A MICROFLUIDIC DEVICE FOR MECHANICAL PROFILING OF HYDROGEL MICROPARTICLES

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ABSTRACT

This paper reports a continuous flow microfluidic device capable of measuring the geometric and viscoelastic properties of hydrogel microparticles by flowing them into a tapered microchannel with an interdigitated microelectrode array patterned underneath. The geometric and viscoelastic properties of microbeads/microcapsules are derived from their motion profiles monitored by the microelectrode array. Different from previous studies, the measurement inaccuracy associated with the negligence of the viscous effect is avoided. This work also eliminates the complicated optical systems used in previous microfluidic approaches, which makes it a truly miniaturized system with great potential for real-time characterization, separation, and sorting of particulate species based on their geometric and viscoelastic properties in a non-laboratory setting.

INTRODUCTION

Hydrogel materials are polymeric networks that contain a significant amount of water [1]. They can be easily engineered into either homogeneous microbeads or core/shell microcapsules with functional materials encapsulated in the hydrogel shell. Due to the softness and biocompatibility, hydrogel microparticles are used extensively in cell therapies, drug delivery, tissue, and food engineering [2, 3]. The geometric (size, shell thickness) and mechanical (stiffness, viscosity) properties of hydrogel microparticles are crucial for their efficacy in respective applications. In particular, the viability of encapsulated cells changes with the size of the hydrogel microcapsules. Studies showed that human cancer cells exhibit a high necrosis ratio while being encapsulated in the microcapsules larger than a certain size (around $200\ \mu\text{m}$) [4-6]. The shell thickness also regulates the cell viability and the drug releasing performance. A thick shell may provide an extended drug releasing profile but may decrease the cell viability [7]. Mechanical properties of hydrogel microparticles are also important. Studies showed that hydrogel microparticles with higher stiffness promote chondrogenesis [8-10], whereas such high stiffness reduces the proliferation rate of stem cells [11]. In cell therapies, the differentiation of the stem cells cultured in the hydrogel microparticles is regulated by the mechanical properties of the hydrogel shells [12].

Since hydrogel microcapsules are often used in batches, fast

screening of a large number of microparticles based on their geometric and mechanical properties is required. The size and shell thickness can be measured using optical microscopy. However, the boundary between the aqueous core material and hydrogel shell may be indiscernible due to low image contrast between the two layers [13]. Although fluorescent dyes can be used to enhance the contrast, the dyes may diffuse into the hydrogel shell and cause inaccurate measurements. Continuous flow microfluidics is a high throughput mechanical profiling approach [14]. Similar as in geometric screening, most continuous flow mechanical measurements rely on the use of high-speed optical systems. The measurements thus cannot be performed in a truly miniaturized setting, limiting the applications in non-laboratory settings. Moreover, in above measurements geometrical and mechanical properties often cannot be decoupled from each other, which can lead to significant measurement errors.

In this study, we report a continuous flow approach to simultaneously measure the geometric and viscoelastic properties of hydrogel microparticles without the use of high-speed optical systems. The size and viscoelastic properties of microbeads were decoupled using a viscoelastic model and measured simultaneously. The shell thickness of hydrogel microcapsules can also be determined. This device provides a high throughput profiling approach for characterizing hydrogel microparticles within a miniaturized setting.

MATERIAL AND METHODS

Device Design and Fabrication

The device consists of a glass substrate patterned with an array of interdigitated microelectrodes and a PDMS substrate that has a tapered channel segment with the linearly changed width (**Fig. 1b**). The width at the channel inlet was greater than the size of the microparticles to be measured, and the width at the channel outlet was smaller than the size of the microparticles to be measured. After bonding the glass substrate and the PDMS substrate together, the microelectrode array was arranged underneath the tapered channel segment. Every two neighboring electrodes worked in a pair and were connected to a reference resistor of $100\ \text{k}\Omega$ (Sparkfun Electronics, CO) and an AC voltage source ($1\ \text{V}$ in amplitude and $3000\ \text{Hz}$ in frequency). There was a total of 15 electrode pairs (**Fig. 1c**). The electrical voltage changes across the reference resistors

Figure 1. Schematic of this approach. a) An assembled device; **b)** Schematic of the tapered channel segment; **c)** The electrical circuit for each electrode pair; and **d)** The illustrative motion profile. The green line represents the motion profile. The intersect point between the linear and non-linear portions represents the contacting point with the channel sidewalls.

were measured using a data acquisition board (National Instrument, TX) at a sampling rate of 30 kHz.

