

MICROFLUIDIC PRODUCTION OF FIBROUS SCAFFOLDS COMPOSED OF ECM PROTEINS FOR 3D CELL CULTIVATION

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ABSTRACT

A microfluidic process is presented to produce cell-adhesive fibrous scaffolds composed of extracellular matrix (ECM) proteins. By utilizing stable multiphase flows in microchannels and the rapidly-gelling nature of alginate, we first formed microfibrils composed of ECM proteins (gelatin or elastin) and Ca-alginate. After chemically cross-linking the ECM proteins and subsequently digesting the alginate polymers using enzymatic reactions, we successfully obtained cell-adhesive microfibrils purely composed of ECMs. As an application, we cultivated mammalian cells in 3D hydrogel matrices together with the fibrous gelatin scaffolds, which resulted in efficient cell adhesion on the scaffolds and cell proliferation within the hydrogel.

KEYWORDS: ECM protein, 3D cell culture, Hydrogel, Microfluidics

INTRODUCTION

Construction of relatively large, functional three-dimensional (3D) tissues *in vitro* is one of challenging topics in tissue engineering. Various types of hydrogels have been used for fabricating 3D cell culture platforms, including collagen [1], alginate [2], agarose [3], PEGDA [4], with various morphologies. However, cells do not significantly proliferate/migrate within the soft hydrogel matrices if solid scaffolds are not embedded together with cells. In this study, we proposed a process to produce microfibril-shaped cell-adhesive scaffolds composed of cross-linked ECM proteins, using microfluidic devices and sacrificial component of alginate. In addition, by using the obtained ECM fibers as the solid scaffolds, we performed 3D cell cultivation within hydrogel matrices.

EXPERIMENTAL SECTION

The process is shown in Figure 1. By continuously introducing a precursor solution, buffer, and gelation solution into multiple-inlet microchannels, fibrous composites of Ca-alginate and ECM proteins are produced. After cross-linking the proteins using glutaraldehyde and enzymatically digesting the alginate matrix, fibrous scaffolds purely made of ECM components are obtained.

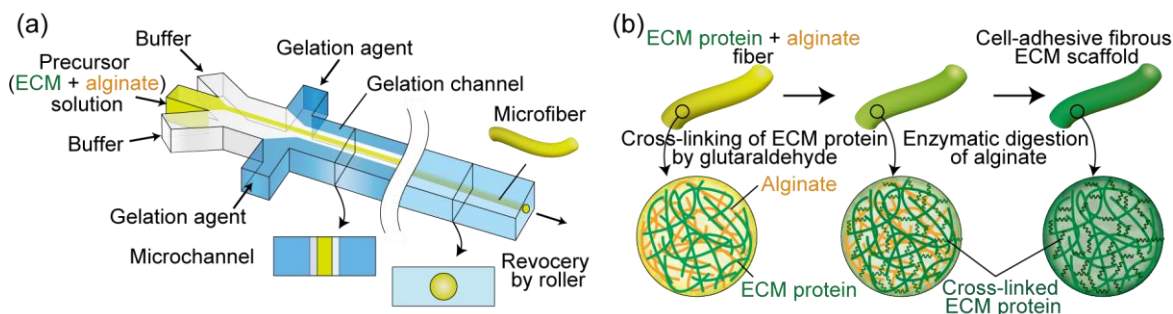


Figure 1: Preparation process of fibrous scaffolds made of ECM proteins; (a) microfluidic device for producing ECM-alginate composite fibers, and (b) subsequent chemical cross-linking and removal of sacrificial alginate gel.

In the experiments, an aqueous solution of 1% sodium alginate (NaA) with 3% gelatin or 1% elastin was used as the precursor solutions. Aqueous solutions of 10% dextran with and without containing 0.1 M CaCl₂ were used as the gelation and the buffer solutions, respectively. These solutions were continuously introduced into the microchannel by using syringe pumps. After recovering the fibers using

a roller, fibers were cut into ~1 mm fragments, cross-linked, and treated with alginate lyase. In the cell culture experiments, we cultivated NIH-3T3 cells in 3D hydrogel matrices together with the fibrous gelatin scaffolds.

RESULTS AND DISCUSSION

Figure 2 shows the preparation of alginate-gelatin composite fibers. We successfully obtained hydrogel microfibers composed of ECM proteins and Ca-alginate with a high production speed (3~10 m/min). Figure 3 shows the obtained alginate-gelatin composite fibers. We were able to control the diameters of the composite microfibers from ~20 to ~50 μm by adjusting the introduced flow rates. After cutting the fiber bundle, cross-linking, and digesting alginate, the fiber diameter was decreased to ~60% (Fig. 4). We confirmed that the presented method was applicable to several types of proteins including elastin and gelatin (Fig. 5).

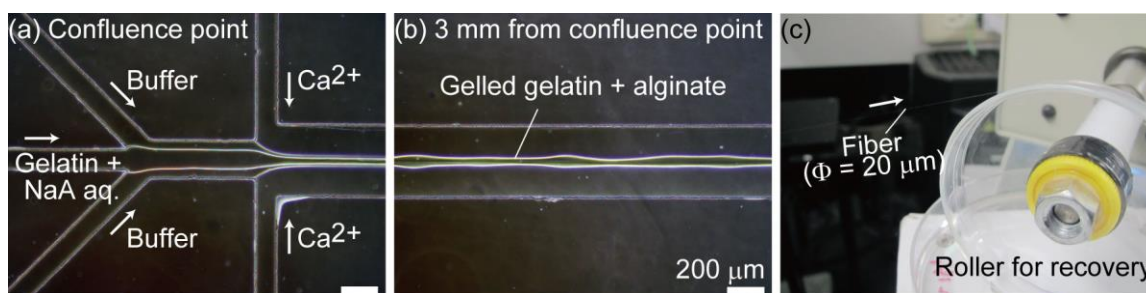


Figure 2: (a, b) Formation of fibrous scaffolds made of gelatin and alginate in a microfluidic device. (c) Recovery of the fiber using a roller.



