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RPMI-1640 laden alginate hydrogel microcapsule produced by a coaxial electrohydrodynamic method

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Abstract. The microencapsulation of biomaterials has a broad application prospects in the field of biomedicine. This paper introduces design and setup of a coaxial microcapsule generator based on electrohydrodynamic (EHD) method. It includes a liquid supply system, a coaxial nozzle, a collector electrode, and a high voltage power supply. The ejection process is studied via LED illumination and a high speed CMOS camera. For the shell and core materials, alginate and RPMI-1640 culture medium are applied respectively. The effects of electric voltage and flow rate on the ejection frequency and volume of micro-droplet are investigated. The generated micro-droplets react in the collector electrode with CaCl₂ solution. Alginate hydrogel microcapsules are produced with RPMI-1640 included. Good encapsulation is confirmed, by fluorescence microscopy, and with RPMI-1640 stained by sodium fluorescein. The produced microcapsule may be used in biological cell encapsulation, in the future.

1. Introduction

Encapsulation of biomaterials such as drugs and biological cells has been widely used in the field of pharmacy and biomedicine [1, 2]. The goal is to achieve efficient encapsulation while maintaining its slow-release properties [2,3,4]. In particular, the semi permeable microcapsules used for cell encapsulation can not only release cytokines, but also protect the cells in the microcapsules from being attacked by, for example, immune systems [5]. For the choice of materials, alginate hydrogel, typically formed through cross-linking reaction with Ca^{2+} solution, is well known for its biocompatibility. As an excellent carrier of biomedical materials such as drugs and cells, it helps to maintain the viabilities of the enveloped viruses, bacteria and cells [6,7].

With the increasing demand for such microcapsules, development of new technology for microcapsule production has become an important research target in the field of biomaterial packaging. The traditional inkjet printing, by squeezing the liquid in the reservoir via for example piezoelectric actuation, usually produces droplets larger than the inner diameter of the nozzle. The most direct way to get smaller droplet is to reduce the nozzle size. However, for smaller nozzle diameter, the amplitude of the actuation required to produce a micro-droplet increases sharply. Moreover, for liquid of high viscosity or liquid containing particles, the nozzle is easily get blocked.

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For micro-droplet generation based on electrohydrodynamic (EHD) method, typically a syringe pump provides a stable liquid supply to the nozzle. A high voltage is applied between the nozzle (conductive) and a collector electrode (also conductive), setting up a strong electric field at the nozzle. Under the stress of the electric field, the liquid forms a conical meniscus (Taylor cone). The liquid at the end tip of the Taylor cone breaks and forms a micro-droplet whose diameter can be smaller than the nozzle. It helps to improve the printing resolution while reducing the risk of nozzle clogging. EHD ejection is a potential method for the preparation of microcapsules [7], and some researches have applied the EHD ejection technology to the encapsulation of biological cells [8].

EHD method has been used to eject alginate micro-droplets. Fukui et al. mixed fluorescent microspheres or albumin-FITC into alginate solution, and prepared alginate hydrogel microcapsules by the EHD method, achieved high encapsulation rate and no serious damage to its internal matrix [9]. In recent years, as a variation of the traditional EHD methods, EHD ejection through coaxial nozzle has attracted lot of attention and has been applied to the encapsulation of micro/nano materials. In a coaxial EHD ejection system, two liquids are supplied to the coaxial nozzle at proper flow rates (usually the flow rate of the outer liquid is far greater than that of the inner liquid). Taylor cone is formed under the action of electric stress, and micro-droplets with core-shell structure are ejected. The coaxial EHD method can realize the preparation of monodisperse microcapsules with high success rate of encapsulation [10].

On the basis of previous studies, we design and build an ejection setup based on the coaxial EHD method. The effects of voltage and flow rate on the volume of the micro-droplet and on the ejection frequency are studied. Stable preparation of RPMI-1640 laden alginate hydrogel microcapsules has been achieved.

2. Experimental setup

The home-build coaxial microcapsule generator based on the EHD method is shown schematically in Fig. 1(a). The whole instrument consists of five parts: a liquid supply system (two syringe pumps), a coaxial nozzle, a collector electrode, a high voltage power supply, and a photographing system.



Fig.1 (a) Schematic representation of the coaxial electrohydrodynamic ejection system. (b)Dimension of the coaxial nozzle.

The liquid supply system is composed of two syringe pumps, one for the inner layer and the other one for the outer layer of the coaxial nozzle. The coaxial nozzle is made of stainless steel, with its dimension shown in Fig. 1(b). The outer layer is supplied with the alginate solution (2wt%) and the inner layer is supplied with RPMI-1640. To collect the droplets, a Petri dish (diameter 50 mm) containing 2wt% Calcium Chloride (CaCl2) solution was placed 18 mm below the nozzle. The CaCl2 not only reacts with alginate to form the hydrogel, but also as a conductive material functions as the collector electrode. The nozzle and the collector are respectively connected to the output and the ground pole of a high-voltage power supply (Dongwen High-voltage Power Supply Co., Tianjin, model DW-P503-1ACDF), with output voltage range 0-50kV, and maximum output current up to 1mA. The photography system is mainly composed of a high-brightness LED and a high-speed CMOS camera (Model mini AX100 from

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Photron). The purpose of the system is to monitor the process of micro-droplet ejection. Photographing is done by using back-lightening configuration, with the nozzle staying between the LED and the camera. The LED brightness is kept constant. The frame rate is set to be 15000 fps, and the exposure time is set to be 50 s.

