

# Mammalian Cells In Nanocapsules: Fiber Production in Co-flowing Ambient Liquid Stream

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## ABSTRACT

Mammalian cell-enclosed in nanocapsules have been investigated as devices for bioproduction, cell therapy and stem cell research. Reduction in the diameter of the vehicles is an important issue as it induces beneficial effects such as higher molecular exchangeability between the enclosed cells and the ambient environment, as well as higher mechanical stability and biocompatibility. The author describes the effectiveness of using a jetting process involving the formation of a stretched thin jet of aqueous polymer solution and its subsequent breakup into droplets in a co-flowing water-immiscible liquid for obtaining droplets of about 100  $\mu\text{m}$  in diameter. The droplet production process and the processes for obtaining gelated microcapsules through a thermal and peroxidase-catalyzed gelation process are also described. In addition, author introduces the production of cell-enclosing hydrogel fibers using the same device developed for the production of cell-enclosing nanocapsules.

*Keywords: nanocapsules, hydrogels, gelation*

## 1 INTRODUCTION

Mammalian cell-enclosing microcapsules are devices for the production of antibodies, enzymes, and therapeutic devices in cell therapy to treat a variety of diseases such as diabetes, liver failure, hemophilia and cancer differentiation pathway of embryonic stem cells between the microenvironment *in vitro* and *in vivo*. The injection of polymer solution into an ambient co-flowing fluid stream can prepare cell-enclosed microcapsules as shown in Fig 1. Smaller microcapsules is a challenge in the field of cell encapsulation. For example, pancreatic islets-enclosing microcapsules of 300-800  $\mu\text{m}$  in diameter. Small microcapsules suffer from the occurrence of fluctuations in the air flow under the flow rate necessary for obtaining such droplets. The gelation of the droplets in a water-immiscible liquid via an enzymatic reaction is focus here.

## 2 DROPLETS PRODUCTION VIA JETTING PROCESS

Using air, small nozzles are produced by microfabrication techniques by droplet breakup in a co-flowing water-

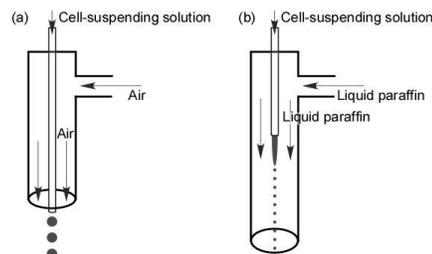


Figure 1. Schematic illustration of droplet production devices using (a) air and (b) liquid paraffin developed as an ambient co-flowing fluid.

immiscible liquid to make emulsion of 100  $\mu\text{m}$  drops (Figure 1b) similar with using air as an ambient fluid (Figure 1a) via jetting (Figure 3). The formation of a jet thinner than the needle diameter is possible by the drag force exerted by the ambient co-flowing fluid. The phenomenon of droplet breakup via jetting is explained by Rayleigh-Plateau hydrodynamic instability in Figures 2 and 4 for droplets obtained from the aqueous solution of 1.0 mPa·s, mean diameter was 655  $\mu\text{m}$ . At a liquid paraffin velocity of 23.5 cm/sec, the diameters of droplets were 202, 110, and 44  $\mu\text{m}$  for aqueous solutions of 1.0, 36 and 194 mPa·s.

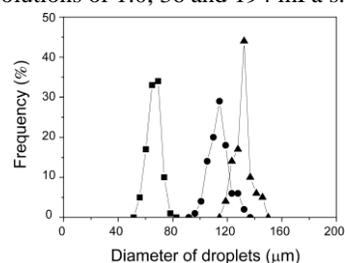


Figure 2. Size distribution of 2.0 wt% sodium-alginate extruded from a needle with 480  $\mu\text{m}$  i.d., 700  $\mu\text{m}$  o.d. at (■) 1.2 cm/sec, (●) 2.6 cm/sec, and (▲) 4.7 cm/sec into a co-flowing liquid paraffin stream with a flow rate of 17.5 cm/sec.

