ALGINATE MICRO-BEAD FABRICATION ON A CENTRIFUGAL MICROFLUIDICS PLATFORM

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ABSTRACT

We present a novel method for the direct, centrifugally induced fabrication of small alginate beads displaying adjustable diameters between 180 µm and 800 µm by polymer-tube micronozzles. The size distribution features a CV of 7 – 16 % for the main peak. Up to 600 beads per second and channel are issued from the micronozzle through an air gap towards a standard lab tube ("Eppi") attached to the rotor spinning and containing a curing agent. At spinning frequencies between 5 Hz and 28 Hz, the tubes align horizontally under rotation and return to a vertical position as soon as the rotor is at rest. The hardened beads are collected within the tube for further processing or characterization. This method is considered as a low cost technology for micro encapsulation technologies.

1. INTRODUCTION

Encapsulation of pharmaceuticals, food flavors or living cells [1] into beads made of gels such as alginate has attracted appreciable attention during the last years. Especially the encapsulation of living cells into biocompatible polymers for in-vivo applications is expected to be a future growth market. An important technical objective is to speed up the diffusive transport of nutrients and oxygen into the beads as well as the reverse transport of the active agents into the body by reducing the bead size below 300 µm [2]. Also a small width of the size distribution, i.e. a high monodispersity, is critical for the therapeutic efficiency.

Three major technical challenges have to be tackled for the production of alginate beads. First, the surface tension and the viscous forces which counteract the droplet break-off at the nozzle have to be overcome. Second, the curing of the alginate beads has to take place without clogging and agglomeration.

Established means for the droplet break-off are the generation of shear stress by an air-jet directed to the forming droplet, a mechanical cutting of the alginate stream or the vibration of the micronozzle. These conventional techniques require a rather complex and costly apparatus. However, a wider acceptance of cell-encapsulation based therapies requires the availability of affordable and reliable in-house fabrication methods.

Microfluidic channel networks have already proven to enable the formation of small and monodisperse micro-beads [3]. However, the processing of the highly viscous bio-polymers like alginate, which are required for a bio-compatible cell encapsulation within small microchannels, still represents a major technical challenge.

In the microfluidic method presented here, the droplet break-off is stimulated by the centrifugal field. To this end, polymer micro-nozzles and standard, commercially available lab tubes are mounted on a rotor.

For the second step performing the hardening, we use the common diffusion method within an aqueous $CaCl_2$ solution containing Ca^{2+} ions. As a direct contact between the curing agent and the alginate at the nozzle would lead to clogging, we dispense through an air gap into the receiving reservoir.

Our simple setup can be designed to fit into any standard lab centrifuge, thus increasing the availability of in-house encapsulation technologies.

2. PRINCIPLE OF OPERATION

Droplet Generation

The formation of a droplet at a nozzle of inner diameter d_n exposed to gravity is depicted in Fig. 1. This quasi-static droplet formation is referred to as "dripping". The droplet breaks off when the gravitational force $\boldsymbol{F}_g = m_{drop}\, g$ exceeds the surface tension induced counter force $\boldsymbol{F}_\sigma = \pi\, d_n\, \sigma_{drop}$ leading to a droplet diameter

$$d_{drop} = \sqrt[3]{\frac{6\, d_n \sigma_{drop}}{\rho_{drop}\, g}} \qquad (1)$$

with the density ρ_{drop} and the surface tension σ_{drop}. Considering an inner nozzle diameter of 127 µm, a minimum bead diameter of 1.76 mm is obtained for a water-like liquid ($\rho_{drop} = 1$ g cm^{-3}, $\sigma_{drop} = 72$ mN m^{-1}). Thus, in order to form droplets in the targeted range of several hundred micrometers, the inner diameter of the nozzle should be even smaller and thus more expensive.

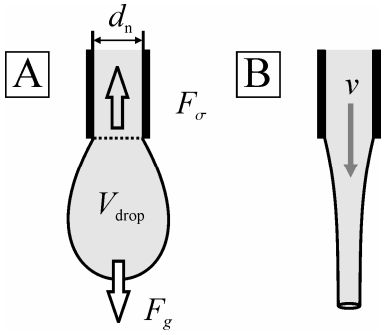

Fig. 1 Dripping out of a micronozzle under the impact of gravity (A). For a higher flow rate *v*, a jet forms out at the nozzle (B).

Another technical problem is the fact that the gravity is not sufficient to propel a flow of the high viscous alginate solution through the thin micronozzle. Thus, an additional pumping pressure has to be applied to induce a flow of velocity *v*. However, if the flow rate becomes too high, the droplet formation changes from the earlier described dripping to the formation of a liquid jet ("jetting") as depicted in Fig. 1, B. Also this jet breaks off into droplets at a greater distance to the nozzle tip due to the Rayleigh instability which is utilized in some micro-bead fabrication apparatus. However, this droplet formation process is of statistical nature and leads to a wider size distribution.

