment, PDMS microchannels were fabricated by standard soft lithography and replica molding (Fig. 2 (a)). The channel depth was uniform, 55 μm. The width of the droplet-generation channel (the first confluence) was 50 μm. The lengths of the water extraction channel and the cross-linking channel were 70 and 75 mm, respectively. Methyl acetate (solubility of water: 8%), collagen type I (rat tendon) dissolved in an acidic solution, and glutaraldehyde in PBS were introduced into the PDMS microchannel as continuous phase (Q_1), dispersed phase (Q_2), and cross-linking reagent (Q_3), respectively. The generated droplets and the synthesized particles were observed by using an optical microscope. To confirm if the obtained particles are composed of the native collagen molecules, particles were stained with Picrosirius Red. To control the morphologies of collagen particles, compositions of the collagen solution, flow rates, and the types of the cross-linking reagent were changed.

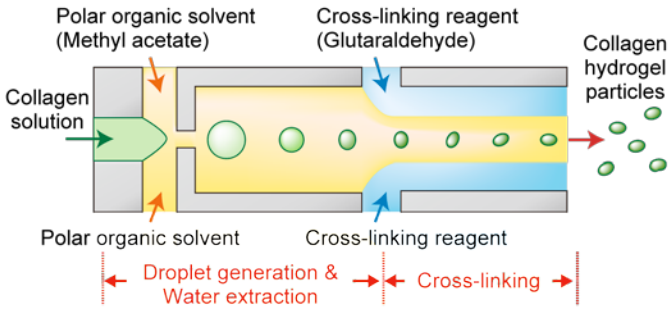

Figure 1: Schematic image showing the production process of micrometer-size collagen hydrogel particles. Droplets of a collagen sol solution are formed in a polar organic solvent (methyl acetate). Water in the droplet is dissolved into the continuous phase, and droplets are shrunk. Concentrated collagen droplets are cross-linked by glutaraldehyde, and hydrogel particles smaller than the initial droplets are obtained.

As an application for cell manipulation, magnetic collagen particles were prepared by suspending paramagnetic beads (Φ: 50 nm) in the collagen sol solution. The obtained magnetic collagen particles and NIH-3T3 or Hep G2

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cells were mixed and incubated in non-adhesive hydrogel microwells, and subsequently cultured for 3 days. The formed cell-collagen particle complexes were moved and recovered by applying a magnetic field.

RESULTS AND DISCUSSION

Fig. 2 (b, c) shows the generated droplets of the collagen sol solution (0.1%) flowing through the microchannel. The droplet volume was gradually decreased, and the final volume of the shrunk droplet was ~0.94% of the initial droplet at the second confluence. Methyl acetate and PBS with glutaraldehyde formed parallel laminar flow in the cross-linking channel, and the shrunk collagen droplets were cross-linked by glutaraldehyde. The obtained collagen hydrogel particles had disk-shape morphology, resembling erythrocytes (Fig. 2 (d, e)). The average diameter and thickness of the collagen particles were 17.0 and 8.0 µm, respectively. This disk-shape morphology would be caused by the localization of collagen molecules in the circumferential region of the droplet during the water dissolution, which was similar to the previous observation in producing toroidal polymer particles [5]. The particles stained by Picrosirius Red exhibited red color, indicating that the obtained particles were made of fibrous collagen, and this particle preparation process did not severely denature the collagen molecules (Fig. 2 (f)). In addition, morphologies of the collagen particles were controlled by changing the flow rates, the types of collagen and the cross-linking reagent (Fig. 2 (g,h)).