Figure 3: (a, b) A bundle of the obtained fiber with diameter of ~20 μm and a total length of ~200 m. (c) Relation between the widths of the fiber and the total flow rate of the precursor solutions.

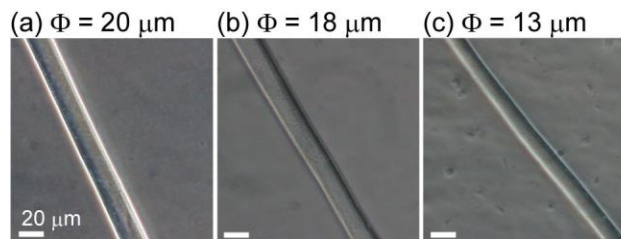


Figure 4: Micrographs showing a gelatin-alginate fiber (a) just after recovery from the microchannel, (b) after chemical cross-linking, and (c) after removal of alginate.

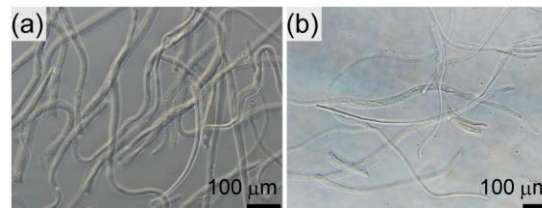


Figure 5: Micrographs showing (a) gelatin and (b) elastin fibers cut into ~1 mm-long segments.

Next, we examined the cell-adhesion characteristics of the obtained scaffolds. NIH-3T3 cells were cultured with the gelatin scaffolds (with and without the alginate-digestion process) under a suspension

culture condition. As a result, cells were adhered only on the surface of the alginate-removed gelatin fibers (Fig. 6), because alginate has a high cell-non-adhesive property.

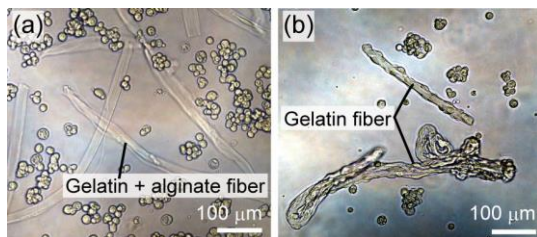


Figure 6: Cell adhesion experiments on the gelatin fiber. Cells and fibers were cultured in a suspension condition. Suspended fibers (a) before and (b) after digestion of alginate. In (b), cells were adhered on the surface of the gelatin fibers.

Finally, the effect of the fibrous scaffolds on the cell proliferation in 3D hydrogel environments was examined. We cultured cells within 3D hydrogels together with the prepared gelatin scaffolds (Fig. 7 (a)); segmented gelatin fibers and cells were suspended in 2% NaA aq., which was gelled into relatively large bulk scale structures (size of ~1 mm) (Fig. 7 (b)). Because of the presence of the fibrous gelatin scaffolds, cells proliferated and formed intercellular networks within the 3D hydrogel matrix, after several days of cultivation (Fig. 7 (c)).

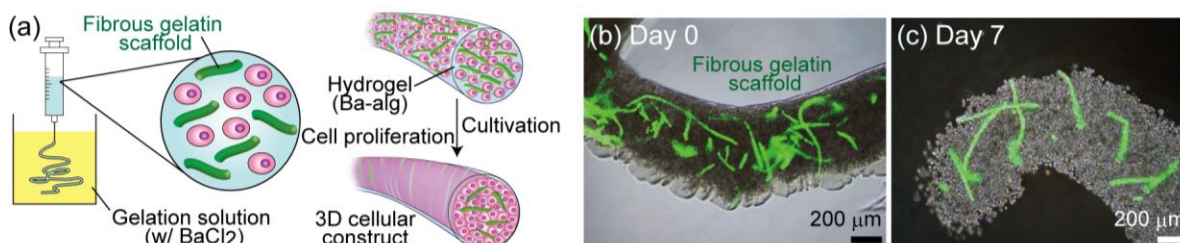


Figure 7: (a) Schematic image showing 3D cell cultivation within bulk-scale hydrogels embedding the obtained gelatin fibers (containing green fluorescent microbeads). (b, c) NIH-3T3 cells cultured within Ba-alginate hydrogel together with the obtained fibrous gelatin scaffolds, (b) 0 and (c) 7 days after cultivation. Cells significantly proliferated in (c).

CONCLUSIONS

We have developed a new technique to produce cell-adhesive fibrous scaffolds composed of ECM proteins. The presented cell-sized ECM fibers would be useful as unique scaffolds to construct relatively large 3D tissues with arbitrary shapes, because of their cell-adhesive characteristics and small sizes comparable to cell size.

ACKNOWLEDGMENTS

This study was supported in part by Grants-in-aid for Scientific Research (23106007 and 25750171) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

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