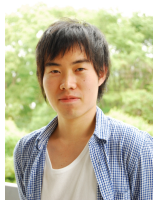
P-018	**Behaviors of tumor cells enclosed in alginate microcapsule with liquid core templated by gelatin microparticles**	

Inamoto K., Sakai S.* and Taya M.*
Chem Eng, Dep Mat Sc & Eng, Graduate School of Eng Sci, Osaka Univ, Osaka, Japan
inamoto@cheng.es.osaka-u.ac.jp

INTRODUCTION AND OBJECTIVES

Three-dimensional multicellular spheroids are increasingly recognized as valuable advanced tools for investigating cell behaviors and intercellular interactions. In cancer research, they are becoming more and more accepted as essential research tools which ideally complement the techniques already available (Friedrich 2007) because they better reflect characteristics of tumors than traditional monolayer cultures. A variety of techniques for preparing tumor cell spheroids (TCSs) has been reported (Friedrich 2007). One conventional technique employs cultivation on dishes with non-adherent surfaces. Hanging-drop culture is also conventional one. Culturing tumor cells in microcapsules is a relatively-recent technique reported for the purpose (Zhang 2005; Zaytseva Zotova 2011). We recently developed original microcapsules with spherical liquid core of around 200 μm in diameter surrounded by on-demand degradable membrane under mild condition for cells with ease (Sakai 2010). The microcapsules were prepared from gelatin and sodium alginate using the method shown in Figure 1 (Sakai 2004). The aim of this study is to evaluate the feasibility of the microcapsules for producing TCSs. For the purpose, we investigated growth profiles of enclosed tumor cells, and behaviors of enclosed cells under the existence of anti-tumor drugs. In addition, we investigated the possibilities of cryopreservation of the tumor cells-enclosing microcapsules, and production of TCSs from single tumor cell. We think that the technique to prepare TCS from one cell will have great potency in cancer research considering the heterogeneity of individual cancer cells even they are obtained from the same tumor mass.

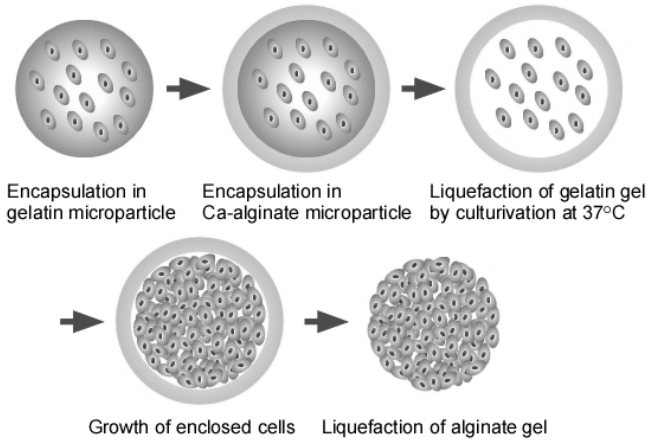


Figure 1: Scheme of TCS production.

MATERIALS AND METHODS

Microencapsulation

A human cervical cancer cell line, Hela cells were suspended in 7.5 % (w/v) gelatin solution at 2.5×10^6 cells/mL and 5.0×10^5 cells/mL for preparing gelatin microparticles containing about 10 cells and one or two cells per microparticle, respectively. Gelatin microparticles of around 200 μm were prepared using the method described previously (Sakai 2004). The resultant gelatin microparticles were suspended in 1.5% (w/v) sodium-alginate aqueous solution, and dropped into 100 mM $CaCl_2$ solution using an electrostatic droplets generator for obtaining Ca-alginate microparticles enclosing cell-enclosing gelatin microparticles. After several times of washing using cell culture medium (DMEM + 10% FBS), the microparticles were incubated at 37°C.

Anti-cancer drug treatment

The medium suspending resultant cell-enclosing microcapsules, 300 μL, was poured into 48-wells dish. Phosphate buffer solution (PBS) containing gemcitabine (pH7.4, 15 μL) at 10 to10,000 nM was added into each well. After 72 h of incubation under usual cell culture condition, the medium was removed and the microcapsules were rinsed using PBS. The viability of enclosed cells was measured using a Cell-counting kit-8 (Dojindo, Japan).

Cryopreservation

At 24 h of culture, the microcapsules were soaked in the medium containing dimethyl sulfoxide at 10% (v/v). Then, the suspension was poured into a cryotube. After 12 h of standing at –80°C, the vessel was transferred into liquid nitrogen. After 2 months of cryopreservation in liquid nitrogen, the microcapsules were put into normal cell culture medium and incubated at 37°C for evaluating growth profiles of the cryopreserved cells in microcapsules.

RESULTS AND DISCUSSION

Anti-cancer drug treatment

Enclosed Hela cells grew and formed TCSs even in the microcapsules containing one cell in one hollow core (Figure 2). The hollow cores of the microcapsules prepared for giving about 10 cells in one hollow core were almost completely filled by cells at day 14. The stationary state of growth of enclosed cells was also confirmed from the plateau values obtained by mitochondrial assay. Figure 3 shows the viability of enclosed cells and monolayer cells after being exposed to gemcitabine for 72 h.