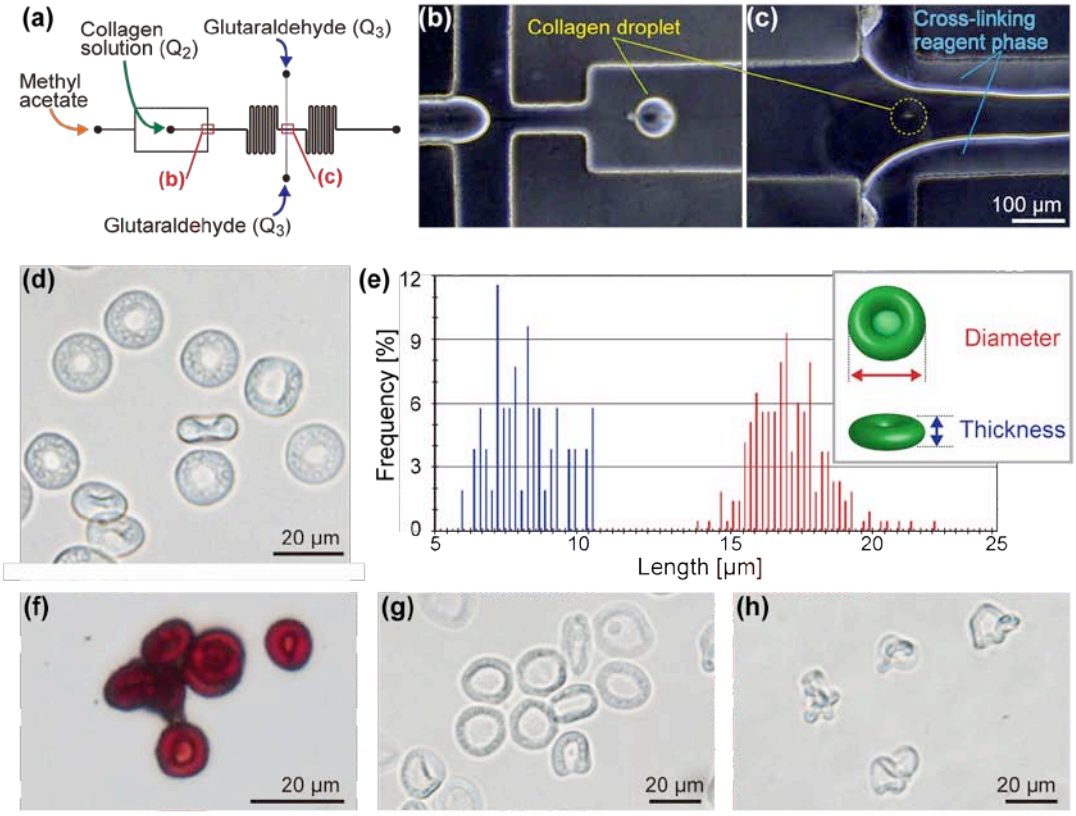

Figure 2:(a) Schematic diagram showing the microchannel design. (b, c) Micrographs of the droplets (b) generated at the confluence (Φ = 71.8 µm, CV = 3.6%), and (c) entering the second confluence after shrinkage. (c) Micrographs of the obtained collagen hydrogel particles having disk-shape morphology. (d) Size distribution of the obtained collagen particles. The average diameter and thickness were 17.0 and 8.0 µm with corresponding CV values of 7.3% and 14.6%, respectively. Methyl acetate, 0.1% collagen I in 0.02N acetic acid, and 2.5% glutaraldehyde in PBS were used as the continuous phase, dispersed phase, and the cross-linking reagent, respectively. Q_1, Q_2, and Q_3 were 30, 0.9, and 15 µL/min, respectively. (f) Collagen particles stained with Picrosirius Red. Red color indicated the presence of fibrous collagen. (g, h) Collagen hydrogel particles prepared under different conditions. Particles with (g) a larger concave area obtained by changing the flow rates and (h) a thinner and crumpled shape obtained by changing the composition of the collagen solution.

As an application for cell manipulation, FITC-labeled spherical collagen particles incorporating magnetic beads were produced (Fig. 3). After incubating NIH-3T3 cells with paramagnetic collagen particles for 12 h, cells were attached on the collagen particles, and it was possible to the move the cell-particle complexes and collected them by applying magnetic fields. As shown in Fig. 3 (e, f), heterogeneous spheroids composed of collagen particles and cells were formed by incubating Hep G2 cells with magnetic collagen particles in non-adhesive circular microchambers made of agarose hydrogel. These heterogeneous spheroids were also recovered by applying magnetic field. The presented micrometer-size collagen particles would be highly useful as building blocks for tissue engineering, model of erythrocytes, and drug-eluting carriers.

