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主　論　文　の　要　旨

論文題目　**Three-Dimensional Fabrication of Hepatic Lobule Model Using Electrodeposition Technology**
(エレクトロデポジション技術による三次元肝小葉モデルの作製)

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論　文　内　容　の　要　旨

3D in vitro tissue models have been used in tissue research as a compromise between 2D cultures of isolated cells and the artificial 3D tissue architecture. Constructing 3D cell models provide a potential alternative to in vivo approaches for whole-organism, and 2D culture with its spatial limitations. Recent advances in tissue engineering have relied upon development of methods to place spatially selective biological components at specific three-dimensional (3D) locations. There is similar interest in developing methods to assemble cells within bio-scaffolds for fabrication of 3D cell structures. Current methods to assemble cells into two-dimensional (2D) or 3D structures include non-spherical polymeric microparticle in situ photo-polymerization, cell patterning on 2D surfaces by using dielectrophoresis technique, 3D bio-printing, cell sheet engineering, and cell encapsulation units. Thus, many fabrication methods have been developed to immobilize and culture cells in 3D formats.

Recently, the development and application of micro/nano devices and automation systems has drawn lots of attention, for example, on cellular mechanobiology, microrobotic manipulation of biological cells and biomedical sensor. In our previous research, we have proposed a thermoresponsive polymer based manipulator to move

micro-scale objects, especially biological cell applications. Later, a microrobotic system was established to fabricate vascular-like microtubes by automated pick-up of cellular micromodules. This microrobotic assembly system can potentially construct 3-D structures by assembly of multiple micromodules. The micromodules were fabricated based on the sensitometric characteristic of the cross-linkable hydrogel, which can be solidified with exposure of UV light. However, such fabricated micromodules bring several issues including: 1) UV exposure of cells may cause the decrease of cell viability. 2) Cells that are encapsulated inside the simple synthetic scaffolds of PEGDA often undergo apoptosis due to a lack of cell-matrix binding sites. Thus, it is difficult to achieve high cell density (similar to that found in vivo: 10^8–10^9 cells cm^{-3}). Therefore, fabrication of basic micromodules with specific shape and high cell density for 3-D cell structure construction is well needed in our group.

