BIOFABRICATION OF LIVING VESSEL STRUCTURES INTEGRATED WITH FLUID PERFUSION

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ABSTRACT

This paper describes a biofabrication method of vessel-like structure with our gel fiber technology. Alginate was modified with gelatin to promote cell adhesion and proliferation, and treated with fibronectin (FN). Vascular endothelial cells (ECs) adhered and proliferated well on the modified alginate fiber. After embedding EC-coated fiber into extracellular matrix (ECM), ECs were transferred to ECM from the fiber, and then the fiber was easily removed with alginate lyase. Fluorescein-labeled materials were flushed into the lumen, and it was observed small-sized molecules were leaked out to ECM. This approach would be applicable for the rapid biofabrication of functional luminal tissues.

KEYWORDS: hydrogel fiber, endothelial cell, vessel structure, tissue engineering

INTRODUCTION

To fabricate large-scaled tissues by using cells in tissue engineering, biodegradable scaffolds are frequently used for implantation or cell-seeding materials. Recently, many modified biomaterials suitable for cell adhesion and proliferation appeared on the scene, and these biomaterials would be prepared for any shape of scaffold rather easily. Alginate is one of such biomaterials used for scaffold. Sodium alginate can form gels rapidly with Ca^{2+}, thus it is useful for biofabrication with microfluidic device. Also, we can fabricate cell fiber easily by using alginate [1].

Cells require nutrients and oxygen to live, however, enough nutrients and oxygen could not be supplied deep inside the tissues with large-scaled scaffold. To fabricate large tissues in vitro, it is quite important to incorporate the vessel network into fabricated tissues. In this point of view, we focused on using our gel fiber to fabricate luminal structures. Alginate can be easily removed with lyase, thus we could prepare vessel-like structure by degrading alginate fibers with lyase after cells adhered onto the surface of fibers.

Alginate gel has high bioaffinity to cells, however, cells cannot attach and grow onto the surface of alginate gel [2]. With that, alginate was modified to have a cell-adhesive property with bonding gelatin covalently.

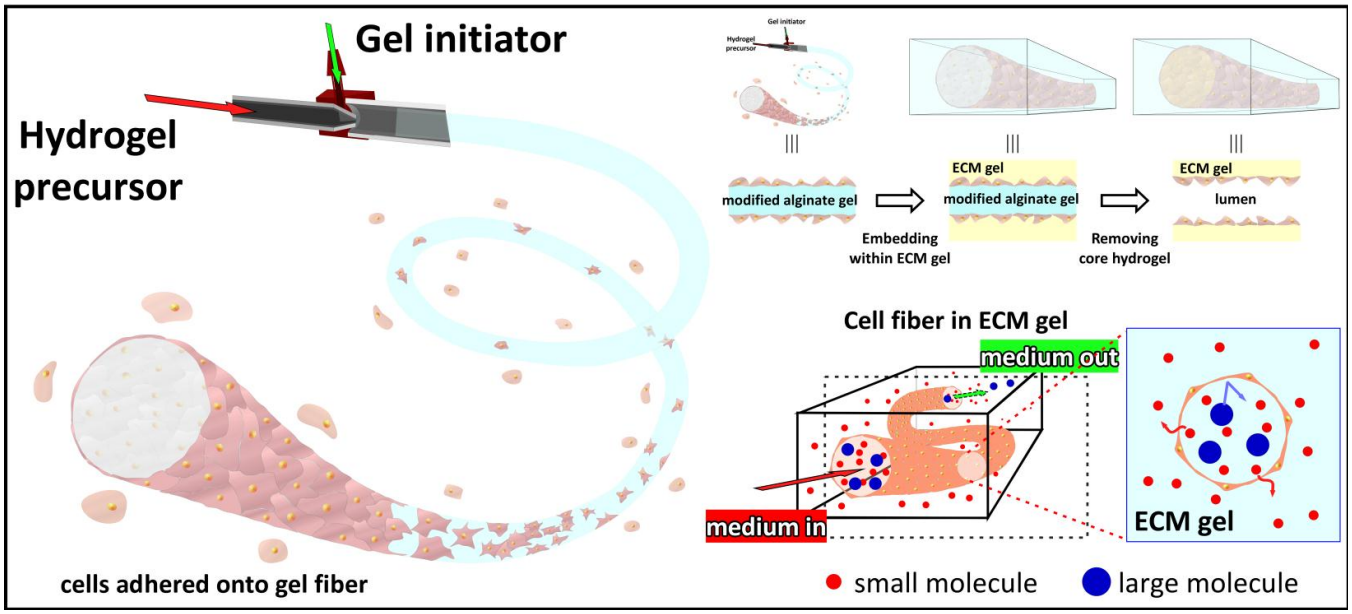

Figure 1. The concept of vessel biofabrication using with gel fiber. After culturing cells onto the surface of modified alginate fiber, the fiber is embedded into ECM gel. Cells are transferred onto ECM gel from alginate gel fiber, and lumen structure can be fabricated by degrading core gel fiber with alginate lyase. The fabricated vessel-like structure is expected to have molecular weight-dependent permeability barrier characteristic of blood vessels.

