THE VIABILITY ENHANCEMENT OF ENCAPSULATED CELLS IN ALGINATE BEADS USING HYDRODYNAMIC REMOVAL OF TOXIC OLEIC ACID

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ABSTRACT

In this paper, we propose a microfluidic chip to improve cell viability after encapsulation in alginate bead used for biological assays such as stem cell differentiation and anti-cancer drug sensitivity. We added a flushing channel to exchange toxic oleic acid with mineral oil to improve cell viability. The flushing of oleic acid after the gelation of alginate dramatically increased the viability of P19 embryonic carcinoma cell up to 90%. The cell viability was proportional to the flow rate of squeezing mineral oil.

KEYWORDS: Encapsulation, Viability, Embryoid body

INTRODUCTION

Microencapsulation is one of the promising strategies for tissue engineering and cell therapy for 3-D culture of cells to mimic a tissue in vivo. Recently many articles have reported the formation of mono-disperse alginate bead using immiscible oil in microfluidic chip [1-3]. However, the gelification in calcium chloride bath was hard to remove tail-shape of bead [4]. Alternative external gelation with oleic acid containing $CaCl_2$ was used to generate spherical microbeads, but the toxicity of oleic acid do serious damage on cells. Therefore, to improve cell viability, we added flushing channel to exchange toxic oleic acid with mineral oil. The mouse embryonic carcinoma cell line, P19, was cultured in the beads and finally the formation of embryonic body (EB) was successful.

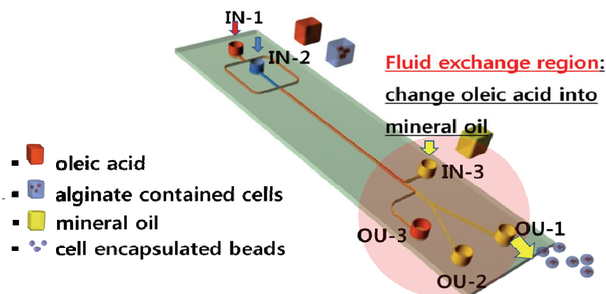

Figure 1. The schematic of cell encapsulation chip with the fluid exchange part to increase cell viability in alginate bead.

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THEORY

Alginate beads were gelified by $CaCl_2$ in oleic acid and then the oleic acid flow was squeezed by mineral oil (A-region). While the squeezed oleic acid was discharged into the waste, the beads were transferred into the mineral oil (B-region). The most of oleic acid was removed by hydrodynamic fluid exchange. The beads in the mineral oil were collected in outlet-1 and the remnant oleic acid surrounding the bead was removed by the difference of hydrodynamic resistance (C-region).

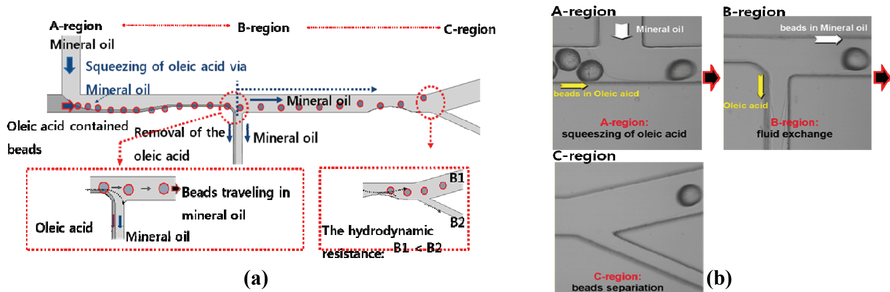


Figure 2. The schematic view and concept of oil-exchange part to increase cell viability in alginate bead; (a) The principle of fluid exchange component (b) The photographs that shows the fluid exchange from oleic acid into mineral oil.

EXPERIMENTAL

Microfluidic devices were fabricated using conventional soft-lithography and replica molding. The formation of droplets, the encapsulation of cells, and the oil-exchange were observed by a high-speed CCD video camera (FASTCAM-ulitma 512 IMAGER, PHOTRON) installed on a microscope (IX2-SLP, PLYMPUS). The images were recorded at 2000 ~ 4000 frame/sec.