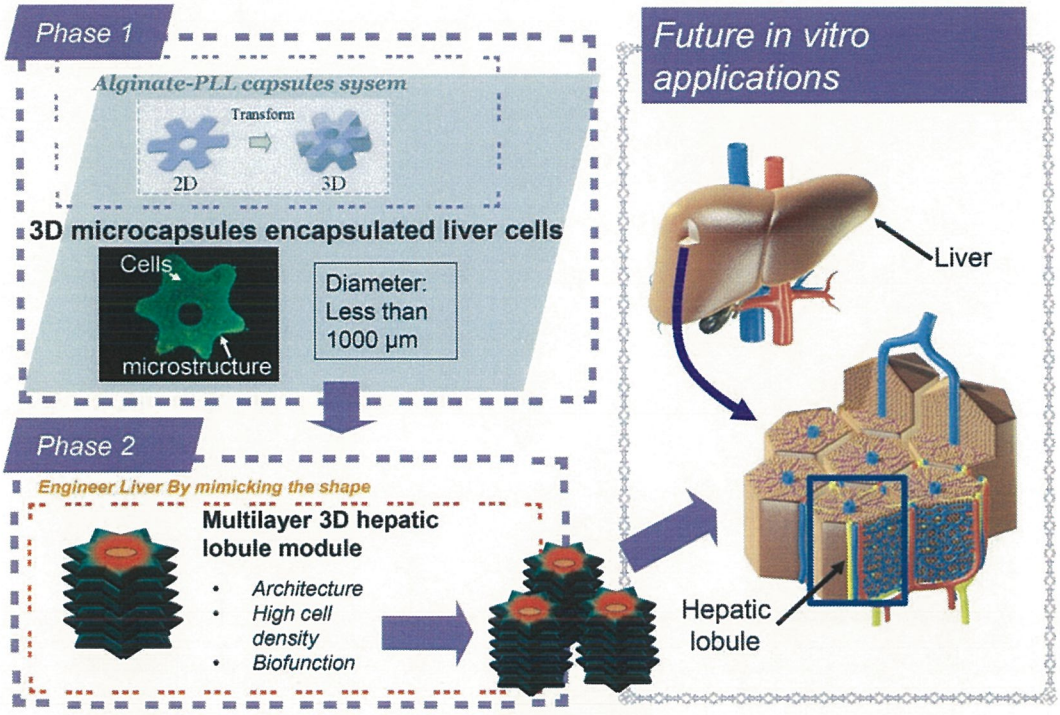

Figure 1 Proposed method: achieving high cell-density structures with hepatic biofunctions

The use of alginate-poly-L-lysine (PLL) microcapsules has shown great potential in fabricating 3D cell structures with high cell density ever since Lim et al. first reported this approach for fabrication of microencapsulated islets for implantation in 1980. Lately, cell-laden Ca-alginate fibres or droplets have been transformed into 3D microcapsules to form tissue-like cell spheroids and cylindroids after long-term cultivation. These microcapsules provide a soft and "liquid-like" platform that mimics the embryonic microenvironment for self-assembly of cells. Small molecular weight

substances like nutrient and oxygen molecules can pass through the alginate-PLL membrane of microcapsules, while cells are blocked by the membrane. However, because of the mechanism of Ca-induced alginate gel formation, it is still difficult to precisely control the gelation process to produce alginate-PLL microcapsules with specific shape. Therefore, despite the commonly used alginate fibres or droplets generated microfluidic devices, there is still a lack of an efficient approach to achieve shape-controlled alginate-PLL microcapsules for 3D cell structure fabrication.

For production of shape-controlled alginate-PLL microcapsules, the electrodeposition method is a promising technique. Electrodeposition of Ca-alginate hydrogels on specific device plays an important role in entrapment and immobilization of biological components, such as cells and bacteria, for studying cell-cell signalling and 3D cell culture. Cheng et al. demonstrated a novel approach for fabrication of alginate gels inside a microfluidic system. In their work, a Ca-alginate composite hydrogel biofilm entrapping bacterial cells was fabricated on gold electrodes inside the microfluidic system, with its shape controlled by using electrical signals. By electrodeposition, Ca-alginate gelation can be triggered by an electrical signal, which enables us to fabricate an in-situ Ca-alginate gel membrane with controllable size and shape on microelectrodes. Consequently, other researchers have also focused on the fabrication of cell structures by depositing cell-laden alginate gel on electrodes. Their results show that cell viability can be maintained during culture, but cells did not spread because of the lack of cell adhesion molecules and spaces within the Ca-alginate gel. Thus, promoting cell proliferation has become the main challenge concerning the electrodeposition method for 3D cell structure fabrication.

Here, we propose a novel method for fabrication of compact microtissue with high cell density by electrodeposition method. Gear shaped microcapsules of RLC-18 rat liver cells were produced for long-term culture to obtain desired morphologies of cell aggregates. The reasons of choosing the gear-shaped in this work are so that we can: 1) prevent tissue necrosis during tissue formation because cell-culture media (i.e., oxygen and nutrients) can be supplied from the cavities of central hollow structure. 2) fabricate the gear-like structure as basic components may have potential for the applications of micro-scale robotic agent. 3) mimic the hepatic lobule in vivo for further studying the properties of the native tissue, since the hepatic lobule is a small division of liver with a central vein in hexagonal shape that similar to a gear-like shape.

