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Microfluidic device utilizing pneumatic micro-vibrators to generate alginate microbeads for microencapsulation of cells

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ABSTRACT

This study reports a new microfluidic device for continuous generation of alginate microbeads. The working mechanism is based on the use of a pneumatically driven micro-vibrator to continuously generate tiny alginate microdroplets into a thin oil layer. The temporarily formed alginate microdroplets soon sink into a sterile calcium chloride solution to form jelled microbeads. By regulating the flow rate of the alginate suspension and the pulsing frequency of the micro-vibrator, the size of the alginate microbeads can be controlled. Experimental results showed that alginate microbeads with sizes ranging from 73 to 302 μm in diameter can be generated at suspension flow rates and vibration frequency ranges of 1.48–9.35 μl/min and 2–16 Hz, respectively. For the aforementioned parameter ranges, the alginate microbeads had reasonable size uniformity with coefficients of variation from 3.8% to 7.8%. Moreover, its application for the microencapsulation of chondrocytes in alginate microbeads has also been demonstrated with high cell viability (94 ± 2%). As a whole, the proposed device has opened up a route to generate alginate microbeads or microencapsulation of cells in a simple, continuous, controllable, uniform, and cell-friendly manner with less contamination.

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1. Introduction

Cell microencapsulation has been widely used in a variety of biomedical fields [1–7]. There are some important technical criteria regarding the appropriateness of cell microencapsulation technology for clinical use including the biocompatibility of encapsulating materials, contamination issues during preparation, and the viability of the resulting encapsulated cells. Regarding the biocompatibility issue, there are many types of natural or synthetic polymers that have been used to encapsulate living cells. Among them, alginate is a frequently used hydrogel for the microencapsulation of cells mainly because of its mild gelling conditions, simple gelling processes under the existence of divalent cations (such as calcium cations) and excellent biocompatibility and biodegradability properties [8,9]. The use alginate or its derivatives has been demonstrated in the literature to encapsulate different cell species for various biomedical applications such as bone [10] or cartilage [11] regeneration, and for treatments for diabetes [12] or cancers [13].

With respect to the strategies for the microencapsulation of cells, conventional encapsulation techniques such as spray drying, spray cooling, extrusion, fluidized beds, coacervation or emulsification may not be suitable for this application. This is because these processes might involve some incompatible operating conditions such as high pressure or temperature, or an organic solvent, which could in turn adversely affect the encapsulated cells or cause contamination. In addition to these strategies, a number of techniques have been recently developed to generate cell-encapsulated microbeads. Briefly, they can be categorized into three major approaches, namely photolithography [14], micro-molding [15] and microfluidic technology [16–19]. Although the approaches of photolithography and micro-molding have been demonstrated to be suitable for encapsulating living cells [14,15], the main shortcomings of these two processes are concerns with ultra-violet (UV) exposure, and the troublesome de-molding process, respectively. With recent advances in microfluidic technology, this has paved an alternative route to produce cell-encapsulated microbeads. The key advantage to using a microfluidic-based strategy for microbead generation or cell microencapsulation is that cell-entrapped microbeads with a high uniformity can be achieved [16,20,21]. Besides, the application of the microfluidic technique can provide an automatic, or continuous way, to generate cell-encapsulated microbeads in contrast to the approaches of photolithography or micro-molding, by which the microbeads are generated in a batch-wise manner. Moreover, a major advantage for clini-

Abbreviations: CCD, charge coupled device; CNC, computer numerical control; CV, coefficient of variation; EMV, electromagnetic valve; PBS, phosphate buffered saline; PDMS, polydimethylsiloxane; PMMA, polymethylmethacrylate.

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cal use, microfluidic technology can generate cell-encapsulated microbeads with a high level of sterility due to the confined microfluidic system.

Recently, several microfluidic systems based on various working principles, including electrospraying [22], the combination of shear force from airflow with a microfabricated nozzle [19], an emulsification mechanism coupled with microchannels with specific geometric designs [18,23–29] or by mechanical agitation [17], have been successfully demonstrated to perform microbead or microsphere generation, or microencapsulation of cells. Although electrospraying provides a useful tool to generate microparticles or to encapsulate biological substances, the impact of an applied high electric field on delicate biological entities should be taken into consideration, which is normally not explored. Alternatively, shear flow generated by a nozzle has been used to continuously generate alginate microdroplets and then microbeads after dipping into a calcium chloride solution [19]. Although this method has proven the feasibility of producing microbeads with a high uniformity, the fabrication and assembly of such devices are, to some extents, technically demanding. More importantly, a concern about the impact of the high shear stress experienced in this process on the viability of the encapsulated cells should be properly addressed. Other types of microfluidic systems make use of two immiscible fluids (e.g. oil [17,18,20,23,27,28] or organic solvents [16,26]) to sandwich an aqueous stream containing the encapsulated substances in a microchannel. The microdroplets (e.g. water-in-oil emulsions) were formed in the oil or organic phase through the design of microchannels with a specific geometry [18] or from mechanical agitation [17]. The microdroplets formed in such processes were subsequently delivered to a container, where the gelation of microbeads occurred. Compared with the generation of microbeads using the principles of electrospraying or airflow-based nozzle design as previously discussed, these microfluidic systems are easier to fabricate, mainly by the well-established soft-lithography technique using polydimethylsiloxane (PDMS) [16,17]. However, these designs involved the use of oil or an organic phase, or mechanical forces to generate water-in-oil emulsions (microdroplets). In the process, these designs may be harmful to the living cells or may cause contamination. For example, the use of an organic solution or mechanical agitation may damage the delicate cell's organelles and thus may affect cell viability. Also, the exposure of gelled microbeads to oil or organic phase could lead to contamination, which may hinder their subsequent applications. Nevertheless, little attention has been paid to these issues.