Preparation of Homogeneous Microbeads and Core/shell Microcapsules

All the chemicals were purchased from Sigma-Aldrich (MO) unless otherwise noted. Na-alginate solution was prepared by dissolving Na-alginate powder in deionized (DI) water and stirring at 900 rpm under 45 °C for 8 hours. CaCl₂ and mannitol solutions were prepared by dissolving CaCl₂ and mannitol powders in DI water and 1.0% w/v cellulose solution separately at the room temperature and stirring thoroughly. Carbon black ink (Yasutomo, Japan) was added to the aqueous core solution (1.0% w/v mannitol) with the volumetric ratio of 1:50 to make the core/shell boundary discernible.

Hydrogel microcapsules were prepared using a co-axial electrospray setup [15]. Briefly, 1.0 % w/v Na-alginate solution (the outer layer) and 1.0% w/v mannitol solution (the inner layer) were pumped through a metallic co-axial syringe needle using two syringe pumps (New Era Pump Systems Inc. NY). The inner diameter of the needle was 0.8 mm, and the outer diameter was 1.4 mm. A 2.0 % w/v CaCl₂ bath was placed under the metallic needle. The distance between the metallic needle and the CaCl₂ bath was 10 cm. The needle was set at +15 kVDC, and the CaCl₂ bath was set at 0 kV. Under such condition, a stable Taylor cone can be formed at the co-axial needle tip. The solutions were sheared off and sprayed towards the CaCl₂ bath. After the bi-layered microdroplets entered the CaCl₂ bath, the Ca²⁺ ion induced gelation occurred in the outer layer. The inner layer remained in the liquid phase. By changing the flow rates of the two solutions, core/shell microcapsules with different diameters and shell thicknesses were obtained. After electrospray, the microcapsules were washed three times and re-suspended in 1.0% w/v mannitol solution. Homogeneous hydrogel microbeads were prepared following a similar procedure, except that a single-axial electrospray process was used [16]. The concentrations of the prepared solid microbeads were 0.5% w/v, 1.0% w/v, 2.0% w/v respectively.

Measurement Principle

Figure 2. The schematic of the microcapsule deformation when it passes through the tapered channel segment. a) The schematic view and b) The free-body diagram of a microcapsule after contacting with the sidewalls (top view).

Once a hydrogel microparticle flows over an electrode pair, the electrical resistance between the two electrodes changes. This is

because the microparticle (Ca-alginate) has a different electrical property from the carrying medium (mannitol). The voltage amplitude across the respective reference resistor changes accordingly. The peak of the voltage amplitude change represents the moment when the center of the microparticle overlaps with the center of the subject electrode pair. The position of the microparticle can be derived from the voltage amplitude changes across all the reference resistors. Continuous scanning can obtain the motion profile of the microparticle.

Before the microparticle contacts the sidewalls of the tapered channel, the displacement changes linearly over time. After the contact, the velocity decreases substantially. The motion profile thus deviates from the linear line. The deviation point (t_c , X_c) indicates the contact point and can be used to determine the size (**Fig. 1d**), expressed as $d = Y_c \cos \alpha$, where Y_c is the channel width at X_c . The curved motion profile (from t_c to t_e) represents the microparticle's viscoelastic behavior while it is squeezed through the tapered channel. A quasi-linear viscoelastic (QLV) model can be used to derive the viscoelastic properties of homogenous microbeads as well as the shell thickness of the microcapsules [17, 18].

