MICROENCAPSULATION OF LIVING CELLS INTO 150 MICROMETER MICROCAPSULES USING MICRO-AIRFLOW-NOZZLE

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Introduction

Microencapsulation of living cells represents a promising alternative nonviral strategy to gene therapy (1,2). A commonly used system for microencapsulation is a polyion complex system based on alginate and poly-L-lysine (PLL) originally developed by Lim and Sun (1). Generally, calcium alginate beads are formed by dripping an alginate solution into a $CaCl_2$ solution. Alginate-PLL-alginate (APA) microcapsules are formed by coating the formed calcium alginate gel beads with a PLL layer and an outer alginate layer. For the use of cell transplantation, smaller microcapsules offer many advantages, such as better transportation of nutrients and oxygen, smaller volume, better dispersion and better mechanical strength.

In order to prepare small microcapsules, different methods have been developed, including the coaxial airflow method, vibrating nozzle method and electrostatic droplet generation (3). Calcium alginate beads with 300 to 800 μm diameters have been successfully prepared by utilizing these methods. Preparation of smaller beads of less than 300 μm with a narrow size distribution has been impractical to date, due particularly to nozzle diameter limitations. We recently proposed a novel method for generating monodisperse emulsion droplets from a microfabricated channel array (4-6). Applying these technologies, we have previously proposed a droplet-droplet reaction system in a water-in-oil dispersion, in which monodisperse alginate droplets and $CaCl_2$ droplets were reacted in soybean oil to form calcium alginate beads smaller than 300 μm (7). However the process using oil phase causes an adhesive oil layer on the surface of the prepared beads. Therefore, the simple calcium alginate beads formation process without using an oil phase is desired.

In this study, we have developed a new micro-airflow-nozzle (MAN), in order to effectively produce 140 to 300 μm APA microcapsules. The device enabled us to prepare calcium alginate gel by simply dripping the alginate solution directly into a $CaCl_2$ solution. Using the MAN, living cells were encapsulated in 150 μm APA microcapsule.

Experimental Part

Figure 1 schematically illustrates the experimental setup. The MANs were fabricated on a silicon plate by photolithography and the multi-step deep reactive ion-etching process (6). The MAN is composed of a nozzle for an alginate solution channel and airflow channels next to the nozzle. The nozzle has a 60 μm internal diameter, 120 μm external diameter and 80 μm height. The airflow channel is composed of 240 μm concentric cylindrical channels.

Chinese hamster ovary (CHO) cells were transfected with a pIRES2-EGFP vector (Clontech Laboratories, Inc., Palo Alto, CA, USA) encoding GFP using Lipofectamine (Invitrogen Corp., Carlsbad, CA, USA). The transfected cells (CHO/NK4-GFP) were cultured in minimum essential medium-alpha (MEM-alpha; Invitrogen Corp., Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen Corp.).

Calcium alginate bead formation procedure was as follows. The sodium alginate (Product name IL-2, Kimica Corp., Tokyo, Japan) solution at 1.5% wt/vol concentration was extruded through MAN with a syringe pump and sheared by the airflow. The resulting alginate droplets fell directly into a 1.1% $CaCl_2$ solution, stirred for 30 min and calcium alginate beads were formed. In order to encapsulate the cells into APA microcapsules, the harvested cells were resuspended in a sodium alginate solution at a final concentration of the 1.0 x 10^7 cells/mL, and calcium alginate beads were prepared as described above. The prepared calcium alginate beads were coated with a PLL (molecular weight 30,000-70,000) layer as described previously (8).

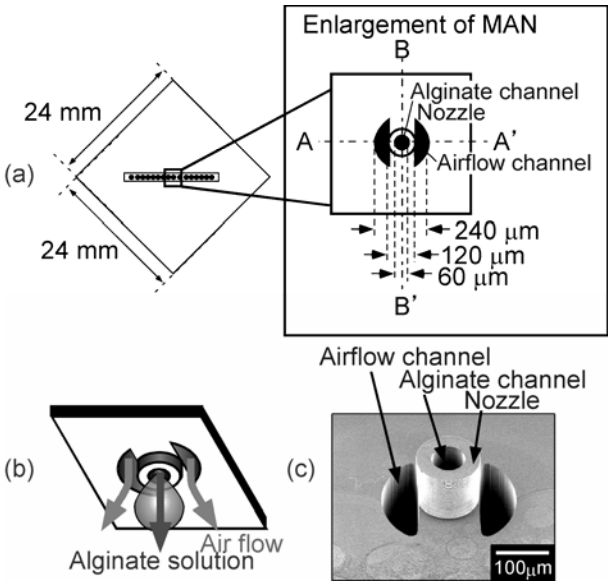

Figure 1. Schematics of MAN (a), schematic flows of alginate solution and airflow (b) and SEM photographs of MAN (c).

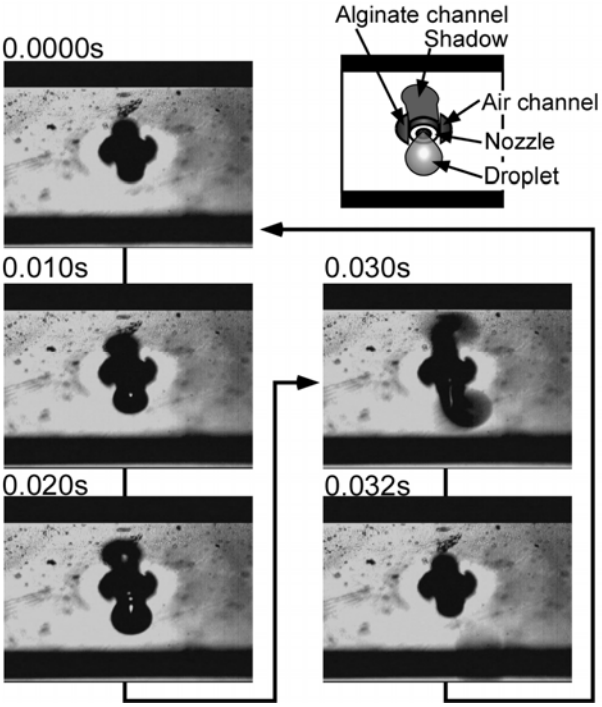

Figure 2. High-speed camera sequence of droplet formation from MAN.

