CONSTRUCTION OF VASCULAR TISSUES VIA MULTILAYER CELL DEPOSITION INSIDE HYDROGEL MICROCHANNELS

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ABSTRACT

This paper reports a new approach for constructing blood vessel-like tissues using microfluidic devices made of calcium-containing agarose hydrogel. Sodium alginate (NaA) solutions with different cell types at high densities were introduced stepwise into the hydrogel microchannel, to deposit cell-containing multiple Ca-alginate hydrogel layers on the channel surface with the help of Ca^{2+} ions diffused through the hydrogel matrix. We successfully obtained capillary-like tissues inside the hydrogel microchannel, which were then recovered from the hydrogel matrix by enzymatically digesting the agarose polymer. The presented technique would pave a new way for preparing capillary-like cell constructs for various tissue engineering applications.

KEYWORDS

Vascular tissue engineering, Microfluidic hydrogel device, Cell assembly, Multilayer deposition

INTRODUCTION

Vascular tissue engineering *in vitro* is an essential process for preparing artificial artery grafts, fabricating relatively large tissues incorporating capillary networks, and studying physiological mechanisms in blood vessel networks such as angiogenesis and thrombosis. Until now, researchers have proposed various methods for building vascular structures, including rolling of cell sheets [1], cell incorporation into fibrous hydrogels [2], and 3D cell deposition by inkjet printing [3]. However, mimicking functional vascular tissues having layered/branched structures is still a significant challenge, mainly because of the lack in proper technologies to assemble multiple cell types into hollow structures. In this study, we propose a simple but highly versatile technique for three-dimensional assembly of cells to construct capillary-like structures utilizing agarose hydrogel microdevices.

EXPERIMENTAL PROCEDURE

The preparation process of vascular tissue models is shown in Fig. 1. A sodium alginate (NaA) solution is introduced into the agarose microchannel containing Ca^{2+} ions. Ca^{2+} ions diffuse through the hydrogel matrix, forming a Ca-alginate hydrogel layer on the microchannel surface, depending on the microchannel geometry. By repeating this deposition process, layered alginate hydrogels incorporating different cell types at high densities are obtained. The formed tissues can be recovered from the hydrogel, by enzymatically digesting the entire hydrogel matrices using agarase.