Our novel centrifugal bead fabrication method enables the formation of smaller droplets while still utilizing the favorable dripping principle with commercially available standard polymer tubes [4]. Therefore, the micronozzle is aligned in radial direction with the tip at the outer diameter (Fig. 2). The highly viscous aqueous alginate solution (2 wt-%) is contained within a reservoir connected to a standard polymer tube constituting the droplet-issuing micronozzle.

This setup is mounted on top of the rotor. The receiving tube is located within a holder which is free to align according to the frequency-dependent centrifugal field ($\boldsymbol{F_\omega}$). Therefore, the free flying alginate droplet impacts on a vertically oriented air-liquid interface within the tube. Upon halting the rotor, gravity prevails again to realign the tube vertically. The tube can be taken out the rotor for further processing, e.g., culturing or analysis.

In this scheme, the centrifugal force induced artificial gravity

$$g_{art} = \omega^2 r \qquad (1)$$

which depends on the rotational frequency ω and the radial position of the micronozzle on the rotor *r* adopts the role of the droplet generating gravity in Fig. 1.

The centrifugal method hence allows the variation of the artificial gravity field, and thus a scaling of the bead diameter via the rotational frequency ω. To generate droplets displaying diameters below one millimeter while avoiding the jetting regime, the process has to occur at a low flow rate and elevated centrifugal field. The flow of the alginate solution through the micronozzle is therefore throttled by its own high hydrodynamic resistance while the strong artificial gravity field "pulls out" the liquid drop at the tip of the nozzle. Using equation 1, this process scheme delivers bead diameters of 328 µm for a nozzle of diameter 127 µm and radial position 5 cm at a frequency of rotation $\omega = 176\ \mathrm{rad\ s^{-1}}$ (28 Hz) as applied in our experiments (calculated using the fluid properties of pure water).

Hardening

The gelification process of the alginate beads initiates in the outer shell at the interface with the $CaCl_2$ solution (100 mM) and continues inbound until the entire droplet forms a solid micro-bead. While the hardening of the outer shell occurs quite rapidly upon contact with the curing agent, the Ca^{2+} ions have to diffuse through an increasingly thick layer of hardened alginate at the later stages. The curing completes within several seconds.

Due to the rapid hardening of the shell, a direct contact between the alginate and the $CaCl_2$ solution at the issuing micronozzle would lead to fatal clogging. One possible solution to avoid clogging is the dispersion of alginate into an intermediate, immiscible oil-phase [5]. However, this method is much slower and it requires an additional washing of the beads before they can be further processed.

A more simple approach for the hardening of alginate beads is presented here. We introduce an air gap as a spacer between the alginate solution issued from the nozzle and the $CaCl_2$ solution stored in a receiving tube. Using a spacer gas instead of a liquid also obviates the need of subsequent washing steps for the retrieval of the beads.

A surfactant (0.1 wt-% Tween-20) supports the swift penetration of the alginate droplet into the liquid bulk to suppress the agglomeration with previously dispensed droplets settling at the liquid meniscus.

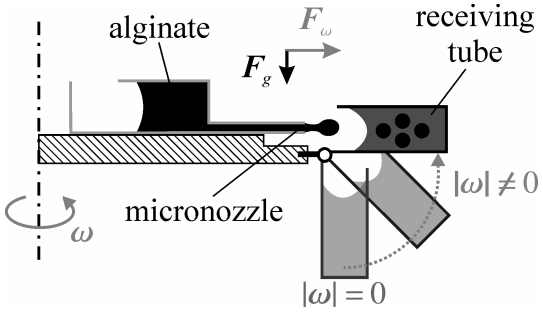

Fig. 2 Functional principle of the rotating micronozzle setup. An alginate solution is dispensed out of a micronozzle under rotation. The droplets "fall" in the artificial gravity ($\boldsymbol{F_\omega}$) into a receiving vessel at a frequency of rotation $\boldsymbol{\omega} \neq 0$.