EXPERIMENTAL

Human umbilical vein endothelial cells (HUVECs) were purchased from Takara Bio Inc. and cultured in EGM-2 medium including FBS and endothelial growth supplements at 37°C and 5% CO_2 atmosphere. Sodium alginate was covalently modified with gelatin using carbodiimide, and dialyzed against distilled water for 10 days to be purified. After dialyzing, modified alginate was lyophilized and stored at 4°C.

First, cell adhesion property onto the surface of modified alginate gel was observed. unmodified alginate and modified alginate gel membranes were prepared by spin-coating and soaking in CaCl2 solution. Cells were seeded onto each surface at 1.0×10^4 cells/cm2, and cultured for 1 day. After 1-day culturing, unattached cells were washed out with fresh medium, and observed under microscopy.

Next, we examined the effect of FN treatment to the alginate gel surfaces. Three types of alginate gel membranes were prepared as same as previously; unmodified alginate, alginate mixed with gelatin and alginate grafted with gelatin. These alginate gel membranes were soaked into FN solution at 37°C for 1 h, and then the amount of adsorbed FN was measured by micro BCA assay.

And then, we evaluated the cell adhesion onto the modified alginate gel surfaces. HUVECs were seeded onto each alginate gel membrane and alginate fiber treated with FN. After culturing for 1 day, unattached cells were washed out with fresh culture medium. Cell adhesion and proliferation on the membrane sheets and the fiber was observed at 1-2 days and 4 days of culture respectively.

Finally, we performed leaking test with fabricated vessel-like structure. Cultured HUVECs fibers were embedded into ECM gels (mixture of collagen and Matrigel), and cultured for another 12 h. Then, in order to fabricate luminal structure of cell fiber, culture medium containing 0.4% alginate lyase was flowed slowly within alginate gel fiber by using glass capillary. The luminal space was perfused for 1 h with fluorescein-labeled moleculules of different molecular weight. And the leakage of each molecule was observed under fluorescent microscopy.

RESULTS AND DISCUSSION

First, cells were seeded onto the surface of unmodified and modified alginate surfaces. Almost cells did not adhere on the unmodified alginate gel. On the other hand, cells adhered and proliferated on the modified alginate surface. However, cell grew slowly and did not reach to confluence. Therefore, we tried to treat the modified alginate with FN. FN is a well-known cell adhesive protein, and has a connective domain to collagen and gelatin. Thus, cell attachment was expected to promote on the modified alginate gel. Actually, the amount of adsorbed FN onto unmodified alginate or mixture of alginate and gelatin was approximately 100 ng/cm^2, whereas that was about 400 ng/cm^2 onto modified alginate (data not shown in this article). In this case, almost gelatin would be maintained within alginate gel thanks to covalent bonding with alginate, whereas gelatin partially might diffuse from mixed gel. Thus it was suggested that more FN could adsorbed onto modified alginate gel than mixed gel.

Since many cells are known to adhere and spread well on the FN coated surface via integrin, cells were seeded onto the alginate gel surfaces after FN treatment (Figure 2). Onto the unmodified alginate surface, almost cells did not attach as same as on the FN umtreated surface. And only small amount of cells adhered and spread onto the mixed gel surfaces. On the other hand, many cells adhered and proliferated onto the modified alginate gel surfaces. Cells require enough FN amount to attach the surface via integrin, and effective amount is more than 100 ng/cm^2 of FN surface density [3]. Thus it may indicate cells didn't adhere and proliferate well onto the mixed gel. These results demonstrated that the alginate modification by gelatin would increase the amount of adsorbed FN onto the surface, and thus cell attachment onto the modified alginate gel surfaces was dramatically improved.

Finally, we examined whether fabricated vessel-like structure had a permeability barrier, one of the critical function blood vessels. Fluorescein-labeled glucose (Mw ~ 340) and FITC-dextran 4000 (Mw ~ 4000) passed outward from HUVEC luminal space, whereas only a little FITC-dextran 20000 (Mw ~ 20000) passed through HUVEC layer within 1 h (data not shown in this article). The molecular permeability of endothelial monolayer become decreasing through over 1.5-nm size of Stokes-Einstein radius [4], and that is converted approximately 5000 in molecular weight of dextran. These results indicated that the fabricated vessel-like structure has a molecular weight-dependent permeability barrier characteristic of living blood vessel.

