Using a CD-like Microfluidic Platform for Uniform Calcium Alginate Drug Carrier Generation

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ABSTRACT

In this paper the manipulation of monodisperse Ca-alginate microparticles using a polymer-based CD-like microfluidic platform and a reaction of external gelation is presented. Our strategy was based on associating the rapid injection molding process for cross-junction microchannel with the sheath focusing effect to form uniform water-in-oil (w/o) emulsions. These fine emulsions, consisting of 1.5% w/v Na-alginate, were then dripped into an oil solution containing 20% w/v calcium chloride ($CaCl_2$) to accomplish Ca-alginate microspheres in an efficient manner. We have demonstrated that one can control the size of Ca-alginate microparticles from 20 μm to 50 μm in diameter (with a variation less than 10%) by altering the relative sheath/sample flow rate ratio. Experimental data showed that for a given fixed dispersed phase flow (sample flow), the emulsion size decreased as the average velocity of the continuous phase (oil flow) increased. The proposed CD-like microfluidic platform is capable of generating relatively uniform micro-droplets and has the advantages of active control of droplet diameter, simple and low cost process, and high throughput.

Keywords: injection molding, alginate, microfluidic, emulsion

1. INTRODUCTION

Ca-alginate is currently gaining a great deal of attention for medical applications as well as for the controlled release of drugs [1]. The success of Ca-alginate beads as carriers is due to the following features: (i) they can dissolve poorly soluble drugs and thus increase their bioavailability, (ii) they have low toxicity and high loading capacity, as well as minimize drug degradation and loss, and (iii) they can be easily produced in large quantities [2]. Of critical importance to their successful implementation as a drug deliverer is their ability to control particle size and size distribution, as this influences the clearance rate from the body which ultimately determines the drug dosage [3]. Basically, an ideal particle size can provide an optimal release rate.

To date, the production of Ca-alginate beads has been accomplished mainly by using external gelation (dripping method). For example, Na-alginate is extruded dropwise through a needle into a solution of divalent cations, which induces crosslinking of the guluronic residues of the alginate polymer [4]. The alternative techniques are (i) atomization (spray-drying) [2, 5], (ii) coacervation [6] (iii) emulsification (internal/external gelation) [7], and other methods [8]. However, the above techniques have well-known drawbacks such as unstable yield, tedious procedures and non-uniform particle sizes with a wide size distribution. It has become imperative for the pharmaceutical industry to develop a reproducible method for generating Ca-alginate microparticles with a uniform particle size and a narrow size distribution in a controlled manner.

A new microfluidic device is developed for size-controlled monodisperse Ca-alginate particles. Microfluidic chip (containing cross-junction microchannel) emulsification is a novel technique for preparing water-in-oil (w/o) and oil-in-water (o/w) emulsions. The mechanism of this type of microfluidic chips in droplet-volume control was well studied recently [9].

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In addition, the polymer-based technologies have widely applied to fabricate the biomedical devices to meet the requirement of the low cost, biocompatibility, flexible process or the capability of mass production [10]. However, no attention has been paid to apply optical disc process to fabricate the polymer CD-like microfluidic chip and use for control the performance of uniform micro-drug carriers. Therefore, this research is introduced a fabrication technique capable of time-saving, low cost, mass production, and multi-functional structures on uni-substrate. Then investigating and comparing the size of the Ca-alginate microspheres obtained by a different ratio of flow rate in the center inlet to the side inlet channels. This proposed CD-like microfluidic platforms are easy to fabricate, set-up, and generate a large set of regular Ca-alginate microspheres.

2. MATERIALS AND METHODS

2.1 Materials

The material of microfluidic substrates in this experiment is optical grade PC (AD5503, Teijin Corporation, Japan). The glass transition temperature is approximately 145°C. The cylinder temperature is controlled from 320°C to 380°C, and the temperature of the stationary side mold is controlled from 100°C to 120°C. Commercial sample of low viscosity sodium alginate (Na-alginate, viscosity 250 cps in 1.5% solution at 25°C; brown algae), was purchased from Sigma Chemical Co. (MO, USA). A sunflower seed oil was purchased from Uni-President Enterprises Corp., Taiwan. All other chemicals (calcium chloride purchased from Panreac Quimica SA) were of analytical grade and used without further purification.

2.2 Microfluidic substrate molding

In this study, the injection molding is conducted on a high-speed injection machine (Sumitomo SD-35E), and the Seiko Giken F type optical disc mold is adopted. The details of the optical disc process were described as follows (Fig. 1). First, a piece of Ni plate with 300 μm thick was electroformed, and then the photoresist (PR) was coated with 20-200 nm thick on its surface. Next, the PR on the Ni was exposed and developed. Finally, the Ni plate was electroformed into 50 μm again. The mold insert was integrated with the mold insert holder and the mold. With the appropriate injection parameter adjusted, the plastic optical disc substrates could be fabricated.