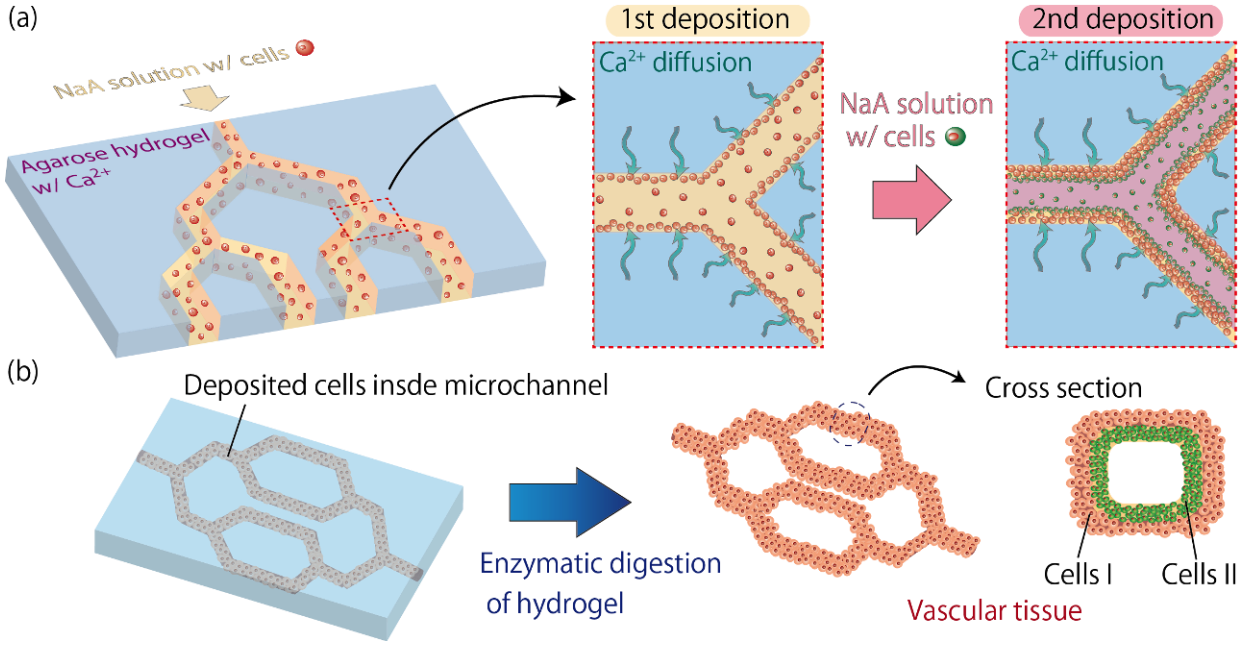

Figure 1: Schematic images showing the procedure of constructing capillary-like multilayer tissues using different cell types inside agarose hydrogel microchannels. (a) Multiple layers of alginate hydrogels incorporating different cell types are formed inside the microchannel with the help of the Ca^{2+} ions diffusing through the hydrogel matrix. (b) Formed tissues are recovered from the microdevice by enzymatically digesting agarose hydrogel matrices. NaA: sodium alginate.

MATERIALS AND METHODS

The microchannel design and photographs of the agarose hydrogel microdevice are shown in Fig. 2. Agarose hydrogel microdevices with Ca^{2+} ions were prepared by using a previously reported molding scheme [4]. An aqueous solution of agarose (5%) with 10 mM $CaCl_2$ was poured onto a silicon mold having SU-8 microstructures and cooled for 2 h to gel the agarose solution. After the complete gelation, the hydrogel plate was peeled off and trimmed. Then, microstructured and flat hydrogel plates were partially melted and bonded, forming microchannel structures. The depth and width of the microchannels were uniform and equal, 500 μm.

Before introducing the NaA solution, the microchannel surface was modified by introducing poly-L-lysine solution containing 10 mM $CaCl_2$ for at least 1 min to improve the attachment of alginate matrices. To control the gelation speed of alginate, trisodium citrate aqueous solution, a chelating agent, was introduced just before introducing the NaA solutions. Then 0.5% NaA solutions with different color fluorescent particles were introduced into the microchannel stepwise by aspiration. For preparing cell-incorporating hydrogel layers, agarose hydrogel microdevices were soaked into a cell culture medium overnight in advance to improve the supply of chemical components into the agarose matrices. Fibroblasts (NIH-3T3) and myoblasts (C2C12) were used, which were stained with red (PKH26) and green (PKH67) dyes, respectively. These cells were individually suspended in isotonic NaA solutions at high densities. The alginate solutions with stained cells were introduced into the microchannel stepwise and the behaviors of the cell deposition were observed under an optical microscope. After finishing the cell deposition, alginate gelation was completed by introducing 10 mM $CaCl_2$ solution.