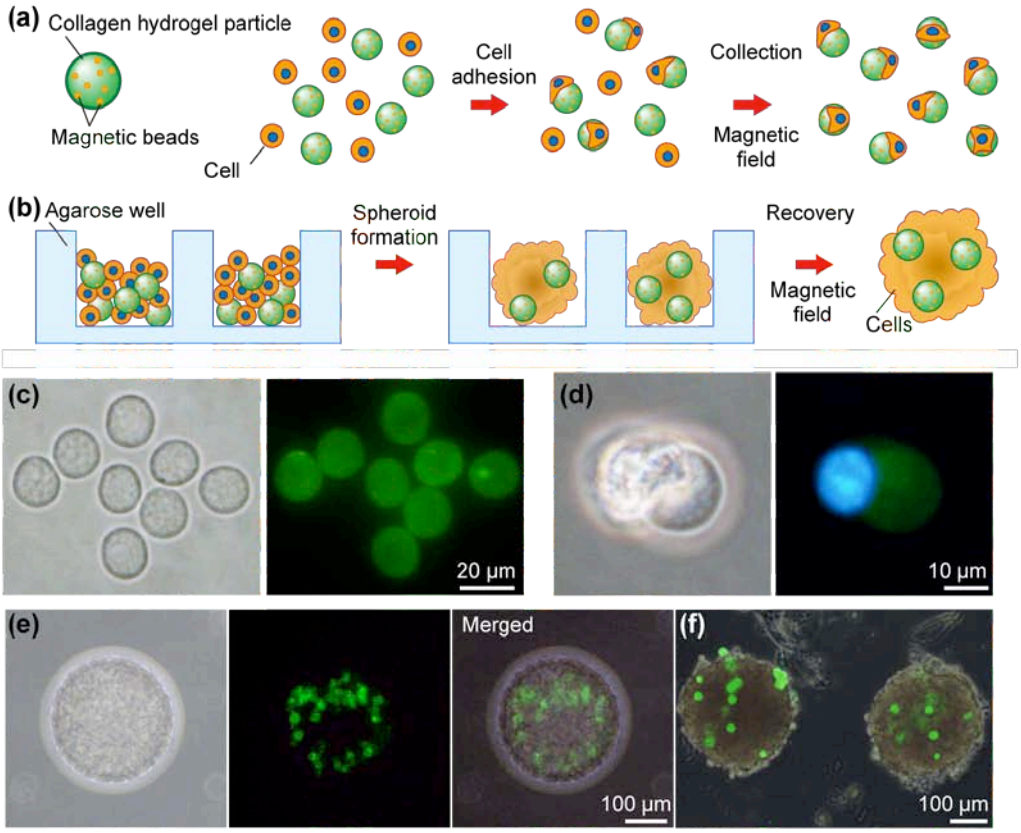

Figure 3: (a, b) Schematic images showing (a) cell adhesion and transportation, and (b) formation of heterogeneous spheroids. (c) Micrographs of magnetic collagen particles containing FITC-labeled collagen. (c) NIH-3T3 cells attached onto a collagen hydrogel particle. Cell nucleus was stained with Hoechst 33342 (blue). (d) Hep G2 cells and collagen particles cultured inside non-adhesive agarose hydrogel microwells at Day 1. (e) Recovered heterogeneous spheroids after 3 days of incubation.

CONCLUSIONS

We have presented a process to produce cell-size collagen microbeads utilizing the non-equilibrium microfluidics, which are difficult to obtain by using conventional techniques. As biological applications, manipulation of cells and cell spheroids were performed utilizing magnetic collagen particles. The presented micrometer-size collagen hydrogel beads would be highly useful as new types of cell handling tools and cell cultivation matrices for tissue engineering.

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