After the microparticle contacts with the sidewalls, its motion is regulated by:

$$F_D - 2F_f \cos \alpha - 2F_N \sin \alpha = m \frac{d^2 x}{dt^2} \quad (1),$$

wherein F_D denotes the drag force due to the leak flow, the velocity difference between the microparticle and the medium, and the pressure difference between the front and the rear surfaces; F_f denotes the friction force between channel sidewalls and the microparticle, F_N denotes the compressive force due to the contact, m is the mass of the microparticle, α is the half oblique angle of the tapered channel segment and x is the lateral displacement along the flow direction (**Fig. 2a**). Because of the low Reynolds number, the flow is laminar and the drag force F_D can be determined by Stokes' Law. Here, we consider the microparticle as a viscoelastic sphere. Since the half oblique angle (α) of the tapered channel is very small ($< 2.4^\circ$), the loading can be approximated as a uniaxial compression. A QLV model can be established and expressed as:

$$F_N = \sigma(\varepsilon) \left[1 - C \left(1 - e^{-t/\tau} \right) \right] \quad (2).$$

The first term on the right side of the equation represents the instantaneous elastic function. The second term on the right side of the equation is a first-order reduced relaxation function expanded using the Prony series, where τ (the time constant) and C (the coefficient of Prony series) denote the constants related to the viscosity of the microbeads. To measure the viscoelastic properties, the first term on the right side of **Eqn. 2** can be approximated using Hertz's theory [19]:

$$\sigma(\varepsilon) = \left[\frac{E\sqrt{d}}{3(1-\nu^2)} (d-y)^{3/2} \right] \quad (3).$$

To measure the shell thickness of microcapsules, the first term on the right side of **Eqn. 2** can be estimated by Reissner's theory [20]:

$$\sigma(\varepsilon) = \left[\frac{4\xi^2 E}{d\sqrt{3(1-\nu^2)}} (d-y) \right] \quad (4),$$

wherein ξ is the shell thickness, E is the instantaneous elastic response of the hydrogel shell, d is the original diameter of microcapsules, and ν is the Poisson's ratio (approximated as 0.5). The friction force has the expression of $F_f = \eta \times F_N$, wherein η is the coefficient of friction (COF) between the hydrogel and the sidewall, approximated as 0.06 [21].

By inserting **Eqs. 2, 3** and **4** into **Eqn. 1**, a second order nonlinear differential equation can be established. The displacement profiles of microparticles can be estimated by solving this equation numerically. The geometric and viscoelastic parameters of microcapsules thus can be determined by fitting the displacement

profiles with the QLV model using the electrically measured data points.

RESULTS AND DISCUSSION

Size Measurement

The sizes of core/shell hydrogel microcapsules and homogeneous hydrogel microbeads determined from electrical voltage measurements were compared with those by the optical measurements. The coefficients of determination between the two measurements are: $R^2 = 0.978$ for core/shell microcapsules and $R^2 > 0.99$ for 1.0% w/v and 2.0% w/v solid homogeneous microbeads (**Fig. 3a**). For 0.5% w/v microbeads, $R^2 = 0.73$. The large deviation is because the 0.5% w/v Na-alginate solution has a relatively low viscosity, causing unstable dripping in the electrospray process. This leads to uncontrolled, non-spherical, and asymmetric shaped Ca-alginate microbeads. However, for 1.0% and 2.0% w/v microbeads, and microcapsules (made of 1.0% w/v alginate), such irregular shapes were not observed (**Fig. 3b**). The results showed the sizes of both homogenous microbeads and core/shell microcapsules can be determined with high accuracy.

Figure 3. Size determination of homogeneous microbeads and core/shell microcapsules. *a)* The comparison of electrically measured sizes versus optically measured sizes. *b)* The representative micrographs of microbeads and core/shell microcapsules.