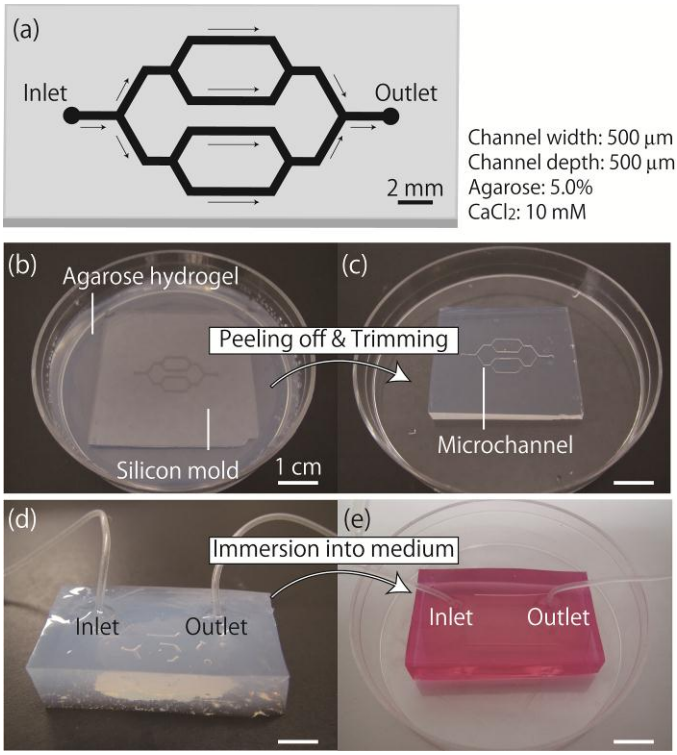

Figure 2: (a) A microchannel design of the hydrogel microfluidic device. (b-c) Photograps showing the preparation process of the hydrogel microdevice: (b) agarose solution with 10 mM $CaCl_2$ was poured onto a SU-8/silicon mold and gelled, (c) hydrogel plate was peeled off and trimmed, (d) microchannel structure was formed by bonding agarose plates by partial melting, with the attached inlet/outlet tubing, and (e) microdevice was dipped in an cell culture medium containing 10 mM $CaCl_2$ for 1 day.

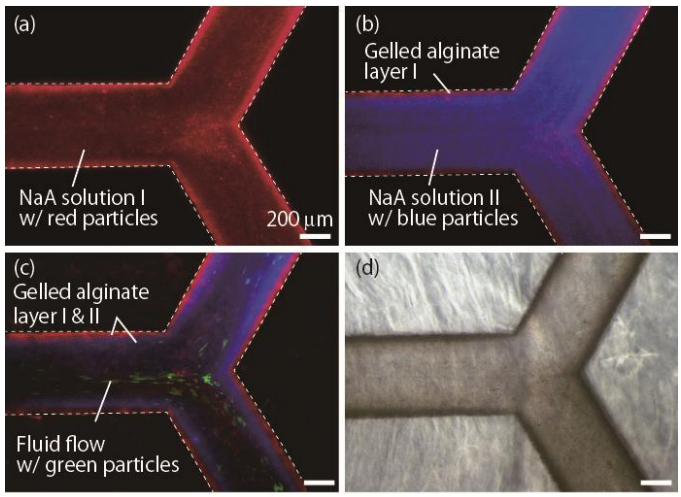

Fig. 3 Formation of multiple alginate hydrogel layers inside the microchannel, using NaA solution containing different color particles. (a,b) Fluorescence micrographs showing (a) formed Ca-alginate hydrogel layer with red fluorescent particles on the microchannel surface and (b) double-layer alginate hydrogels formed after repeating the deposition process. (c) Fluorescence micrograph showing green fluorescent particles flowing through the layered hydrogel structure. (d) Phase contrast image of the formed alginate hydrogel layers.

RESULTS AND DISCUSSION

We first demonstrated the stepwise deposition of multiple hydrogel layers inside the branched microchannel. When 0.1 M trisodium citrate solution was introduced for 1 min and the flow rate of NaA solution was 20 μL/min, alginate hydrogel layer with a thickness of 150 μm was formed on the microchannel surface after 3 min of flow introduction. After the introduction of the second NaA solution, double-layer alginate hydrogels were formed. We observed the flowing particles through the formed hydrogel, indicating the formation of hollow hydrogel structures corresponding to the microchannel geometry (Fig. 3). In addition, we confirmed that both the flow rate and the introduction period of the NaA solution were critical to control the thickness of the deposited layers (Fig. 4). When the flow late of the NaA solution was lower than 20 μL/min, alginate gelation rapidly proceeded after 2 min from the introduction, and the microchannel was completely filled with the formed hydrogel at ~ 7

min. On the other hand, when the flow rate of alginate solution was higher than 50 μL/min, alginate gelation was extremely slow mainly because of the removal of the formed alginate layer by the applied high shear stress on the microchannel surface.