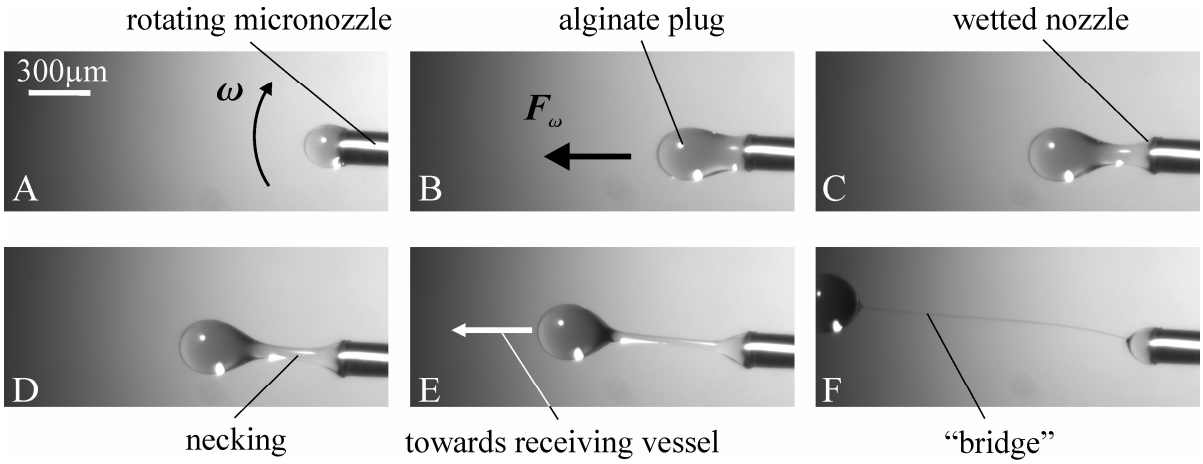

Fig. 3 Stroboscopic sequence visualizing the droplet break-off under rotation at the 127 µm (165 µm) micronozzle. As can be seen in C, the entire face surface of the micronozzle is wetted by the alginate solution at the tip. Hence, the inner as well as the outer nozzle diameter govern the droplet size.

3. EXPERIMENTAL RESULTS

The droplet generation process of an alginate solution at the tip of a rotating micronozzle (polymer tube, inner diameter: 127 µm) is depicted in Fig. 3. The alginate plug breaks off as soon as the centrifugal force $\boldsymbol{F_\omega}$ dominates the counteracting surface tension. During the droplet formation, the full face surface of the nozzle tip is wetted by the alginate solution (Fig. 3, C). Thus, the inner diameter (ID) as well as the outer diameter (OD) of the micronozzle impact the droplet size. During the break-off process, a liquid bridge emerges between the nozzle and the detaching droplet, subsequently leading to the formation of minute satellite droplets (Fig. 3, F).

We have used two different micronozzle geometries of 511 µm (562 µm) and 127 µm (165 µm) (ID (OD)), respectively. Fig. 4 shows beads with a diameter of 400 µm immersed in aqueous environment on the left (511 µm (562 µm) micronozzle, 11 Hz) and smaller beads of 240-µm diameter placed on a plane surface on the right (127 µm (165 µm), 22.5 Hz). The surface topology of one of these 240-µm beads is shown the center. Apart from the droplet generating and hardening process, this topology also reflects the drying phase after being exposed to environmental air.

To demonstrate encapsulation, a fluorescently labelled oligonucleotide is added to the alginate solution and the dry bead is captured by a fluorescence reader (Fig. 4, right). The clear fluorescent signal shows the successful encapsulation of fluorescently labeled DNA molecules. The variation of the fluorescent signals over the three beads reveals a still rather limited reproducibility of the amount of encapsulated molecules.

The mean bead size (of the primary peak, see below) as a function of the frequency of rotation is displayed in Fig. 5, left. Due to the scaling of the artificial gravity over the frequency of rotation (eq. 2), the bead size shrinks towards elevated frequencies of rotation. The measured bead diameter for the narrow nozzle at 28 Hz is much smaller than calculated in section 2 (calculated: 328 µm, measured: 180 µm). This deviation may be explained by differing material parameters of the alginate solution in the experiment (higher density and lower surface tension) and the additional influence of dynamic effects which are not considered in the quasi-static model.

The bead generation rate depends on the length and the diameter of the nozzle, the frequency of rotation and the viscosity of the dispensed solution. A high throughput of

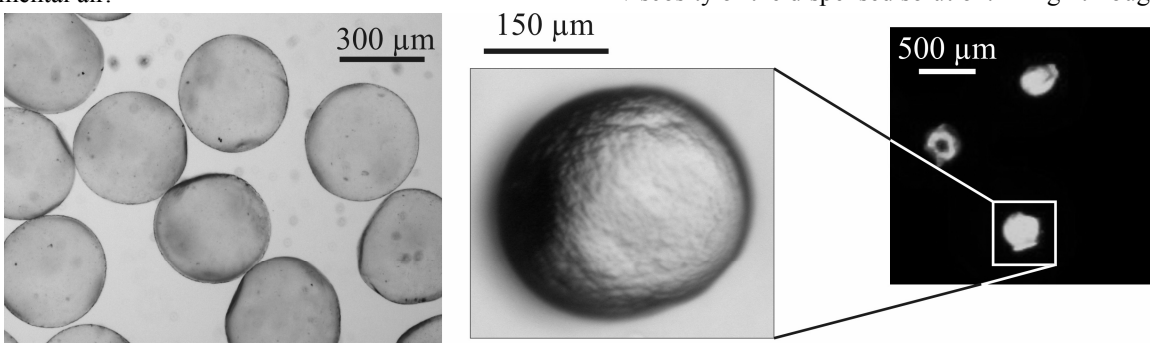

Fig. 4 Left: photographs of 400 µm alginate beads in water. Three 240 µm diameter beads as depicted in the center have been dispensed, containing 0.1 µM Cy3-labeled ssDNA (60-mer oligonucleotide). Afterwards, the beads are read out in a standard fluorescent reader (right).