Results

A sodium alginate aqueous solution was extruded through an MAN at a 5 mL/hr flow rate. Airflow was supplied to two side air-channels at a 1.2 L/hr flow rate for each single air-channel. Figure 2 shows a droplet formation process observed with microscope equipped with a high-speed camera. The same-sized droplets were sequentially prepared every 0.32 s. The measured average droplet diameter was 176 μm. The prepared droplets fell directly into the $CaCl_2$ solution and calcium alginate gel beads were formed. Figures 3a and 3b represent the resulting calcium alginate beads at different airflow rates. Airflow rates in each single air-channel were

1.2 L/hr for Figure 3a and 0.9 L/hr for Figure 3b. The diameter of the prepared beads decreased with increasing airflow rate as known for concentric airflow method. Figure 3c shows diameter distribution of prepared calcium alginate beads. Number-average diameters and coefficients of variation of the prepared calcium alginate beads were 139 μm and 7.3% for a 1.2 L/hr airflow rate, and 301 μm and 4.5% for a 0.9 L/hr airflow rate. Spherical and monodisperse calcium alginate beads were prepared in both cases.

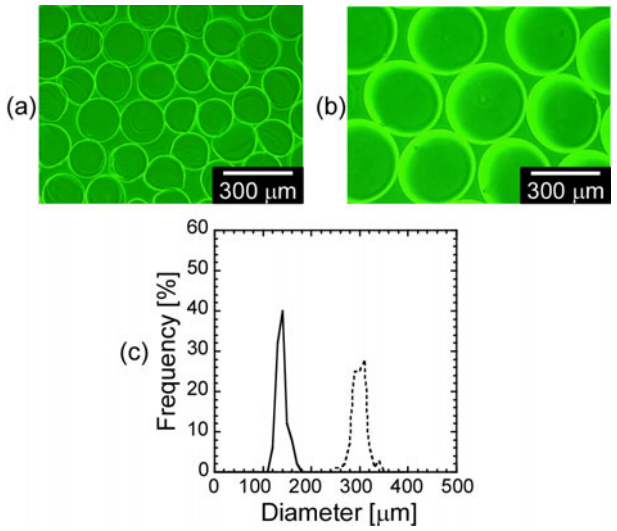

Figure 3. Microscope photographs (a, b) and diameter distribution (c) of prepared Calcium alginate beads. Airflow rate of each single air-channel was 1.2 L/hr (a, solid line in c), and 0.9 L/hr (b, broken line in c).

The bead formation system proposed in this study can be applied for encapsulation of living cells, since the system works under sterile conditions. CHO/NK4-GFP cells were encapsulated into the calcium-alginate beads using the MAN. The prepared calcium alginate beads were coated with PLL. APA microcapsules with 150 μm diameter containing CHO/NK4-GFP cells were successfully prepared using MAN as depicted in Figure 4. The prepared APA microcapsules were easily dispersed in the aqueous solution. Green fluorescence from the GFP produced by the cells in the microcapsule is depicted in Figure 4b, indicating that living cells were encapsulated while maintaining their viability.

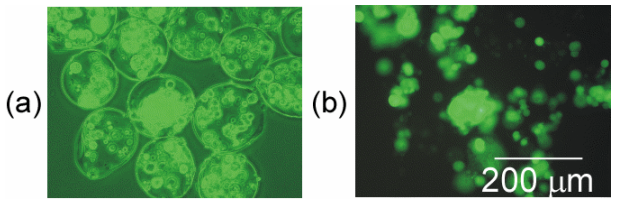

Figure 4. Cell encapsulation into APA microcapsules. Microscope photographs of APA microcapsules containing CHO/NK4-GFP cells observed in a bright field (a) and fluorescence mode (b).

Discussion

We have presented a novel device for production of small APA microcapsule. It has a microfabricated nozzle with a 60 μm internal diameter. Calcium alginate beads were formed by a simple process, in which an alginate solution was sheared by airflow and the prepared droplets were directly dripped into the $CaCl_2$ solution. The device enables us to prepare 140 to 300 μm APA microcapsules and encapsulate living cells into 150 μm APA microcapsules. The microcapsules were formed under sterile conditions and without chemical irritants such as surfactant, which enabled successful encapsulation of living cells in the APA microcapsules.

The microfablicated nozzle is advantageous in terms of precision and low pressure loss (short nozzle) compared to the conventional needle-based system. The size of the prepared beads was controlled by the airflow rate. Smallest limit is restricted by the external diameter of the nozzle. In order to prepare smaller beads, a thinner nozzle should be fabricated. From the point of microfabrication technology, fabrication of a thinner nozzle is possible, however, droplet formation would be unstable if the nozzle size is similar to the cell size.

Smaller capsules had the advantages including better transportation of nutrients and oxygen, high mechanical strength and better dispersion. In addition, smaller microcapsules enable injections with a

thinner needle and infusions through a selectively placed catheter. This would provide local delivery of recombinant proteins into thinner blood vessels. These characteristic physicochemical and biological features of smaller microcapsules create the new prospects for microencapsulation technology due to their various advantages.

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