The percentages of the undamaged cells enclosed in the droplets of 110 and 44  $\mu\text{m}$  in diameter [defined as the viability of cells enclosed in the droplet]/[viability of the cells before extruding in co-flowing liquid paraffin  $\times 100$ ] were more than 95% (Figure 5a). In addition, cells retrieved from these droplets had same growth profiles in cell culture dishes as normal subculture protocol (Figure 5b).

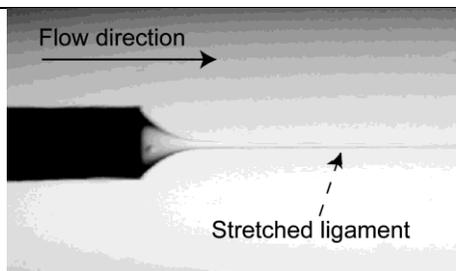


Figure 3. Sodium-alginate aqueous solution (194 mPa·s) extruded from a needle of 300  $\mu\text{m}$  i.d. and 480  $\mu\text{m}$  o.d. into the ambient liquid paraffin stream. The velocities of the sodium-alginate aqueous solution and liquid paraffin were 1.2 cm/sec and 23.5 cm/sec, respectively.

The drag force is necessary for droplet breakup in water-immiscible liquid to make droplets of less than 100  $\mu\text{m}$  in diameter and insufficient to damage cell viability and growth activity.

## 2 CELL ENCAPSULATION IN MICROCAPSULES

Liquid paraffin as an ambient co-flowing fluid makes gelled capsules or droplets of polymer solution with a diameter of less than 100  $\mu\text{m}$  to damage the cells. Agarose and alginate microcapsules are introduced in following.

### 2.1 AGAROSE MICROCAPSULES

Agarose is a natural polysaccharide. Agarose gel is formed by cooling aqueous agarose solution, which is obtained by heating an aqueous suspension of agarose powder until a clear solution is formed.

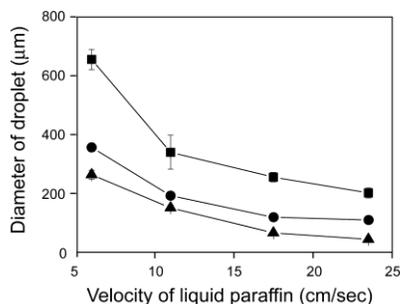


Figure 4. Droplet diameter as a function of liquid paraffin for three aqueous solution differing in viscosity: (■) 1.0, (●) 36, and (▲) 194 mPa·s. Aqueous solutions were extruded at a velocity of 1.2 cm/sec from a needle with a 300  $\mu\text{m}$  i.d. Error bars represent SDs.

Using agarose with a low gelling temperature (26-30°C at 1.5%), mammalian cells can be suspended in the solution at 38°C and kept viable. Cell-enclosing agarose microcapsules of about 100  $\mu\text{m}$  in diameter can be obtained by extruding a cell-suspending agarose solution from a needle with an inner diameter of 300  $\mu\text{m}$  into the ambient liquid paraffin stream

with laminar flow at 38°C, and subsequently cooling the resultant emulsion system in an ice bath.

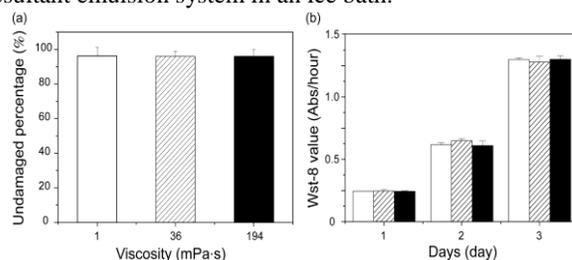


Figure 5. (a) % undamaged human tongue squamous carcinoma cells enclosed in droplets in aqueous solution with different viscosities: (white) 1, (diagonal line) 36, and (black) 194 mPa·s. (b) Proliferation profiles of the cells cultured in tissue culture dishes (white) before and after being retrieved from droplets obtained from aqueous solutions of (diagonal line) 36 and (black) 194 mPa·s.

The gelled microcapsules are collected via centrifugation after adding biological buffer into the emulsion system. Enclosed genetically modified cells express the cytochrome P450 2B1 enzyme (CYP2B1) in the agarose microcapsules of about 90  $\mu\text{m}$  in diameter (Figure 6). Ifosfamide is a prodrug that is metabolized into acrolein by CYP2B1 in the liver. Cell-enclosing microcapsules into tumors using a 26-gauge syringe after suspending them in saline. Significant regression of tumors in the recipients implanted agarose cell-enclosing microcapsules shows than those implanted with the empty agarose microcapsules (Figure 7).

