Encapsulation of tissues in cage-like living cell layer using microcapsules

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INTRODUCTION

Cell-enclosing microcapsules have been studied for more than 40 years as tools for treatment of diseases, production of useful biomolecules, and pharmaceutical assays. In the present study, we demonstrate the feasibility of the bio-device as a platform for preparing spherical tissues covered with an additional cell layer for tissue engineering applications (Fig. 1).

Covering non-autologous small tissues with a recipient's own cells prior to transplantation is reported to be effective for improving histocompatibility of grafts. In addition, covering with endothelial cells would enhance vascularization to grafts. This technology will also be useful for the study of cellular movements stimulated by the interactions of heterogeneous cells, e.g., tumour cell migration and angiogenesis in tissue. Here, we propose a method for preparing spherical tissues covered with an additional living cell layer. The main feature of our method is that the small tissues are wrapped with a cell layer templated by a microcapsule degradable on-demand using non-proteolytic enzyme under mild condition for cells. The microcapsule membrane was made from alginate derivative possessing phenolic hydroxyl (Ph) moieties (Alg-Ph) cross-likable through horseradish peroxidase-catalysed reaction (Sakai 2007). Due to the existence of the Ph moieties, the surface of the resultant hydrogels can be modified with other polymers possessing Ph moieties for functionalization via the HRP-catalysed reaction (Sakai 2010). In the present study, we used gelation derivative possessing Ph moieties (Sakai 2009a) for preparing the cell adhesive surface on the cell-enclosing Alg-Ph microcapsules. We demonstrate the effectiveness of our method for wrapping two types of aggregates, one formed prior to encapsulation and other grown from individual cells in spherical cavities within the microcapsules (Fig. 1).

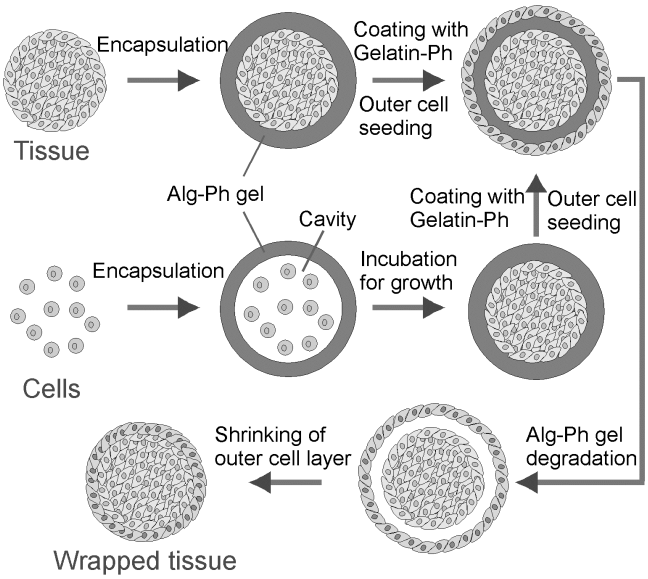

Figure 1. Schematic of tissue wrapping with a shrinking living cell cage.

MATERIALS AND METHODS

Tissue and cell-encapsulation

Multicellular spherical tissues of human hepatoma cell line HepG2 cells prepared by culturing them on an ultra-low cell attachment dish were suspended in 1.5% (w/v) Alg-Ph solution (pH7.4). The suspension was dropped into 100 mM $CaCl_2$ solution using an electrostatic droplet generator. The diameter of the resultant Alg-Ph microcapsules was ca.500 μm.

Spherical tissues were also prepared in microcapsules having spherical cavities through the growth of individual cells encapsulated within the cavities. HepG2 cells were enclosed in gelatin microparticles of about 200 μm in diameter using an axisymmetric flow-focusing droplets generation devise (Sakai 2009b): 7.5% (w/v) Gelatin aqueous solution containing individual HepG2 cells was extruded into a co-flowing stream of liquid paraffin containing lecithin at 3% (w/w). The resultant emulsion system was cooled in an ice bath for allowing hydrogelation of gelatin solution. Subsequently, the gelatin microparticles were suspended in 2.5% (w/v) Alg-Ph solution. Alg-Ph microcapsules of ca.300 μm in diameter containing cell-enclosing gelatin microparticles were prepared by extruding the Alg-Ph solution containing the gelatin microparticles and 40 U/mL HRP into liquid paraffin stream containing H_2O_2. By incubating at 37°C, spherical cavities templated by the gelatin microparticles formed in the Alg-Ph microcapsules due to the gel-to-sol transition of gelatin gel. The Alg-Ph microcapsules were incubated in medium until the enclosed HepG2 cells fulfilled the cavities.

