ELECTRODEPOSITED ALGINATE HYDROGELS FOR FABRICATION OF CELL SHEETS
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ABSTRACT
In the present study, we present a method of electrodeposition of hydrogels to fabricate cell sheets. In the method, calcium-alginate hydrogels are electrodeposited by water electrolysis. 3T3 cells were cultured on the hydrogels to form cell sheets, and the cell sheets were collected by dissolving the hydrogels. Intact cell sheets can be collected, indicating that the method is useful to create cell sheets.

KEYWORDS: Electrochemical method, cell sheet, alginate hydrogel

INTRODUCTION
In this study, we show a novel method for fabrication of cell sheets using hydrogels. Hydrogels have been widely used for tissue-engineering scaffolds. Among them, alginate hydrogels are widely used because the hydrogels are easily fabricated by adding a sodium alginate solution into a solution containing bivalent cation, such as Ca²⁺. Recently, Rubloff and coworkers reported an electrochemical method to fabricate calcium-alginate hydrogels [1-4]. In the method, Ca²⁺ is produced by water electrolysis on an electrode, and calcium-alginate hydrogels are electrodeposited onto the electrode. The mechanism is described in a former section. In our previous researches, the method has been applied for fabricating tubular structures and microwell arrays of calcium-alginate hydrogels, and these hydrogels were used for three-dimensional tissue organs [5, 6]. The method also applied for an electrochemical hydrogel lithography using scanning an electrode, and drawing of calcium-alginate hydrogels are achieved [7].

In the present study, we propose a method for fabrication of cell sheets using the electrodeposited calcium-alginate hydrogels. Cell sheets have a contiguous sheet-like construct containing cell-cell interactions and extracellular matrixes, and cell sheets are widely used fabrication of retina, skin, liver, cardiac muscle and so on. An intelligent thermoresponsive surface have been widely used for cell-sheet fabrication [8].

In the present study, mammalian cells were cultured for several days on calcium-alginate hydrogels electrodeposited by patterned electrodes to fabricate cell sheets on the hydrogels. To improve cell adherence on hydrogels, collagen was mixed into calcium-alginate hydrogels during the electrodeposition. After fabrication of cell sheets, the collagen/alginate hydrogels were dissolved by EDTA to collect intact cell sheets from the culture surface.

THEORY
Figure 1 shows the basic mechanism of the method. Electrodes are inserted into a sodium alginate solution with CaCO₃ particles. Water electrolysis by the electrode provided H⁺, and the CaCO₃ particles

![Figure 1: Schematic of the electrodeposition method of calcium-alginate hydrogels on electrodes. Ca²⁺ is produced by reacting CaCO₃ particles with generated H⁺ by water electrolysis. The generated Ca²⁺ is reacted with alginate near the electrodes, thereby fabricating calcium-alginate hydrogels on the electrodes.](image-url)
are reacted with \( \text{H}^+ \) to release of \( \text{Ca}^{2+} \) in the sodium alginate solution, resulting in electrodeposition of calcium-alginate hydrogels on the electrode.

**EXPERIMENTAL**

Figure 2 shows the mechanism of cell-sheets fabrication using electrodeposited collagen/alginate hydrogels. Briefly, a sodium alginate solution containing collagen and \( \text{CaCO}_3 \) particles was introduced onto an ITO electrode. Proper potential was applied to the electrode to induce water electrolysis, and a thin collagen/alginate hydrogel film was electrodeposited. 3T3 cells were cultured on the film to form cell sheets. After fabricating cell sheets, the film was dissolved by chelate effect to collect the cell sheets.

**RESULTS AND DISCUSSION**

Figure 3 shows that 3T3 cells were successfully cultured on collagen/alginate hydrogels. After the 7-day incubation, the cell sheet-like construction of 3T3 cells formed on the collagen/alginate hydrogels. To collect the cell sheets, the hydrogels were treated with an EDTA solution. Since the hydrogels were dissolved within 5 min, the cell sheets were successfully released from the electrode as an intact sheet. The shape of the cell sheet was similar to that of the electrode. After transferring the cell sheets into a fresh medium, the cell sheets were collected using an EDTA solution from the culture surface. After introducing the cell sheet into a solution, an optical image of the cell sheet was acquired (A). After staining the cell sheet with calcein-AM and PI, phase contrast microscopic (B) and fluorescent images (C) of the cell sheet were acquired.

Figure 2: Schematic of cell-sheet construction. After depositing calcium-alginate hydrogels containing on electrodes by the electrodeposition method, cells are seeded onto the hydrogels and cultured for several days to form cell sheets on the hydrogels. The hydrogels is dissolved by an EDTA solution to collect cell sheets from the electrode.

Figure 3: Cell culture of 3T3 cells on calcium-alginate hydrogels containing collagen.

Figure 4: Collection of cell sheets. 3T3 cell sheets were collected using an EDTA solution from the culture surface. After introducing the cell sheet into a solution, an optical image of the cell sheet was acquired (A). After staining the cell sheet with calcein-AM and PI, phase contrast microscopic (B) and fluorescent images (C) of the cell sheet were acquired.
construction was still maintained (Fig. 4A). Thus, cell sheets were rapidly collected without enzymatic and physical treatment. Live-dead staining using calcein-AM and PI showed that the cell viability in the sheets were high, indicating little influence of the detachment process for cell viability (Fig. 4B, C).

Although we used collagen to improve cell adhesion in the present study, there are many candidates to improve cell adhesion. For example, RGD peptides are widely used for this purpose [9]. Now, a RGD peptide-conjugated alginate are being used for cell-sheet fabrication using the electrodeposition method [10].

CONCLUSION

In conclusion, the novel method for construction of cell sheets was developed using the electrodeposition method of hydrogels. The method is a simple and inexpensive method. Since cell sheets were rapidly collected by dissolving the hydrogels without enzymatic and physical treatment, the method is useful for cell-sheet engineering. In the present study, collagen was mixed into calcium-alginate hydrogels during the electrodeposition to improve cell adhesion on hydrogels. We believe this technique can be applied to construct various cell sheets.

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REFERENCES


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