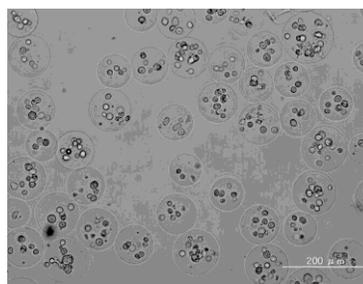


Figure 6. Photograph of CYP2B1-expressing cells-enclosing agarose microcapsules after 1 day of culture *in vitro*

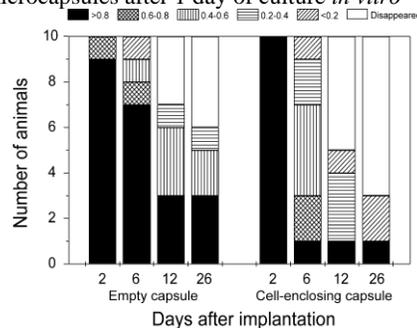


Figure 7. Preformed tumor size changes of the recipients transplanted with empty and CYP2B1 cell-enclosing microcapsules. The nude mice each were used as recipients of empty and cell-enclosing microcapsules. Tumor size just before

capsule implantation was set to 1 and each block represents the proportional size of the original tumor.

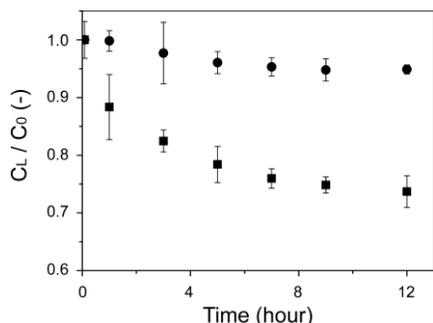


Figure 8. Time-course of diffusion of bovine serum albumin from 10 mL Krebs Ringer Hepes buffer solution (pH 7.4) into 5 mL of microcapsules at 37°C.  $C_0$ ,  $C_L$ : initial concentration and concentration of bovine serum albumin at each time point. (■): Non-treated alginate-agarose microcapsules, (●): Alginate-agarose microcapsules soaked in 0.05 wt% chitosan-acetic acid aqueous solution (pH 6.3) for 10 min

## 2.2 ALGINATE-AGAROSE COMPOSITE: AGAROSE-GELATIN CONJUGATE MICROCAPSULES

Common method is coalescence between droplet(s) of aqueous sodium-alginate solution and droplet(s) of an aqueous divalent cation solution such as calcium ions. Alginate-agarose composite microcapsules involves two steps: First, the droplets are obtained via extrusion of the mixture solution into a co-flowing liquid paraffin flow which is cooled for the gelation of agarose. After formation of spherical microcapsules via gelation of agarose, a 100 mM  $\text{CaCl}_2$  solution is added to the suspension for the gelation of alginate. The diffusion of bovine serum albumin into the microcapsules after soaking in aqueous chitosan solution show microcapsules (Figure 8). Gelatin is a natural polymer derived from collagen, and has been used for medical applications such as wound dressings and as scaffolding material for tissue engineering. With the agarose and alginate-gelatin microcapsules, cell-enclosing microcapsules can be made via the droplet breakup process using liquid paraffin and the subsequent thermal gelation process makes agarose-gelatin conjugate microcapsules.

## 2.3 ENZYMATICALLY-CROSSLINKED ALGINATE MICROCAPSULES

For enzymatic polymerization illustration, peroxidase for obtaining microcapsules. Peroxidases function as oxidoreductases that catalyze the oxidation of donors using  $\text{H}_2\text{O}_2$  resulting in polyphenols linked at the aromatic ring by a C-C and C-O coupling of phenols.