In order to generate cell-encapsulated microbeads in a simpler, continuous, controllable, cell-friendly manner with less contamination, this study proposes a new microfluidic device for the microencapsulation of cells in alginate microbeads. The working mechanism is based on the manipulation of a pneumatically driven micro-vibrator to create droplet spotting for generating alginate microdroplets in a thin oil layer. The temporarily formed alginate microdroplets soon sink into a sterile calcium chloride solution located below, to become gelled microbeads. By regulating the flow rate of the alginate suspension (delivered by a spider-web micropump) and the pulsing frequency of the micro-vibrator, the size of the alginate microbeads can be controlled. The application of the proposed device for the microencapsulation of chondrocytes in alginate microbeads is also explored.

2. Materials and methods

2.1. Design

A photograph of the prototype microfluidic device for alginate microbead generation is shown in Fig. 1(a). It is comprised of five

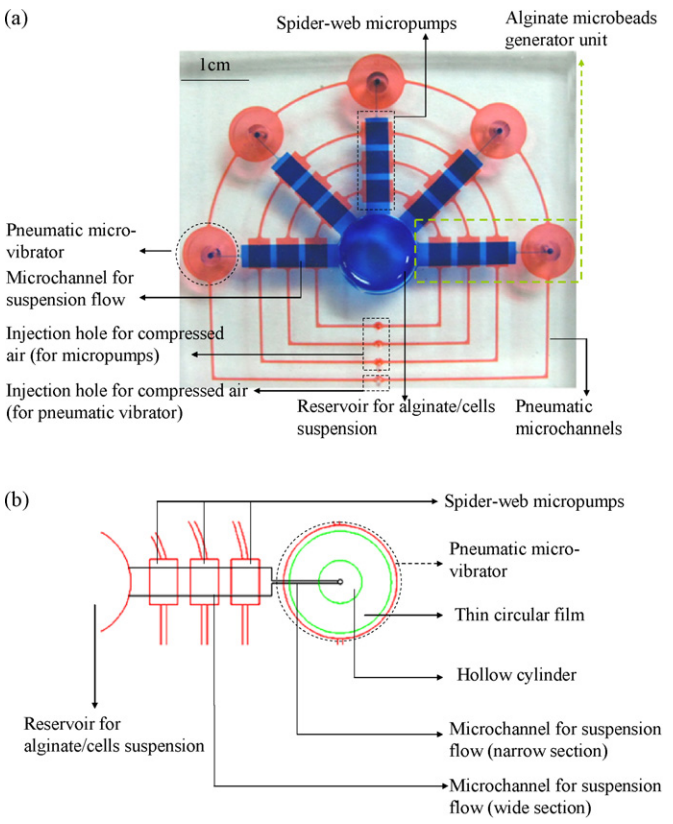

Fig. 1. (a) A photograph of the microfluidic device consisting of five alginate microbead generators. (b) A close-up illustration of a single alginate microbead generator (top view).

identical sets of alginate microbead generators radiating from one reservoir for alginate/cell suspension loading. For each unit of the alginate microbead generator (Fig. 1(b)), a microchannel containing one wider (length: 17,500 μm, width: 4000 μm, depth: 300 μm) and then another narrower (length: 5800 μm, width: 200 μm, depth: 100 μm) sections is designed to transport alginate suspension flow from the alginate/cell suspension reservoir to the center of the circular pneumatic micro-vibrator with the aid of the integrated spider-web pneumatic micropump. In this work, the design of the membrane-based pneumatic micropump is based our previous study [30], in which three rectangular pneumatic chambers (length: 4500 μm, width: 4000 μm, depth: 200 μm) were used to actuate the pumping effect. The suspension flow transported to the center of the circular pneumatic micro-vibrator is then connected to a needle (32G, ID: 100 μm, OD: 200 μm, length: 6500 μm) which is installed perpendicular to the circular pneumatic micro-vibrator (the opposite side of Fig. 1(b)), and acts as an outlet for the microfluidic system. In this study, micropumps and micro-vibrators are driven pneumatically. In this microfluidic device (Fig. 1(a)), four circular pneumatic microchannel loops (width: 500 μm, depth: 100 μm) are designed to serially connect the rectangular and circular pneumatic chambers, allowing three applied pneumatic sources to simultaneously actuate five micropumps. Similarly one applied pneumatic source can actuate five micro-vibrators.

The assembly of the microfluidic device for alginate microbead generation is schematically illustrated in Fig. 2(a). Briefly, it consists of three microfabricated PDMS layers (layer A, B, and C) and five needles connected to the pneumatic micro-vibrator parts of the five alginate microbead generators, respectively (Section D). Structurally, layer A, with multiple microfabricated rectangular and circular pneumatic chambers, and the pneumatic microchannel loops are designed to actuate by deforming the elastic PDMS mem-

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