Viscoelastic Measurement of the Solid Microbeads

The mechanical properties were estimated using both a linear elastic model (where the reduced relaxation function on the right of **Eqn. 2** equals to 1) and the QLV model. It is found that although the linear elastic model (denoted by the green line) can match the total transmit time ($t_e - t_c$), a good fit with the trajectory of a microbead (denoted by red dots) cannot be reached (**Fig. 4a**). The QLV model (denoted by the blue line) can reach a much better fit with the experimental data and is expected to yield more accurate results.

The instantaneous elastic response E , the time constant τ , and the coefficient of the Prony series C can be determined by fitting the experimental data with QLV model. The results showed that microbeads with the highest concentration have the highest E , the lowest τ and C , and vice versa (**Fig. 4b-d**). Statistical analysis showed that the viscoelastic parameters vary significantly with the alginate concentration of the microbeads. The C values for 2.0% w/v microbeads were very small (0.04 ± 0.01). The reduced relaxation function is approximately 1. The 2.0% w/v microbeads can thus be regarded as linear elastic bodies. For 0.5% and 1.0% w/v microbeads, the viscous behaviors cannot be neglected. The results also showed

that the viscoelastic properties do not show significant difference at different flow rates. This indicates that the geometric and mechanical measurements using the QLV model are independent of input flow rates. This makes it possible to design devices to accommodate the needs for various throughputs.

Figure 4. Estimation of the viscoelastic properties of homogeneous microbeads. *a)* The comparison of the QLV model and the linear elastic model using a representative trajectory of a 1.0% w/v and 332 μ m microbead under 60 ml/h (the circles and the short-dashed line). The fitted curves using the linear elastic model is denoted by the long-dashed line and the QLV model is denoted by the solid line. *b-d)* The viscoelastic parameters of homogeneous microbeads derived after the data fitting. *** denotes $p < 0.005$.

Shell Thickness Measurement of the Core/shell microcapsules

Similar as for homogeneous microbeads, the size and the shell thickness of core/shell microcapsules were determined from electrically measured displacement profiles. The comparison between the QLV model and the linear elastic model showed that the I/O ratios estimated by the QLV model agree better with those by the optical measurements ($R^2 = 0.878$ versus $R^2 = 0.649$) (**Fig. 5**). The larger errors associated with the linear elastic model indicated that the viscous behavior of the core/shell microcapsules with a hydrogel shell and an aqueous core is not negligible.

Figure 5. Determination of the shell thickness of core/shell hydrogel microcapsules. The calculated I/O ratios compared with the observed I/O ratios using *a)* the linear elastic model and *b)* the QLV model.

CONCLUSION

In this work, a continuous-flow device was developed and validated for geometric and viscoelastic profiling of hydrogel microparticles (*i.e.* homogeneous microbeads and core/shell microcapsules). Homogeneous Ca-alginate microbeads with different concentrations (0.5% w/v, 1.0% w/v and 2.0% w/v) were prepared using a single-axial electrospray system. Ca-alginate core/shell microcapsules (1.0% w/v Ca-alginate as the shell, 1.0% mannitol as the aqueous core) with different shell thicknesses were prepared using a co-axial electrospray system. The microcapsules and microbeads were pumped through a microfluidic microchannel with a tapered channel segment under a constant flow rate. The displacements of the microparticles were recorded by an array of microelectrodes patterned underneath the microchannel. A viscoelastic model was developed to determine the viscoelastic parameters, shell thickness, and diameter of these microparticles from their displacement profiles. The results showed that viscoelastic parameters and size of homogeneous alginate microbeads, the shell thickness, and the size of core/shell hydrogel microcapsules can be determined simultaneously by fitting their displacement profiles with a viscoelastic model. This work demonstrates a high-throughput (up to 584 microparticles per minute) geometric and viscoelastic profiling method for hydrogel microparticles. Higher throughput is possible by increasing the flow rate, changing the microelectrodes layout, and increasing the sampling rate. It also eliminates external high-speed optical systems and shows potential for high throughput measurements towards subsequent real-time microparticle/microcapsule sorting and separation within a truly miniaturized non-laboratory setting.

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