P-025 **Development of *in situ* gellable and fast degradable hydrogel under the existence of serum for cell-enclosing microcapsule**

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INTRODUCTION AND OBJECTIVES

Encapsulation of mammalian cells has been studied since the 1960s. We recently reported the methods for preparing mammalian cell-enclosing microcapsules with spherical liquid core of around 200 μm in diameter (Sakai 2008a, 2009a, 2010). The spherical liquid core structure was effective for facilitating growth of enclosed cells. In the previous reports, we used alginate-based gel microparticles and gelatin gel microparticles as templates of the spherical liquid cores. After coating the core microparticles with another polymer hydrogels, spherical liquid core structures were formed by degrading the core microparticles using alginate lyase (Sakai 2008a), and liquefying gelatin gel through thermal treatment (Sakai 2009a), respectively. Drawbacks of the systems were the necessity of adding chemicals into medium, and retention of liquefied gelatin molecules in microcapsules for a certain period of time due to a slow release of large gelatin molecules through surrounding hydrogel layer, respectively. In this study, we attempted to develop novel core microparticles suitable as template of the spherical liquid cores. The core microparticles were prepared from the derivative of amylopectin bearing phenolic hydroxyl moieties (AP-Ph). It has been reported that the polymers bearing Ph moieties were crosslinkable through horseradish peroxidase (HRP)-catalyzed reaction (Kurisawa 2005; Sakai 2009b). We expected that the AP-Ph microparticles obtained through the enzymatic reaction would be degraded by amylase contained in serum, the common component of cell culture media (Figure 1).

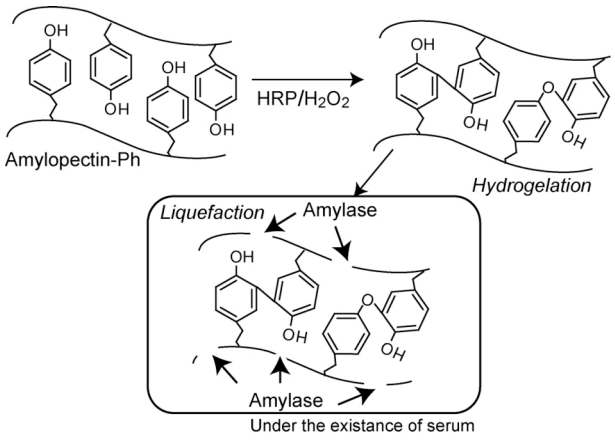

Figure 1: Schematic illustration of hydrogelation of AP-Ph by HRP and liquefaction of AP-Ph gel by amylase contained in serum.

MATERIALS AND METHODS

Synthesis of AP-Ph

AP-Ph was synthesized by conjugating amylopectin with tyramine using 1,1-carobonyldiimidazole in anhydrous dimethyl sulfoxide. The AP-Ph used in this study contained 3×10^{-6} mol-Ph/g-amylopectin.

Microcapsule production

An axisymmetric flow-focusing droplet generation device designed in our laboratory was used for preparing microcapsules. AP-Ph was dissolved in saline at 10 % (w/v). For allowing HRP-catalyzed hydrogelation of AP-Ph solution, HRP was dissolved in the solution at 10 U/mL. The AP-Ph solution was extruded from a 26-gauge needle at 0.05 mL/min into the flow of liquid paraffin containing H_2O_2 and lecithin. The liquid paraffin suspending partially gelated AP-Ph droplets were collected in a plastic tube for 6 min of flowing and stood for 4 min for allowing further cross-link formation. Cell enclosing AP-Ph microparticles were prepared by using the AP-Ph solution containing a human cervical cancer cell line, Hela cells at 1.5×10^7 cells/mL. After rinsing, the microparticles were suspended in the solution of sodium-alginate derivative bearing Ph moieties (Alg-Ph). The Alg-Ph solution containing AP-Ph microparticles and HRP (10 U/mL) was extruded into the flow of liquid paraffin containing H_2O_2 and lecithin Alg-Ph microparticles were obtained through the same processes with that for AP-Ph microparticles. After several time of rinsing, the Alg-Ph microparticles were suspended in DMEM medium containing fetal bovine serum (FBS) at 10% (v/v).

AP-Ph microparticle degradation

The degradability of AP-Ph microparticles under the existence of serum was determined by soaking AP-Ph microparticles in the medium (DMEM) with and without FBS at 37°C. Microphotographs of the microparticles in each medium were taken every 10 min. From the microphotographs the time spent until complete degradation of the AP-Ph microparticles were measured.

RESULTS AND DISCUSSION

Controlling AP-Ph microparticle diameter

Figure 2 shows the effect of flow rate of liquid paraffin on the diameter of AP-Ph droplets. The diameter decreased from 169 to 128 μm with increasing liquid paraffin flow rate from 0.5 to 4.2 mL/min. The trend was similar to those reported for Alg-Ph microparticles (Sakai 2007).

