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Fabrication of Multilayered Hepatic Lobule Tissues Using Ca-Alginate Hydrogel Platforms

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***Abstract*— Artificial cell sheet is utilized as a useful method for tissue engineering. We proposed a novel approach to fabricate the Ca-alginate gel sheet embedded liver cells to mimic the liver lobule tissue. Ca-alginate sheet with hepatic lobule shaped pattern was firstly deposited on a micro-electrode device based on the electrodeposition method. Viability of embedded cells was checked to be maintained more than 90%. What's more, we further stacked the fabricated cell sheets into a pre-designed PDMS mold. The stacked multi-layered 3D cell structure can contribute the possibility for the applications of artificial liver fabrication.**

I. Introduction

In vitro bio-fabrication of cell tissue is a promising way for the alternative of animal experiments and pharmacological assays, also realizing the replacement of damaged tissues or organs. To duplicate the native body conditions, cell-to-cell and cell-to-extracellular matrix (ECM) interactions of the cellular microenvironment are the important parameters for artificial tissue fabrication. Hydrogels are attractive scaffolds for 3D cell culture and tissue engineering due to their tissue-like water content, injectability, and tunable properties [1, 2]. Extensive efforts have been made to control various formations of hydrogels such as thermoresponsive gel, photo-crosslinkable gel and chemical-crosslinkable gel, for the purpose of drug release [3], cell assembly In vitro [4, 5], tissue formation [6-8] and subsequent transplantation [9].

Recently, cell sheet fabrication as an important technology for 3D tissue has been well utilized [10, 11]. For example, regeneration of cornea is engineered by culturing the desired cells on a thermo-responsive polymer surface [12]. Thus, lots of biomaterials such as nature polymer and synthesis polymer were utilized as scaffold for cell-containing sheet fabrication. Among these hydrogels, an acidic polysaccharide of sodium alginate, which can ionically cross-linked with multivalent cations (e.g., Ca^{2+}, Fe^{3+}) is widely used to entrap and immobilize cells [13], bacterial [14] and other bio-components. In our previous work, we propose a novel method for fabrication of compact microtissue with high cell density by electrodeposition method [15]. This method is also used for fabrication of 3D-multilayer hepatic lobule-like tissue

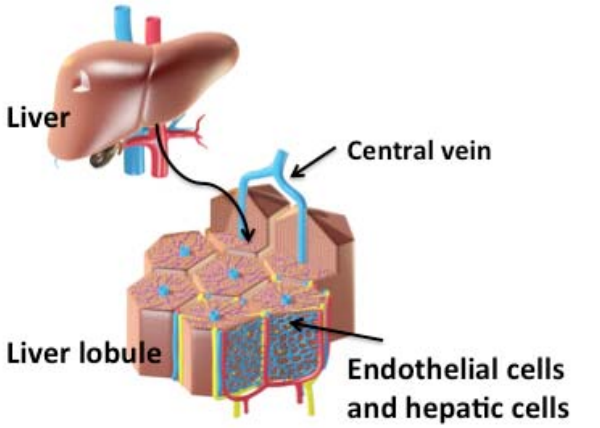

Fig. 1 Hepatic lobule is one of the small vascular units composing the substance of the live in a hexagonal shape with portal triads at the vertices and a central vein in the middle.

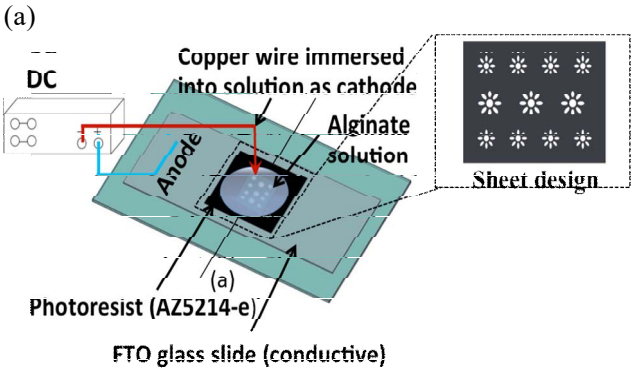

Fig. 2(a) Development of micro-electrode device with hepatic lobule patterns. (b) Assembly of cell sheets into multilayered hepatic lobule tissue using a PDMS mold.

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constructs. cells encapsulated within the microcapsules eventually fully occupied all spaces to achieve hepatic lobule micro tissue within 2 weeks [16]. However, there is still lack of an efferent method for cell sheet fabrication with precise shape-control ability using Ca-alginate hydrogel.

In this paper, we propose a novel method to fabricate cell sheet for artificial liver tissue engineering. Ca-alginate hydrogel cell sheet with hepatic lobule shape were successfully deposited using an electrode-device. The viability of the embedded cells was maintained than 90%. The cell sheets were further stacked together to form 3D multilayered structure within a PDMS mold.