Next, by introducing NaA solutions incorporating different cell types stepwise, we successfully constructed capillary-like tissues with double-layer hollow structures, through which fluorescent particles flowed (Fig. 5). The initial cell densities were 7.2×10^7 cells/mL (C2C12) and 4.2×10^7 cells/mL (NIH-3T3), respectively. The cells were stably trapped stepwise in the alginate hydrogel matrices on the microchannel surface. Finally, we tried to recover the formed vascular tissues inside the microchannel by enzymatically digesting the whole agarose hydrogel matrices using agarase. After soaking the agarose hydrogel microdevice in an agarase solution in PBS, the entire agarose matrices became fragile and were easily broken, and the formed tissue structures were successfully recovered from the microdevice. In addition, we were able to handle the formed tissues by using tweezers because of the high mechanical strength of the alginate hydrogel. These results show that it is possible to fabricate functional vascular tissue models and assemble cells into 3D structures by using the presented method.

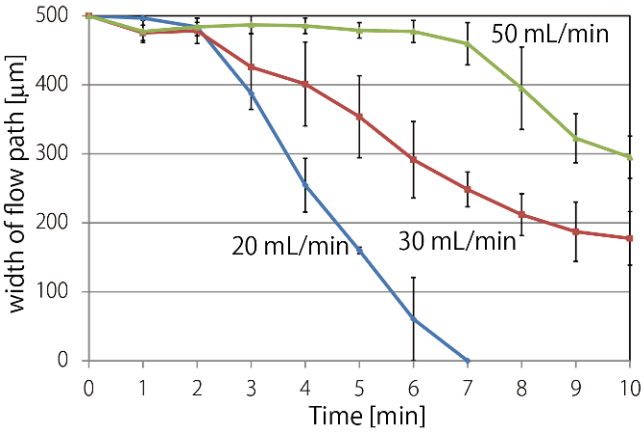

Fig. 4 Relation between the width of the flow path and the flow rate of the NaA solution. The x-axis indicates the time from the introduction of the 0.5% NaA solution.

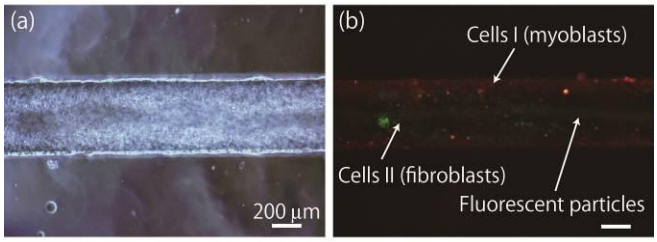

Fig. 5 (a) Phase contrast and (b) fluorescence micrographs showing the deposited cell layers inside the microchannel, through which green particles were flowing. Myoblasts and fibroblasts were stained with red (PKH26) and green (PKH67) fluorescent dyes, respectively.

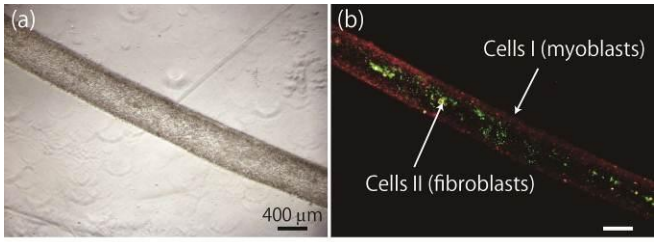

Fig. 6 (a) Phase contrast and (b) fluorescence images showing the recovered tissues by enzymatically digesting the agarose hydrogel matrices.

CONCLUSIONS

A new process has been developed for easily and accurately preparing vascular tissue models. It would be possible to prepare vascular tissue models with various configurations by changing the microchannel geometry, the flow rate and the introduction period of the NaA solution. We expect that the presented method would become a highly useful approach for vascular tissue engineering.

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