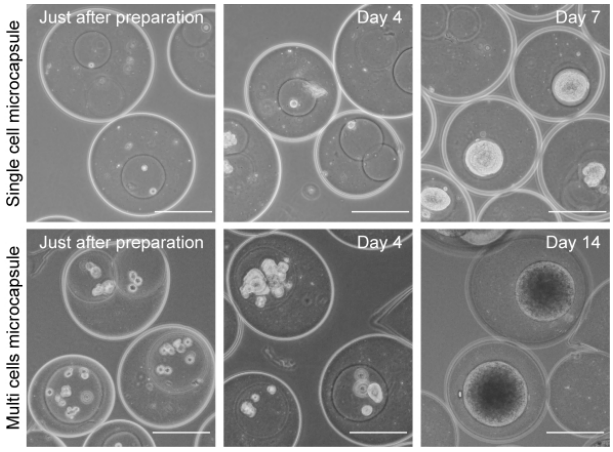


Figure 2: Transition of morphologies of Hela cells enclosed in microcapsules. The microcapsules in upper photos contain 1 cell in one hollow core. Bars: 200 μm.

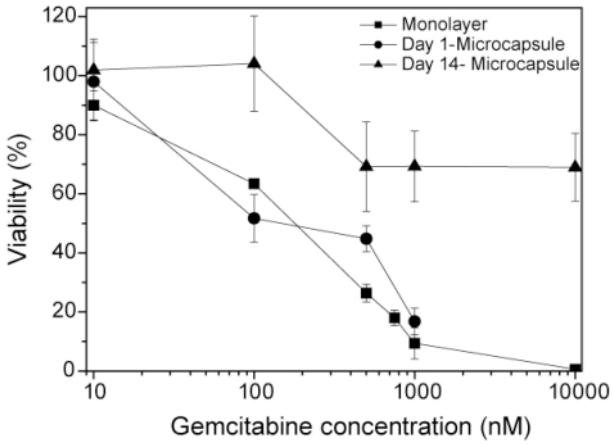


Figure 3: Effect of gemcitabine concentration on viability of enclosed cells. Bars: SD (*n* = 3).

The degree of decrease in viability of the cells after being exposed to gemcitabine at 1 day of incubation was almost the same as those in monolayer culture. The concentration of gemcitabine resulting in 50% decrease in viabilities was about 200 nM. In contrast, the enclosed cells at 14 days of incubation had a significant high tolerance to gemcitabine: The degree of viability decrease was about 30% even at 10,000 nM gemcitabine. At this concentration, almost all the cells in monolayer culture were dead.

The useful point of our microcapsules is that the microcapsule membrane is on-demand degradable without applying external forces given by pipetting and so on. The TCS-enclosing microcapsules were degraded within 30 s by soaking in sodium citrate solution (pH7.4). We measured gene expression of the cells in the TCSs collected through the process at 18 day of culture using a qRT-PCR. The expressions of multidrug resistance (MDR1) and monocarboxylic acid transporter 1 (MCT1) genes strongly correlating tolerance of cells to anti-cancer drugs were 4.6 and 12.4-times higher than those detected for the cells in monolayer culture (internal control gene: GUSB).

Cryopreservation

Next, we investigated the possibility of cryopreservation of the cell-enclosing microcapsules. Considering practical application of the tumor cells-enclosing microcapsules, the possibility of cryopreservation would be helpful for supplying homogeneous TCSs at the time of need by stocking the microcapsules produced in large quantity at one time. As seen in Figure 4, the cells, which were cryopreserved at 1 day of culture after encapsulation in microcapsules and stored for 2 months, grew successfully after thawing. The viability of enclosed cells just after thawing was 85.3%. These results clearly indicate the feasibility of our microcapsule as vehicles for cryopreservation of tumor cells for cancer researches using TCSs.

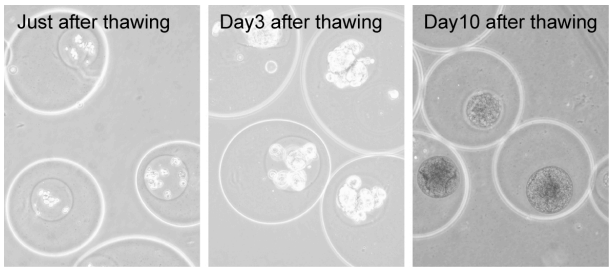


Figure 4: Transition of morphologies of enclosed cells after 2 months of cryopreserving.

CONCLUSIONS

We evaluated the feasibility of our microcapsules having well-ordered sized hollow cores and on-demand degradable membrane as a tool for preparing TCSs. The TCSs obtained from Hela cells using the microcapsules had higher tolerance to gemcitabine and higher gene expression relating to tolerance to gemcitabine than those in monolayer culture. These results demonstrate the feasibility of our microcapsule as a tool for supplying TCSs to a variety of cancer researches.

REFERENCES

- Friedrich J. et al. (2007) *Experimental anti-tumor therapy in 3-D: spheroids--old hat or new challenge?*. Int J Radiat Biol 83, 849-871.
- Sakai S. et al. (2004) *Preparation of mammalian cell-enclosing subsieve-sized capsules (<100 μm) in a coflowing stream*. Biotechnol Bioeng 86, 168-173.
- Sakai S. et al. (2010) *Calcium alginate microcapsules with spherical liquid cores templated by gelatin microparticles for mass production of multicellular spheroids*. Acta Biomater 6, 3132-3137.
- Zaytseva Zotova D.S. et al. (2011) *Polyelectrolyte microcapsules with entrapped multicellular tumor spheroids as a novel tool to study the effects of photodynamic therapy*. J Biomed Mater Res B Appl Biomater 97, 255-262.
- Zhang X. et al. (2005) *Development of an in vitro multicellular tumor spheroid model using microencapsulation and its application in anticancer drug screening and testing*. Biotechnol Prog 21, 1289-1296.