For the fabrication process, briefly, 2D Ca-alginate gel structures were formed by housing an alginate aqueous solution containing $CaCO_3$ particles and RLC-18 cells onto a microfabricated gear-shaped microelectrode. By the conventional electrodeposition

method, the gelation of Ca-alginate can be triggered by an electrical signal, and it enables us to fabricate in situ Ca-alginate gel structures with controllable size and shapes onto the microelectrodes. Electrodeposition of alginate gel provides a programmable method for the spatiotemporally controllable assembly of biomaterials. Thus, we can achieve 2D cell-laden Ca-alginate gel structures on the microelectrode with predefined gear-shape. However, electrodeposition of Ca-alginate gel membrane has its own limitation to achieve 3D microstructure with high cell density due to the lack of adhere molecular in alginate gel. Therefore, the novelty of this work is to induce alginate-Poly-L-lysine (PLL) microcapsule system to overcome this limitation for 3D microtissue fabrication.

In order to transform the 2D gel structures into 3D microcapsules for 3D tissue fabrication. The fabricated gel structures were detached from the microelectrode surface and treat with PLL and sodium citric solution in order. By reacting with PLL solution, a semi-permeable alginate-PLL complex will be formed around the gel structures. This alginate-PLL layer, with a membrane thickness of 4-5 μm, will allow oxygen and nutrient to enter inside while blocking the encapsulated cells to go outside. Next, by reacting with sodium citric solution, 3D microcapsules will be formed since the sodium citric solution can go through the alginate-PLL layer to dissolve the alginate gel inside to form a hollow structure. Thus, the cells within the microcapsules will adhered to the inner surface of alginate-PLL layer and occupied all of the space inside the microcapsules by culture.

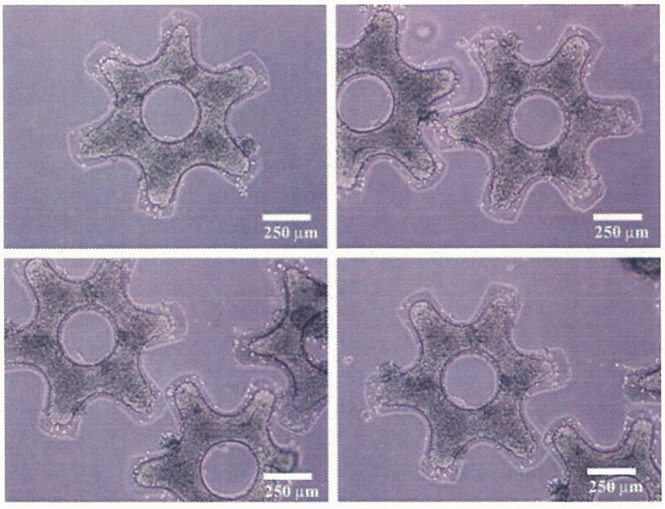

Figure 2 The culture results of the fabricated six-tooth gear-like tissue using RLC-18 (rat liver) cells in day 9

In this paper, an array of gear-shaped Ca-alginate gel structures were successfully generated onto the microelectrode. Followed by detachment and treatment with PLL and sodium citric solution, the gel structures were transformed into 3D cell-laden

alginate-PLL microcapsules while maintaining gear shape. Finally, the cells within the microcapsules were further promoted into gear-like compact microtissue by proliferating and occupying all the space of the microcapsules. Cell viability was checked and the characterization of the fabricated gear-like tissues were demonstrated.

In the current results, RLC-18 cells were successfully encapsulated within 3D microcapsules for long-term culture to form microtissue with high cell-density (similar to that found in vivo: 10^8–10^9 cells cm^{-3}) in hepatic lobule shape. Cell viability in 9-day cultured 3D cell structures assessed using the live/dead assay kit; the viability was approximately 94%. No necrotic area, caused by high cell-density due to cell proliferation and tissue contraction, was found in the central tissue because the microcapsule size was optimal for nutrient transportation. The main advantages of our approach including: 1) Gear-like tissue fabrication with predefined shape 2) High cell density while maintaining the viability 3) Movable structures that can be transported and manipulated.

We believe that our method is highly compatible with the formation of the 3D complex tissues, since the cells can be easily molded with different types of microcapsule units. In our future work, such gear-like microtissue can be assembled to build macroscopic 3D tissue architecture such as hepatic lobule model for tissue engineering. In addition, the presented 3D microcapsule system could be applicable to various combinations of cell types for the study of cell-cell interaction.