3. Experimental results

3.1. Droplet spray process

2wt% alginate solution (from Sinopharm) was supplied to the outer layer of the nozzle at a flow rate of 3.77mL/h. RPMI-1640 (from ThermoFisher) is supplied to the inner layer of the nozzle at a flow rate of 0.47mL/h. A voltage of 5.85kV is applied between the nozzle and the collector. A typical ejection process is shown in Fig. 2(a). With the help of image processing software [11], the length of the meniscus at the nozzle is plotted versus time, shown in Fig. 2(b). For the early stage of the ejection cycle, the meniscus length at the nozzle increases slowly and linearly. When it reaches a certain length, the liquid quickly forms a Taylor cone, and then the Taylor cone breaks and a micro-droplet is generated. After the droplet is produced, the liquid at the nozzle recoils rapidly and the meniscus length decreases sharply. It can be seen that the ejection process is highly repeatable and stable. Although not shown here, the long tail will break away, and the micro-droplet becomes spherical before reaching the collector. Therefore the volume of the micro-droplet can be easily estimated.



Fig. 2 (a) Ejection process for a RPMI-1640 laden alginate (2wt%) micro-droplet. (b) Time dependence of the meniscus length at the tip of the nozzle.

3.2. Volume of micro-droplet and ejection frequency

The droplet volume and ejection frequency are apparently two key parameters to characterize the ejection process. First, the effects of the applied voltage on the droplet volume and ejection frequency are studied, with all other conditions fixed. Specifically, the respective flow rates of the inner (RPMI-1640) and outer layer (alginate solution) are Qi=0.47mL/h and Qo=3.77mL/h, the distance between the nozzle and collector is h=18mm.

For voltage V less than about 5.6kV, the droplet diameter is large (up to 1-2mm), and the droplet ejection frequency is less than 1 Hz. When the applied voltage is above 5.6kV, as shown in Fig. 3, with the increase of voltage, the frequency of droplet ejection increases, the droplet volume decreases. The distribution of droplet volume is relatively narrow, as indicated by the error bars. It is confirmed that the average volume of droplets multiplied by the ejection frequency matches the total flow rate of the two liquids.





Then, for voltage V=5.85kV, distance h=18mm, and with the inner flow rate fixed at Qi=0.47mL/h, the ejection process are examined at a set of outer layer flow rates (or equivalently, total flow rates). As shown in Fig. 4, with the increase of the total flow rate of liquid supplies, both the ejection frequency and the volume of micro-droplet increase. When the flow rate is sufficiently large, the volume of micro-droplet stabilizes.

Experiments are also performed with alginate solution of lower concentrations, 1.5wt% and 1wt% to be specific. Results similar to those shown in Fig. 2, 3 and 4 are observed. Although not shown in this short paper, as the alginate concentration is reduced, the ejection frequency increases, while the volume of the micro-droplets gets smaller. This is likely due to that the viscosity is smaller for alginate solution of lower concentration, and also to that less viscous liquid would deforms more quickly under the electric stress.



Fig.4 Droplet volume and ejection frequency at a set of total flow rates .

3.3. Microcapsule and encapsulation of RPMI-1640

After reaching the collector, as the outer material of the micro-droplets, alginate quickly reacts with CaCl2, and becomes alginate hydrogel. And the RPMI-1640 can be trapped inside. This core-shell structure might be of potential applications in the field of stem cell encapsulation. In order to examine if the encapsulation is successful, the inner RPMI-1640 was mixed with sodium fluorescein (with its absorption and emission maxima at 460nm and 515nm respectively). Keeping the inner flow rate of Qi=0.47mL/h, the encapsulation of RPMI-1640 in the microcapsules are compared under two flow rates of the outer layer liquid (alginate solution for this experiment).

Figure. 5 (a) shows fluorescence photo of microcapsules (Eclipse600 fluorescence microscope, Nikon), prepared under the condition of Qo=8Qi. The microcapsules show clear core-shell structure, the core region in green color indicates the encapsulation of RPMI-1640 stained by sodium fluorescein. Figure 5 (b) and (c) are fluorescence and bright field photos of a typical ruptured microcapsules prepared under the condition of Qo=4Qi. Compared with Fig. 5(a), it can be seen that there is no obvious core-shell structure, and the internal fluorescence dye has been completely lost. The experimental results show that increasing the flow rate of the outer layer liquid is beneficial to the encapsulation of inner liquid.

Similar encapsulation experiments are also performed with alginate of lower concentration (1.5wt% and 1wt%). As mentioned earlier, as the alginate concentration gets lower, the sizes of the microcapsules would be reduced. This might be an approach if smaller microcapsules are expected. However, although not shown here, the success rate of the encapsulation deteriorates as the alginate concentration is reduced.



Fig. 5 (a)(b)photograph of microcapsule in fluorescence field.(c) photograph of microcapsule in bright field.

4. Conclusion

A home-build coaxial EHD ejection system is used for the production of microcapsule, with alginate hydrogel as the outer shell and RPMI-1640 cell medium as the core. The ejection process is studied by using a visual monitoring system. Both the voltage and the flow rates have their impacts on the volume of micro-droplet and on the ejection frequency. It is also shown that increasing the external flow rate is beneficial to the encapsulation of the inner fluid. Although reducing alginate concentration allows us to make smaller microcapsules, the success rate of encapsulation would deteriorate.

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