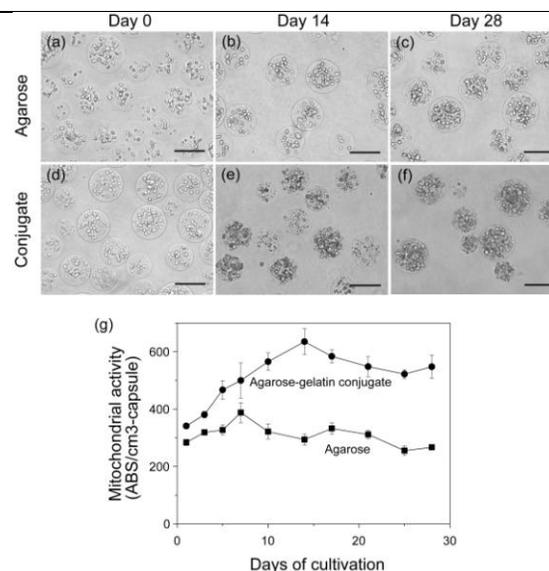


Figure 9. Microphotographs of cell-enclosing (a-c) unmodified agarose and (d-f) agarose-gelatin conjugate microcapsules (a, d) immediately after encapsulation, at (b, e) 14 d, and (c, f) 28 d of cultivation (Bars are 150  $\mu\text{m}$ ). Transition of mitochondrial activity of cells enclosed in (■) unmodified agarose microcapsules and (●) agarose-gelatin conjugate microcapsules.

The alginate incorporated hydroxyl phenol moieties into about 3% of the original carboxyl groups (Alg-Ph) and was used as the material of microcapsules. The liquid paraffin containing  $\text{H}_2\text{O}_2$  is mixed by vigorously stirring an aqueous  $\text{H}_2\text{O}_2$  solution with liquid paraffin followed by a centrifugation step to separate the liquid paraffin and non-dissolved aqueous  $\text{H}_2\text{O}_2$  solution. 5 mL of aqueous  $\text{H}_2\text{O}_2$  solution (31 wt%) was added into 1000 mL liquid paraffin. Cell-suspending 1.5 w/v% Alg-Ph solution containing 1.6 units/mL of horse radish peroxidase (HRP) was extruded into a co-flowing stream of liquid paraffin containing dissolved  $\text{H}_2\text{O}_2$  (Figure 10a). The liquid paraffin suspension with partially gelled Alg-Ph microcapsules was collected in a plastic tube. After 10 min of standing to allow for further progress of the enzymatic crosslinking reaction (Figure 10b), Alg-Ph microcapsules were collected via centrifugation. The resultant microcapsules had high sphericity (Figure 11).

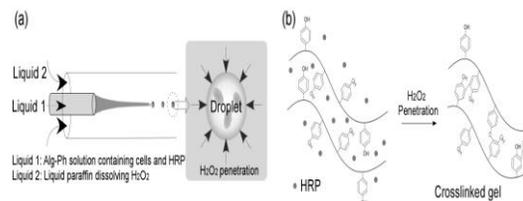


Figure 10. Schematic illustrations of (a) breakup of cell-enclosing droplets in a co-flowing stream of liquid paraffin containing  $\text{H}_2\text{O}_2$  and HRP-catalyzed gelation and (b) HRP-catalyzed crosslink formation using  $\text{H}_2\text{O}_2$  penetrating from the ambient liquid paraffin.

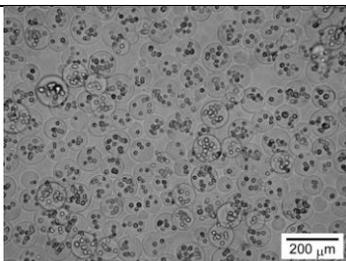


Figure 11. Micrograph of feline kidney cell-enclosing Alg-Ph microcapsules of 84.8  $\mu\text{m}$  in diameter .

Peroxidase-catalyzed encapsulation process shows cytotoxicity of  $\text{H}_2\text{O}_2$  on viability and growth profiles of the cells (Figure 12). Cell-enclosing microcapsules can be prepared 400  $\mu\text{m}$  in diameter using the peroxidase-catalyzed cell-encapsulation process using liquid paraffin containing dissolved  $\text{H}_2\text{O}_2$  is more suitable for the production of the microcapsules of about 100  $\mu\text{m}$  in diameter.