CONCLUSION

In this study, we fabricated vessel-like structure using with modified alginate gel fiber. Alginate was covalently modified with gelatin, and FN robustly adsorbed to modified alginate. HUVECs attached and proliferated well onto the modified alginate surface treated with FN. Fabricated vessel-like structure had a molecular permeability as similar to blood capillary vessel. Thus, it is indicated that functional luminal structure would be easily fabricated with our method. We believe this fiber technology would be great helpful to fabricate large and functional 3D reconstructed tissues in near future.

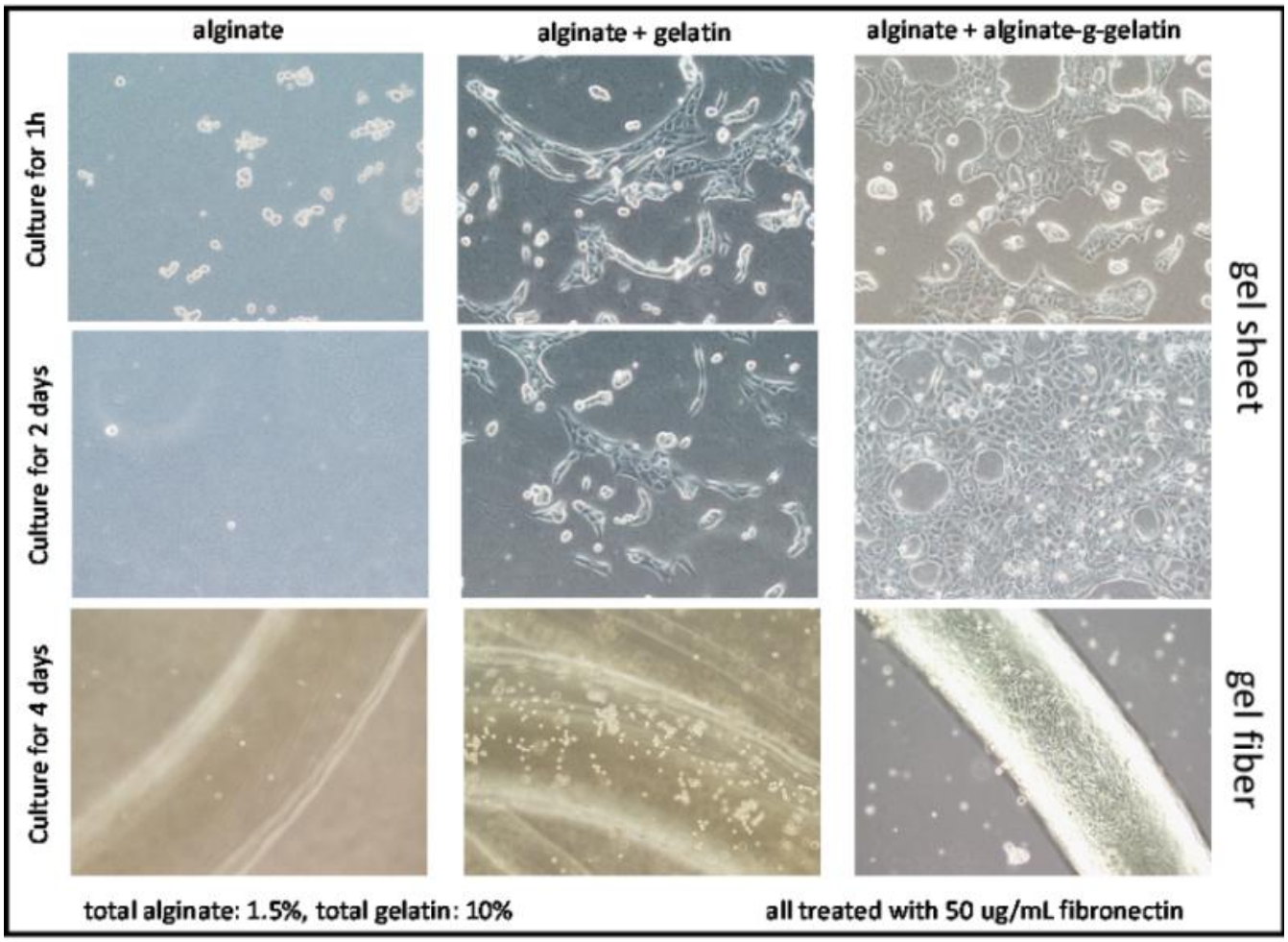

Figure 2. HUVECs were cultured on each alginate gel surface treated with fibronectin. Modification with gelatin to alginate increased FN adsorption onto the gel surface. And thus, cell attachment and proliferation onto the modified FN-treated surface were promoted.

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