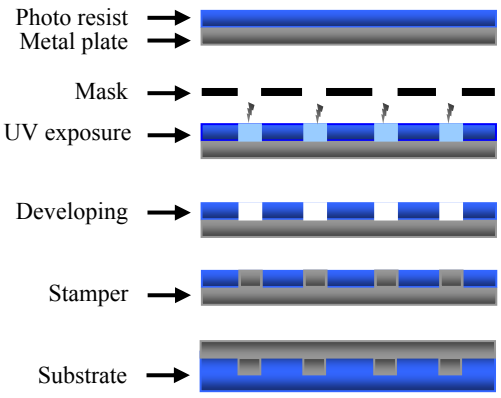

Fig. 1. The optical disc fabrication process for CD-like microfluidic substrates.

2.3 Principle

In this study we reported the use of microfluidics to elicit control over the spontaneous self-assembly of w/o emulsions from a solution of dissolved Na-alginate. These semi-products (emulsions) were then dripped into a solution containing calcium (II) ions, resulting in the instantaneous formation of Ca-alginate microspheres. The mechanism of Ca-alginate microspheres synthesis was that the calcium (II) ions released by calcium chloride were then undergoing crosslinking with Na-alginate micro-droplets to produce Ca-alginate microparticles (external gelation, as shown in Fig. 2). The

generation of a narrow size distribution of self-assembling emulsions was based on a focusing force in the cross-junction channel (Fig. 2a). The mechanism of this type of CD-like microfluidic platform was that by varying the ratio between oil and water flow rates provides finer control of the droplet sizes [9]. Based on the outstanding performance of the CD-like microfluidic platform, we utilized it in this work for pharmaceutics (e.g. Ca-alginate particle generation).

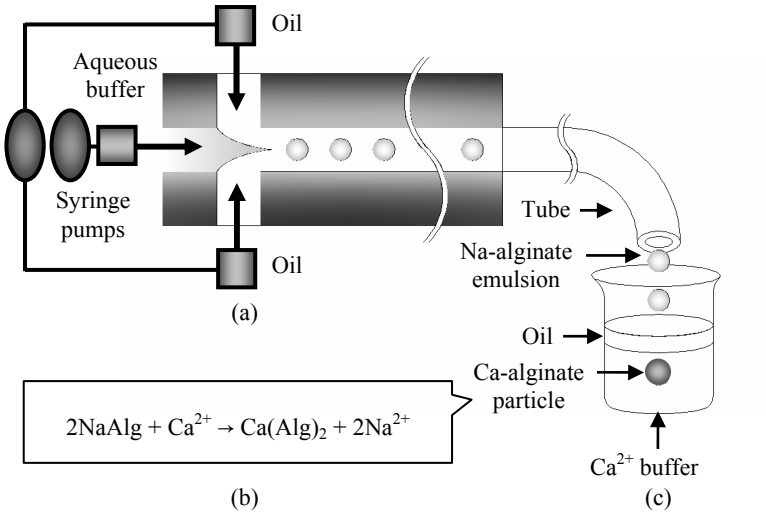

Fig. 2. Illustration of system mechanism: (a) schematic drawing of Na-alginate emulsion generator in a cross-junction microchannel, (b) the mechanism of Na-alginate polymerization: the chemical reaction in the reservoir is that the sodium ions of alginate are substituted by calcium ions, indicating the formation of Ca-alginate particles, and (c) a reservoir.

2.4 Experimental procedure

Figure 3 shows an overview of the experimental setup used. The procedure is as follows. First, the fluids of the center and side inlet channels were connected to Na-alginate solution (sample flow) and oil (sheath flow), respectively. Generally speaking, the material to be encapsulated is mixed with an alginate solution. Second, the fluids are then injected into the CD-like microfluidic platform by syringe pumps (Kdscientific KDS230) programmed by a PC. In this work we hydrodynamically focus a stream of aqueous solution (Na-alginate solution, dispersed phase) at a cross-junction microchannel by two oil streams (continuous phase), enabling the construction of w/o Na-alginate emulsions along the microchannel. Finally, the Na-alginate emulsions were then undergoing gelation by dripping them into a calcium (II) ion solution to formed Ca-alginate particles.