AP-Ph microparticle degradation

Figure 3 shows transition of the morphology of AP-Ph microparticles of about 150 μm in diameter after soaking in the medium containing FBS at 10% (v/v). The microparticles disappeared within 90 min of incubation. In contrast, those soaked in the medium free from FBS remained their shape during 16 h of study. These results clearly demonstrate AP-Ph microparticles were fast degradable under the existence of serum. The time for degradation decreased with increasing the content of FBS in medium: In the medium containing FBS at 50% (v/v), the AP-Ph microparticles disappeared within 20 min.

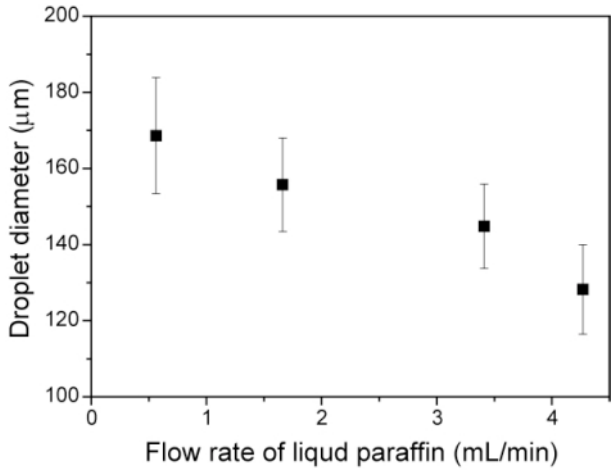

Figure 2: Effect of flow rate of liquid paraffin on the diameter of AP-Ph droplets.

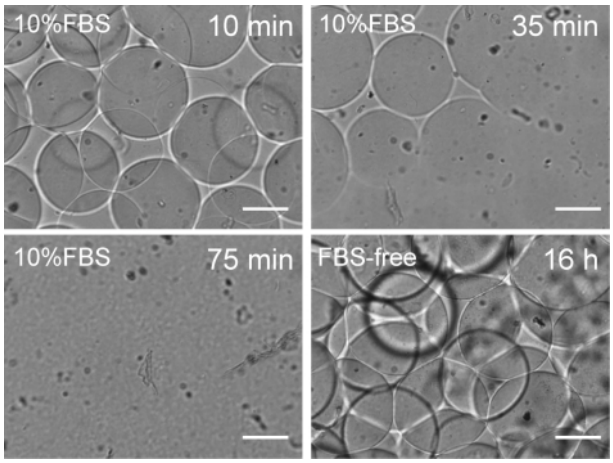

Figure 3: Microphotographs of AP-Ph microparticles after soaking in medium. Bars: 100 μm.

Cell-encapsulation and growth of enclosed cells

Figure 4 shows cell-enclosing AP-Ph microparticles and Alg-Ph microparticles enclosing the AP-Ph microparticles. No specific harmful effect of using AP-Ph was observed compared to previously reported microparticles prepared from polymers bearing Ph moieties through the HRP-catalyzed cross-link formation in liquid paraffin dissolving H_2O_2 (Sakai 2007). The viabilities of the enclosed cells in AP-Ph microparticles and subsequent Alg-Ph microparticles were 91.6 and 86.7%, respectively. Furthermore, the enclosed cells grew in the Alg-Ph microcapsules and filled the spherical liquid core within 2 weeks of encapsulation.

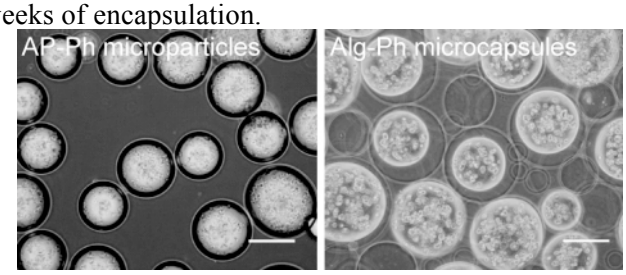

Figure 4: Microphotographs of Hela cells-enclosing AP-Ph microcapsules and Alg-Ph microcapsules. Bars: 100 μm.

CONCLUSION

We developed the novel microparticles of around 150 μm from AP-Ph through HRP-catalyzed cross-links. The microparticles were degradable within 60 min in the medium containing FBS at 10%. After subsequent coating of the microparticles with additional alginate gel layer, spherical liquid core structure formed only by incubating in the medium containing FBS. The enclosed cells grew and filled the spherical liquid cores after 2 weeks of culture. These results demonstrate the great potential of AP-Ph microparticles as templates of cell-enclosing spherical liquid cores of microcapsules.

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