MICROFLUIDIC METHOD FOR THE PRODUCTION OF MONODISPERSE ALGINATE MICROBEADS AND IN SITU ENCAPSULATION OF CELLS
Chang-Hyung Choi, Jae-Hoon Jung and Chang-Soo Lee
Department of Chemical Engineering, Chungnam National University, Daejeon, Korea

ABSTRACT
This study presents a simple microfluidic method for the production of monodisperse alginate hydrogels using chaotic mixing. Droplets comprising of alginate and calcium as cross-linking agent were formed as an immiscible continuous phase, and then the alginate and calcium in the droplet came into contact and were rapidly mixed. Gelation was achieved in situ by the chaotic mixing of the droplets in the microfluidic device. By controlling flow rate, viscosity and interfacial tension, the size of hydrogel microbeads could be easily manipulated. Furthermore, the proposed method can be applied to in situ encapsulation of yeast cells in monodisperse alginate hydrogels.

KEYWORDS: Microfluidic, Alginate, Monodisperse, Mixing, Encapsulation

INTRODUCTION
Spherical hydrogel microbeads are attractive materials for imaging aids, drug delivery systems, and cell delivery systems due to their biocompatibility, wetting, nonimmunogenicity. Recently, microfluidic system clearly showed the feasibility of synthesis of alginate hydrogels. However, gelation of alginate microbeads was performed by transferring non-polymerized alginate droplets into a calcium solution from a microfluidic device [1]. This approach could provide the problem of merging between non-polymerized alginate emulsions before transferring into exterior calcium solution. Another approach showed alginate microcapsules could be produced by controlled diffusion of calcium dissolved in continuous phase [2]. Although they successfully generated microcapsules, relatively long time was required for achieving stable gelation. Here, we present a microfluidic approach involving chaotic mixing that was used to synthesize alginate hydrogels having a narrow size distribution in situ in microfluidic channels.

EXPERIMENTAL
The microfluidic devices depended on the use of two aqueous flows including alginate and calcium chloride, and hydrophobic hexadecane as continuous phase (Fig. 1). The two aqueous flows were continuously injected into a flow of water-immiscible hexadecane in the main microchannel, where they spontaneously pinched off at the junction. In our experiment, two types of microchannel were applied to generating alginate beads and encapsulating cells in situ, separately (Fig. 2).
RESULTS AND DISCUSSION

As shown Fig. 3, typical phase diagram of the microfluidic system in Ca-Q\textsubscript{aq} coordinates. Three kinds of flow formation were observed in microchannel: Unstable (I), Uniform droplets (II), and laminar flow (III). We could find the optimized condition of monodisperse alginate gel through this phase diagram.

Figure 3. (a) The flow patterns observed in microfluidic device (b) A image of the three regions

Figure 4 shows optical microscopy image of produced alginate microbeads in our system. The alginate beads had a very narrow size distribution.

Figure 4. (a) The optical images of the monodisperse alginate beads produced using microfluidic device, scale bar=100 μm (b) The size distribution of generated microbeads (CV=1.1%)
As shown Fig 5(a), the microbead size was decreased with a decrease in the interfacial tension as varying concentration of surfactant in hexadecane phase because thread of fluid becomes thinner. Figure 5(b) demonstrates the effect of the alginate viscous property on the average microbead size for various concentration of alginate solutions.

To demonstrate the in situ encapsulation of cell in our microfluidic device, GFP-yeast cell was loaded into additional injection line (Fig. 1(b)). Figure 6 shows typical images from the encapsulated cell in alginate beads, and confirms that the cells were successfully encapsulated into alginate gel with our approach.

CONCLUSIONS
A microfluidic approach for the in situ production of monodisperse alginate hydrogels has been demonstrated. As a proof of concept, the size of alginate beads was manipulated by changing concentration of surfactant and viscosity. In addition, we provide versatile tools for encapsulation of cell and protein for drug delivery and cell transplantation and microreactors.

ACKNOWLEDGEMENTS
This study was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (Project No: A062254).

REFERENCES