II. Experimental Procedure

A. Materials and solution preparation

We used sodium alginate (Medium viscosity, A2033), fluorine doped tin oxide coated glass slide (surface resistivity ~7 Ω/sq, 735140) (Sigma-Aldrich) and HEPES (346-01373) (Wako Pure Chemical Industries). Calcium carbonate ($CaCO_3$) (0.97 μm, #2300) were kindly supplied from Sankyo-seifun Ltd (Japan). Photoresist (AZ5214-E) were purchased from AZ electronic material GmbH.

Deposition solution

The deposition solution was prepared by dissolving 1% w/v alginate sodium in solution containing NaCl (126 mM), KCl (2.7 mM), $Na_2HPO_4 \cdot 12H_2O$ (8.1 mM), KH_2PO_4 (1.47 mM) and HEPES (21 mM). The pH was adjusted to 7.3 by adding 0.5 M NaOH solution. $CaCO_3$ (0.5% w/v) was uniformly dispersed in the solution using magnetic stirrer for 24h.

HEPES buffer solution

The HEPES buffer solution was prepared by dissolving HEPES (5g/L) in solution containing NaCl (8 g/L), KCl (0.37 g/L), Na_2HPO_4 (1.076 g/L) and glucose (1 g/L). pH was adjusted to 7.3 by adding 0.5M NaOH solution.

Calcium chloride solution

To prepare 1.1% calcium chloride solution, 0.55 g of $CaCl_2$ (anhydrous) is dissolved in 50 ml of distilled water.

Cell viability test solution

The cell viability solution was a mixture of 0.8 μL calcein AM (1 mg/mL, Wako), 2.8 μL propidium iodide (PI) (1 mg/mL, Wako) and 1 mL HEPES buffer solution.

Rat liver (RLC-18) cells were cultured for experiments with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin streptomycin in a 10 cm tissue culture dish at 37 °C in a humidified 5% CO_2 incubator. When reaching 90% confluence, cells were detached using trypsin/EDTA (Invitrogen) and gently pipetted to break aggregates. The cells were then centrifuged, re-suspended, and counted for further passaging or experimental use.

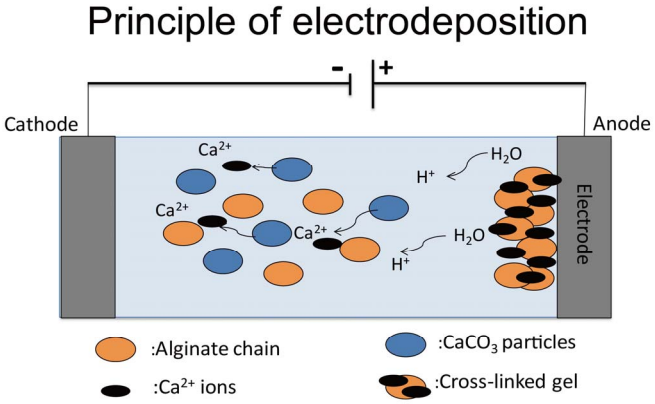

Fig.3 The principle of electrodeposition for depositing the Ca-alginate gel film onto micro-electrode surface.

B. Design of hepatic lobule module

Hepatic lobule is one of the small vascular units composing the substance of the liver. The hepatic lobule is roughly hexagonal in shape with portal triads at the vertices and a central vein in the middle as shown in Fig. 1. We designed the micro pattern to mimic the liver lobule tissue. Comparing with the liver histology, the center of one unit means central vein. Around the central vein, it is composed of petals. The petals mean hepatic cords. Between the petals, lined with endothelial cells and hepatic phagocytic cells. The design of the sheet is about 12 mm in length and 9.5 mm in width. The microelectrodes were on the outside of the flower-shape as shown in Fig. 2(a).

C. Fabrication of cell sheet

In the approach, we propose a novel method for fabrication of tissue structure by electro-deposition method and the mechanism was shown in Fig. 2(a). To obtain designed cell sheets, firstly, a patterned photoresist layer onto Fluorine-doped tin oxide (FTO) glass was prepared through photolithography technique. The photoresist AZ (5214-E) is coated on the surface of FTO layer and its thickness is about 1.2 μm. Secondly, the electro-deposition method was applied. We put one electrodes (cathode) into deposition solution (a mixed solution of alginate, $CaCO_3$ particles and cells) and on electrode (anode) onto the FTO glass surface to build the system as shown in Fig 2(a). Then, a DC potential is applied to the anode electrode for a short time to triggers H^+ generation by the electrolysis of water to form an acid environment around the anode surface ($2H_2O = O_2+4H^++4e^-$). Thus, Ca^{2+} ions are released from $CaCO_3$ particles due to the protons encounter with the $CaCO_3$ particles ($2H^++CaCO_3 = Ca^{2+}+H_2O+CO_2$). Finally, these Ca^{2+} ions will immediately react with alginate to form Ca-alginate hydrogel membrane onto the anode electrode [17]. By this process, Ca-alginate gel sheet structures are fabricated which embedding with the liver cells. In the next steps, the sheets on the FTO glass are cultured in the deposition solution (Dulbecco's Modified Eagle's Medium (DMEM) which is supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin) at 37°C in a humidified 5% CO_2 incubator. In the next day, the cell sheets are detached from the FTO glass by shaking the