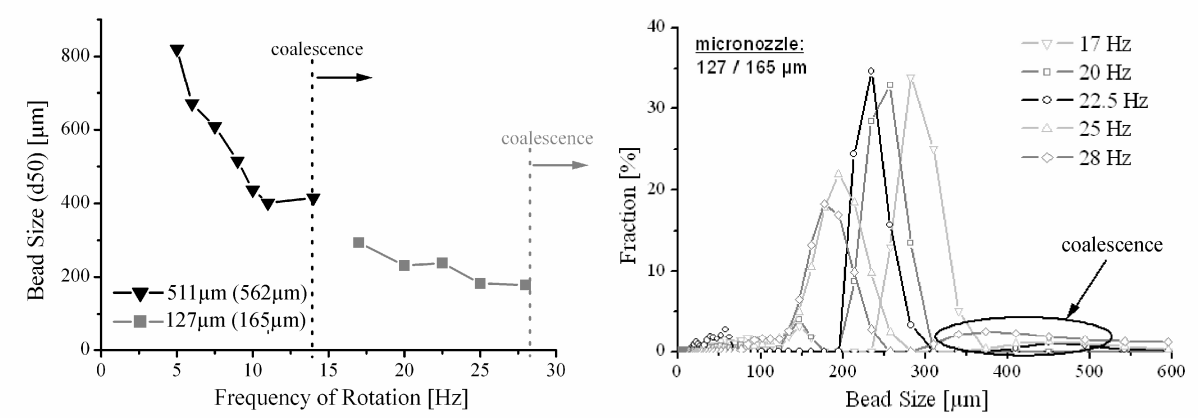

Fig. 5 Left: Size distribution of the bead diameter fabricated using the 127 µm (165 µm) micronozzle. The sizes have been measured using a laser diffractometer (Beckman Coulter, LS230). Right: Size of the micro-beads vs. the frequency of rotation. The size shrinks towards elevated frequencies due to the increasing impact of the artificial gravity field.

600 beads per second has been measured for the 127 µm (165 µm) micronozzle at 28 Hz. For more concentrated alginate solutions, also durations of several seconds for the formation of a single droplet have been observed. So, besides the high throughput encapsulation of large amounts of beads, also the fabrication of just a few beads is possible for small scale encapsulation experiments.

With decreasing size, the beads are increasingly stabilized by the interfacial tension at the liquid surface instead of penetrating into the liquid bulk. As also the frequency of bead generation increases with the rotational frequency, the beads tend to coalesce at the surface, thus setting a practical upper limit for the frequency of rotation and hence defining a minimum bead diameter for a given nozzle geometry. The two different nozzles in Fig. 5 exhibit different upper limits of the working range when coalescence occurs at the liquid gas interface in the receiving tube (14 Hz for the 511 µm (562 µm) and 28 Hz for the 127 µm (165 µm) nozzle respectively).

The size distribution of the beads using the smaller micronozzle at different frequencies is depicted in Fig. 5, right. All bead size measurements have been done using a laser diffractometer setup (Beckman Coulter, LS230). The beads exhibit a narrow distribution width (CV between 7% and 16% of the main peak) around a pronounced maximum. We (partially) attribute the narrow size distribution to the pulse-free centrifugal droplet generation mechanism. At frequencies above 20 Hz, the above described coalescence of the droplets sets in which is reflected by second peak at the upper end of the size distribution plot (black arrow).

4. CONCLUSION AND OUTLOOK

We added micro-bead fabrication capabilities to our recently introduced centrifugal multiphase microfluidic platform [6,7]. By adjusting the spinning frequency and nozzle geometry, the main peak of the alginate solution can be pushed below 200 µm, exhibiting a CV down to 7 %, only. This size of the alginate micro-beads enables their application for therapeutic cell encapsulation. Compared to existing methods, our novel centrifugal scheme offers a well reproducible pulse-free droplet generation and the capability to handle highly viscous liquids. Future work will focus on the processing of high-viscosity alginate solutions which is not possible with the current (pressure driven) technologies. We expect an increased mechanical stability of the beads and thus reduced stress and higher vitality of the encapsulated cells.

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