Surface modification wit Gelatin-Ph

The Alg-Ph microcapsules containing spherical tissues of HepG2 cells were suspended in Krebs Ringer Hepes buffered solution (pH7.4) containing 0.1% (w/v) Gelatin-Ph, 10U/mL HRP and, 0.3 mM H_2O_2 for 1 min and then rinsed with medium.

Wrapping tissues in endothelial cell layer

The microcapsules coated with Gelatin-Ph were suspended in medium containing human aortic endothelial (HAE) cells. The medium was gently mixed every several hours to allow homogeneous attachment of the HAE cells on the microcapsules. After an appropriate incubation of the microcapsule covered with HAE cells, they were soaked in the medium containing alginate lyase at 2.0 mg/mL.

RESULTS AND DISCUSSION

Firstly, the possibility of wrapping tissues with the HAE cell layer by degrading the Alg-Ph microcapsule membrane was evaluated using the Alg-Ph microcapsules enclosing agarose microparticles. The HAE cells that lost their anchoring substrate before completely covering the surface of the microcapsules dissociated into small fragment (Figure 2). In contrast, the cells that lose their anchoring substrate after completely covering the surface of the microcapsules could wrap around the agarose microparticles. The HAE cells on the microcapsules began to shrink after exposure of the microcapsules to alginate lyase. The decrease in the size of the cage-like HAE cell layer lasted until it came into contact with the agarose microparticles. No fragmentation was observed during shrinking. This result means that the HAE cell layer shrank while maintaining cell-to-cell connection.

The possibility of wrapping of enclosed tissues by the outer HAE cell layer was also observed for the both the tissues prepared prior to encapsulation in Alg-Ph microcapsules (Figure 3) and formed in the cavity of microcapsules (Figure 4). Furthermore, the method was also effective for wrapping multiple spherical tissues within a single cage of HAE cells when multiple tissues existed in a microcapsule. The HAE cells attached on the HepG2 tissues easily detached and dissociated into small fragments when they were manipulated using a pipette within 30 min of wrapping. However, dissociation did not occur after 1 h of wrapping, meaning the HAE cells established cell-to-cell connection with the inner HepG2 cells within 1 h.

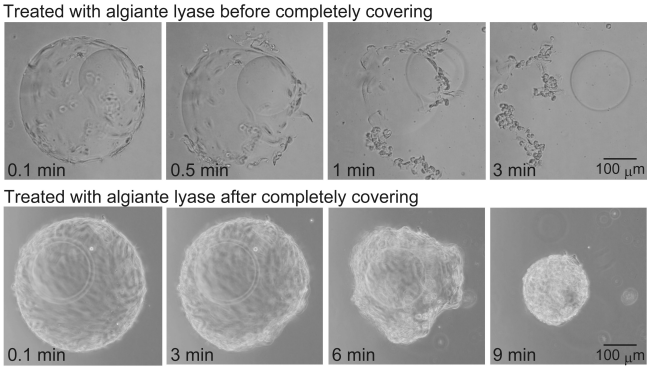

Figure 2. Transition of morphologies of HAE cells on microcapsules after treatment with alginate lyase. (Sakai 2012)

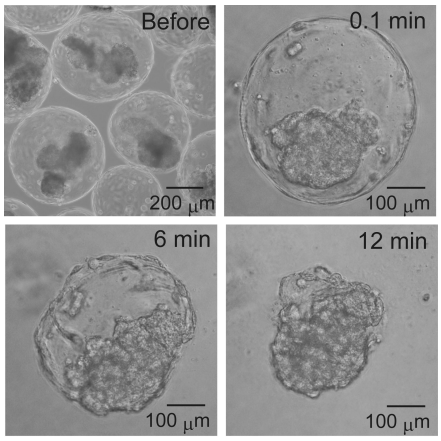

Figure 3. Wrapping tissues prepared prior to encapsulating Alg-Ph microcapsules with HAE cell layer. (Sakai 2012)

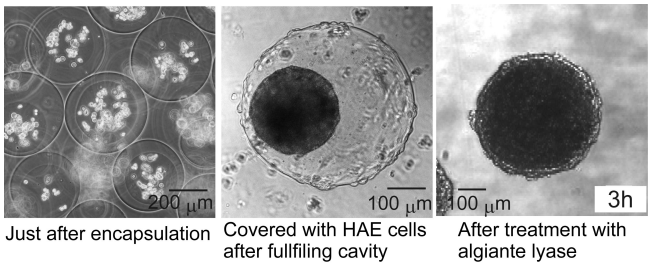

Figure 4. Growth of HepG2 cells in cavity of microcapsule and wrapping the resultant tissues with HAE cell layer. (Sakai 2012)

CONCLUSION

We proposed a method of using cell-enclosing microcapsules as a platform for preparing spherical tissues covered with an additional cell layer for tissue engineering applications. The tissues in Alg-Ph microcapsules covered with HAE cells could be wrapped with the HAE cell layer by degrading the microcapsule membrane with alginate lyase.

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