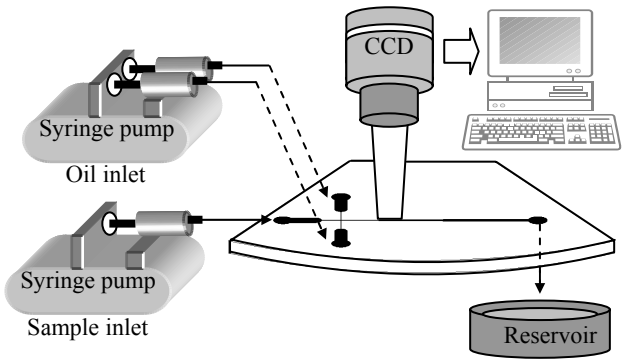

Fig. 3. Schematic representation of a Ca-alginate microparticles generator set up

2.5 Microspheres size measurement

A fluorescence microscope is used to observe the experimental results. The image and detection system consist of an optical microscope (BX60, Olympus, Japan) and a digital camera (DP70, Olympus, Japan). The diameter of each microsphere was measured and averaged. A total of 30 microspheres were measured to provide an average size.

3. RESULTS AND DISCUSSIONS

3.1 CD-like microfluidic platform

This CD-like microfluidic platform was low cost, easy to fabricate, easy to set up, as well as easy to organize and program. It consisted of four layers from top to bottom (an expanded view was shown in Fig. 4a): the cover layer (contained one outlet port and three inlet ports), the meso-layer (prevented from the fluids leaking out), the CD-like microfluidic substrate (cross-junction channel, depth/width: 50 μm/50 μm) and the bottom layer (the disk structure, 55.0 mm in radius, was designed in a modular specification for placing on an inverted fluorescent microscope), respectively. The cover and bottom layers were both laid out on a conventional poly methyl methacrylate (PMMA) using a CO_2 laser machine (LaserPro Venus, GCC, Taiwan), and the meso-layer was fabricated with PDMS by PMMA molding. Finally, these four layers were integrated by screws (a photo image was shown in Fig. 4b).

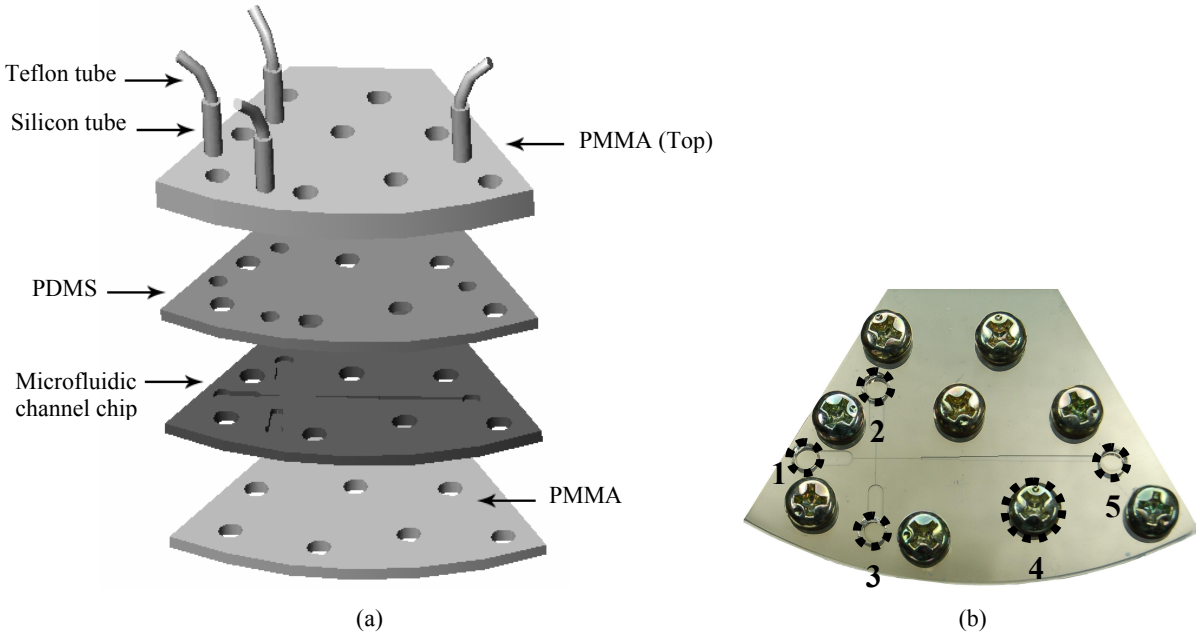

Fig. 4. (a) The expanded view and (b) the photo image of a CD-like microfluidic platform: 1-3. inlet ports, 4. screws, 5. outlet port.

3.2 Formation of monodisperse emulsions

For the uniform w/o Na-alginate emulsions generation, 3 mL 1.5% (w/v) Na-alginate solution and 3 mL sunflower seed oil (40 mPa s (cP)), were employed as the sample-phase fluid and oil-phase fluid, respectively. A water-soluble dye (red ink) was dissolved in the sample solution for immediate (real-time) observation. This viscous solution was then fluidified by shear forces in the CD-like microfluidic platform equipped with a cross-junction channel, and the resulting uniform semi-products (Na-alginate emulsions) are observed and characterized by an inverted fluorescent microscope.