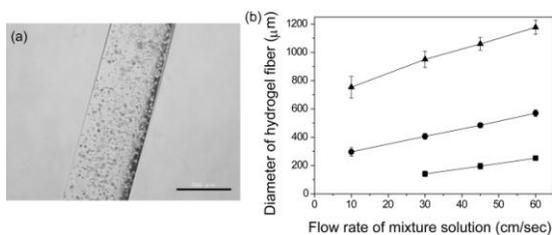


Figure 13. (a) BEC-enclosing Ca-Alg/gelatin fiber. Bar: 500  $\mu\text{m}$ . (b) Diameters of Ca-Alg/gelatin hydrogel fibers as a function of the flow rate of the Na-Alg/gelatin mixture and the diameter of the needles, (■) 270, (●) 480 and (▲) 940  $\mu\text{m}$  in diameter) from which the mixture was extruded. The flow rate of the 100 mM  $\text{CaCl}_2$  solution was fixed at 33.9 cm/s.

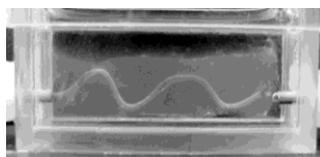


Figure 14. Photograph of the wavy-shaped channel developed in collagen gel by degrading the cell-enclosing alginate fiber

### 3 CELL ENCAPSULATION IN CA-ALGINATE GEL FIBERS

Injected cell-suspending aqueous alginate solution into a co-flowing 100 mM  $\text{CaCl}_2$  solution (Figure 13a). Similar to the droplet production in liquid paraffin, the diameter of the fiber is controllable by changing the flow rate of ambient liquid and the diameter of the needle from which the cell-suspending alginate solution is extruded (Figure 13b). Due to the flexibility of alginate gel fibers and their ability to degrade using alginate lyase, it is possible to develop a tubular construct with a straight configuration as well as a

3D configuration such as the wavy configuration observed in hydrogels (Figure 14).

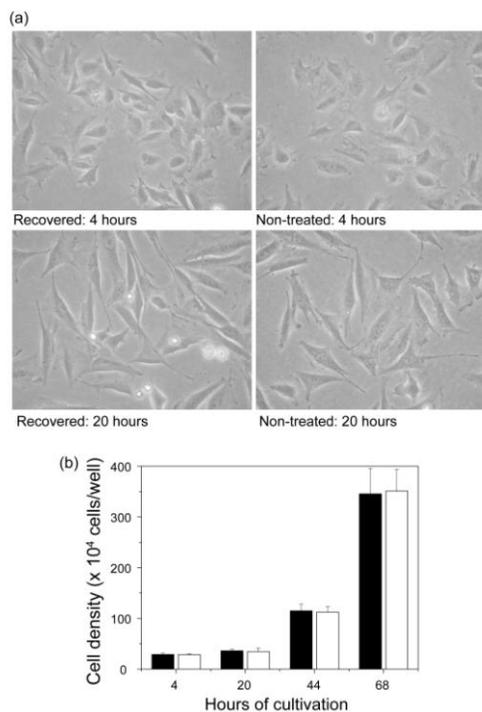


Figure 12. (a) Micrographs of CRFK cells cultured on cell culture dishes of 60 mm in diameter at 4 and 20 hours after seeding. (b) Proliferation profiles of CRFK cells on tissue culture dishes. Columns indicate mean densities of (black) recovered cells and (white) non-treated cells. Error bars: SDs

### 4 CONCLUSION

Production of cell-enclosing microcapsules of about 100  $\mu\text{m}$  in diameter can be made by jetting a cell-suspending aqueous polymer solution from a needle with a diameter of several hundred micrometers. The gelation of the droplets suspended in a water-immiscible liquid was achieved via a thermal gelation process using agarose and agarose-based materials and an enzyme-catalyzed gelation process. The small size of the microcapsules is an art to encapsulate pancreatic islets, useful devices in the field of cell therapy and bioproduction in genetic engineering of cells as reactors to produce desired proteins.

### 5 REFERENCES

1. Sakai, S. Hashimoto, I. Ogushi, Y. & Kawakami, K. (2007). *Biomacromolecules*, 8, 2622-2626.