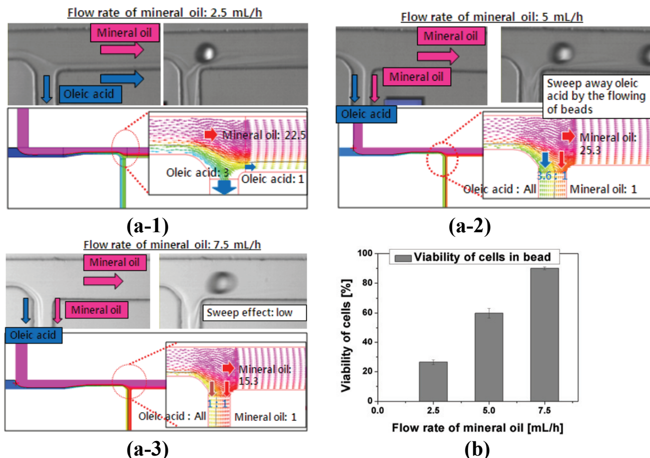
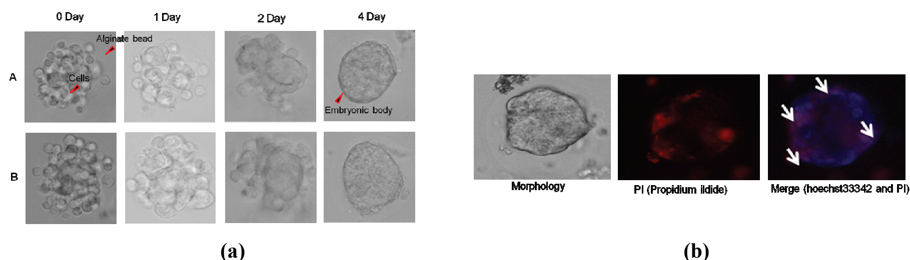


Figure 3. (a) The proportion of oleic acid flow with the variation of mineral oil flow rate (experiment and simulation) (b) The cell viability with the flow rate change (2.5, 5.0, 7.5 mL/h).

RESULTS AND DISCUSSION

Oleic acid, sodium alginate solution and cell suspension in alginate were injected from inlet-1 (350 μL/h) and inlet-2 (80 μL/h) via the syringe pump respectively. The cell viability was tested with the variation of the flow rates of mineral oil. When the flow rates of mineral oil were 2.5 mL/h, 5 mL/h, and 7.5 mL/h, the percentages of viable cells were 26.4±1.6 %, 59.8±3.3 %, and 90±1.0 % respectively. This result shows that the cell viability was directly associated with the portion of toxic oleic acid. The simulation study of fluid exchange part shows that more than 30 % of oleic acid was not removed when the flow rate was 2.5 mL/h. Increasing the flow rate to 5 mL/h removed all the toxic oil flow, but the oleic acid at the boundary of bead was not flushed thoroughly. Therefore, the flow rate was to be more than 7.5 mL/h to attain more than 90 % cell viability.

P19 embryonic carcinoma cells, encapsulated in the beads, were cultured to form EB for stem cell differentiation study. The cells were aggregated strongly to form an EB for 4 days (Figure 4). The EB was stained by propodium iodide (PI) to assess the cell viability. The dead cells in EB was observed only at the boundary of EB and most of the inner cells were alive.



(a) **(b)**

Figure 4. The formation of embryonic body in bead (a) Embryonic body formation during 4 days culture in a bead (2 samples) (b) The viability assay of embryonic body using PI staining.

CONCLUSION

The enhancement of cell viability in the alginate microencapsulation of mammalian cells was demonstrated by using oil-exchange microfluidic chip. The encapsulated EB is being transferred to a microfluidic perfusion culture chip to study the effect of alginate bead on the differentiation of stem cells.

ACKNOWLEDGEMENTS

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REFERENCES

[1] X. D. Liu et al, Journal of Applied Polymer Science, 87, 848 (2003).
[2] J. H. Xu et al, Lab Chip, 6, 131 (2006).
[3] Y Piotr Garstecki et al, Lab Chip, 6, 437 (2006).
[4] L. Capretto et al, Lab Chip 8, 617 (2008).