In the initial experiments, the flow rates of the sample-phase and the oil-phase fluids were set to 1.000 μL/min. We found that the sample-phase fluid was compressed by a shear force to an arrow shape (Fig. 5) and then separated into

emulsions of about 50 μm in diameter. In addition, the diameter distribution of the emulsions formed was quite uniform (50 ± 5 μm), and the gap between each emulsion was stable (75 ± 5 μm). The flow rates of the oil and the sample solution were adjusted to control the degree of hydrodynamic focusing and the width of the center stream, resulting in the generation of size-controlled Na-alginate emulsions. Therefore, we conclude that the hydrodynamic focusing can perform the emulsification in a size-controlled manner. In order to take these emulsions out for advanced applications, we solidify the Na-alginate emulsions by the gelation.

3.3 Formation of Ca-alginate microparticles

The semi-products (Na-alginate emulsions) were formed in the continuous oil flow. The continuous oil flow could prevent these semi-products from fusing together, and could transport them to the calcium carbonate solution through a Teflon tube; they precipitated spontaneously at the bottom of the oil due to their higher density than that of the oil. Therefore, Na-alginate emulsions could react with calcium (II) ion at the interface between the oil phase and the water phase. The water-soluble Na-alginate emulsions after they had undergone crosslinking were gelled into solid spheres upon contact with calcium (II) ion by external gelation, result in water-insoluble Ca-alginate microspheres.

The Ca-alginate microspheres prepared as described above were separated from the calcium chloride solution and oil by vacuum filtration. They were washed twice with 30 mL *n*-hexane/ether, and then cleaned with 10 mL 50 mM Tris–HCl buffer (pH 7.2). All the Ca-alginate microspheres were then subject to freeze-drying. After being dipped in liquid nitrogen, they were dried at -70°C under vacuum (0.1 mmHg) for 10 hours and then vacuum-dried at room temperature for 1 hour. We found the shapes of most Ca-alginate microspheres remained spheroid after the gelation and the freeze-drying process.

3.4 Influence of flow rate

To gain further understanding, the relationship between size and flow speed were studied. The emulsion size was easily varied by changing the flow conditions in the microchannel. Fig. 5 showed the relationship between the average flow speed of the phases and the emulsion size (diameter), it was evident that the size and gap of the emulsions, generated in the cross-junction, were controllable and reproducible by using the CD-like microfluidic platform.

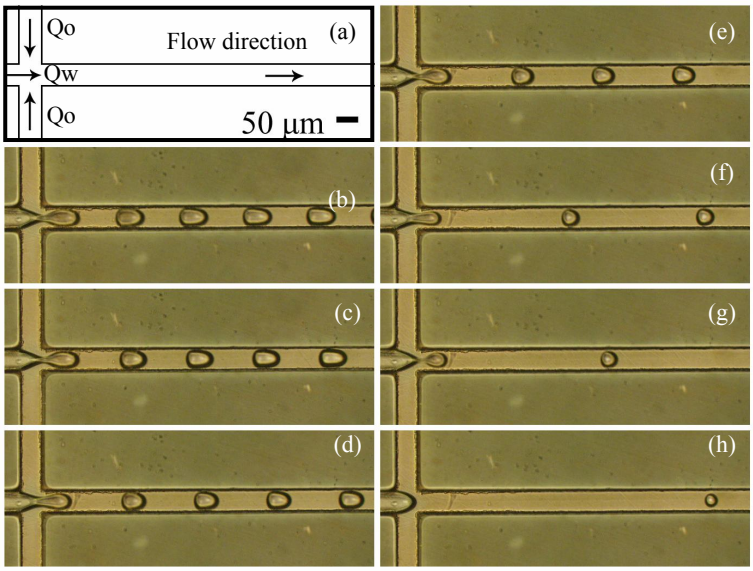

Fig. 5. Water in oil emulsions generation: (a) chart of flow directions, (b) to (h) show the emulsions generation at fixed oil flow rate (Qo) and the water flow rate (Qw) is reduced from (b) to (h).

4. CONCLUSION

We demonstrate in this study a CD-like microfluidic platform that utilizes cross-junction microchannel, enabling to produce 20-50 μm Ca-alginate beads with a narrow size distribution (<10%). This method has turned out to be one of the most efficient methods for the production of monodisperse Ca-alginate microparticles. We designed a simple and cost-effective platform for manipulation of Ca-alginate microparticles by the immiscible property of sample and oil solutions in the microchannel and in-situ external gelation. We found that the oil-phase fluid (the pressure gradient conditions were created by the syringe pump) can pose a focusing/shear force on the sample-phase fluid in the microchannel, it can be used for the dynamic control of emulsion size. Our platform is very attractive from a practical point of view, since it easily emulsifies and yields extremely uniform microemulsions with a very high loading capacity. The approach in manipulation of Ca-alginate microspheres will provide many potential usages for pharmaceutical applications.

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