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Alginate microgels created by electrohydrodynamic jetting

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Abstract - Developing a facile process that can produce small hydrogels of a well-defined shape with diameters in the order of 100 μm is of great interest in the field of biomedical applications including drug delivery, tissue engineering, and cell manufacturing. We attempted to employ electrohydrodynamic (EHD) jetting process to fabricate such microgels made of sodium alginate. To achieve the controlled sizes and shapes of the microgels in a reproducible manner, the solution parameters (sodium alginate concentration, calcium chloride concentration) as well as operational parameters of EHD jetting (flow rate, voltage, distance between the jetting nozzle to the substrate) have been studied and optimized to yield a jetting condition with the sustained Taylor cone-jet mode. The morphology and the size of the resulting microgels for varying conditions were characterized by optical microscopy. The developed microgels can be employed as a biological reactor within which immune cells such as B cells can be manufactured for cellular immunotherapies for infections or autoimmune diseases.

I. Introduction

Electrohydrodynamic (EHD) jetting is used in order to produce micro- and nano-materials for several fields such as medicine, pharmaceutics, and bioengineering (Xie et al., 2015). In EHD jetting, a high electric voltage is applied to a jetting liquid, and the resulting electrical field drives a charged jetting liquid to travel towards the grounded electrode. When a positive voltage is applied to a polymer solution as the jetting liquid, positive charge accumulates on the surface of the meniscus of the liquid droplet, which changes the shape of the meniscus look like a cone (Taylor cone). Above a certain maximum electric potential, the electrostatic repulsion forces eventually break the surface tension of the droplet, and a jet of the solution is ejected and moves towards the electrode (Kim et al., 2009).

Sodium alginate is derived from seaweed and is a natural polysaccharide and is a structural polymer in the cell wall of seaweed. The alginate polymer, when in contact with divalent cations, forms hydrogels as a result of ionic cross-linking (Hench and Jones, 2005). When sodium alginate is in contact with a solution containing divalent cations such as Ca²⁺, a single calcium ion conjugates with two carbonate ions from neighboring repeating units of alginate polymers. This can happen throughout a mixture of multiple polymer chains in a solution, which is called cross-linking (Rowley, Madlambayan and Mooney, 1999).

When the solution of sodium alginate, our choice of polymer in the jetting solution, leaves the jetting capillary, the resulting jet falls into a collecting calcium chloride (CaCl₂) solution that is placed directly below the jetting capillary. The microgels are formed in the calcium chloride solution due to ionotropic crosslinking mentioned above (Park et al., 2012). In order to achieve a stable cone-jet mode, various concentrations of alginate solutions as well as several operational parameters such as voltage, diameter of jetting capillary, flow rate, and the distance between the jetting capillary and the collector with CaCl₂ solution were tested. The controlled jetting mode would be required to create microgels of defined shapes. The defined shapes could be either a conventional spherical shape or some non-spherical shapes. The non-spherical microgels could be interesting due to their anisotropic responses to the external forces as well as their unconventional surface profiles (Hu, Azadi and Ardekani, 2015).

The alginate microgels is advantageous for various biomedical applications due to its biocompatibility and biodegradability. They are useful as a means of delivery for bioactive macromolecular compounds as well as living cells (Kikuchi et al., 1999).

II. Experimental Procedure

Materials
Sodium alginate from brown algae (Mₐ=80-120 kDa, M/G = 1.56) was purchased from Sigma-Aldrich and used without any further purification. Corresponding amount of alginate polymer powder was dissolved in ultrapure water, phosphate-buffered saline (PBS) buffer, or saline solution (0.9 % (w/v) of sodium chloride) at least 24 hours before jetting experiment.
Characterization

The resulting microgels were examined under an optical microscope (EVOS FL Auto, Life Technologies). The diameter of the microgels was measured from the optical microscope images. A ruler was placed directly on the computer monitor and the measurements of the diameters were recorded in MS-Excel. The measured values were calibrated using the scale generated by the microscope. The average diameter was calculated by observing a sample size of minimum 20 microgels. The viscosity of jetting solutions was measured by Mars 60 Rheometer (HAAKE, Thermo Fisher) equipped with titanium cone and plate (20 mm diameter and 2° angle) (Sánchez-Morán et al. 2019). All measurements were carried out at 37 °C.

EHD Jetting

The EHD jetting system is composed of a programmable syringe-pump (SyringePumpPro), a high voltage power supply (Gamma High Voltages), a charged coupled device (CCD) camera (DinoLite), a set of hypodermic needles (19 gauge), a light source, and a computer (Figure 1).

For a typical EHD jetting, the jetting solution of sodium alginate (0.5%w/v - 4.0%w/v) are supplied through a capillary at a flow rate (1.5mL/h - 4.5 mL/h), while a high voltage (12.3 kV - 24.7kV) is applied to the jetting capillary. The distance (8.0 cm -9.0 cm) between the jetting capillary and the collecting substrate is adjusted as well. As a collecting substrate, a square (5.0 cm x 5.0 cm) piece of aluminum foil or 0.1M CaCl₂ solution in a grounded metal container was used.

III. Results and discussion

EHD jetting in dripping mode

Effect of alginate concentrations.

The effect of alginate concentration and microgel size created by EHD jetting in dripping mode was investigated, as shown in Figure 2 and 3. Figure 2 shows the representative microscope images of microgels made from alginate solutions of different concentrations employed as jetting liquids. The EHD jetting in dripping mode was achieved when 1.5 mL/hr flow rate, 8 cm height between the needle and the collecting substrate with calcium chloride solution, and the voltage of 12.3 kV were employed as critical working parameters. Different morphologies of microgels were observed. It is clear to observe that increasing alginate concentration leads to an increment of microgel diameter (Figure 3), which agrees well with the previous findings (Gryshkov et al., 2014). A more unstable fluctuation in the dripping mode was observed at 0.5 % alginate solution, causing a wider size distribution of microgels. It was also interesting to observe that non-spherical microgels were fabricated from jetting of 0.5 and 1.0 % alginate solutions. The resulting microgels demonstrated unique morphologies: mushroom-like (Figure 2A), bowl-like (Figure 2B), or spheres (Figure 2C and 2D). The mushroom-like particles are anisotropic in their shapes and dimensions (Figure 2A and 2B). The mushroom-like particles have two different sides of longer axis (y-axis in Figure 2A), one side with smooth periphery (like the cap of mushroom), and the other side with a slightly bulging stalk.
bowl-like shape is very similar to the mushroom-like, except that one side of longer axis is simply flat without the stalk (Figure 2B). For the reporting purpose, the minimum diameter (the shortest axis) of the mushroom-like and bowl-like particle was measured (arrows in Figure 2A and 2B).

Figure 2. Optical microscope images of microgels that are created by EHD jetting in dripping mode using different concentrations of alginate solutions as jetting liquids. (A) 0.5, (B) 1.0, (C) 2.0, and (D) 4.0 % (w/v) alginate solutions. The scale bar represents 1,000 µm. Red lines are added rendering to indicate the corresponding axis (A and B).

Figure 3. Effect of alginate concentration on the diameter of resulting microgel made by EHD jetting in dripping mode.

Effect of flow rates.

We evaluated how the flow rates in EHD jetting affect the size of the resulting microgels. Jetting solutions of a constant alginate concentration of 2.0 % (w/v) were employed in EHD jetting in dripping mode at four different flow rates, while all other working parameters remained constant, i.e., the collecting height of 8.0 cm and voltage of 12.3 kV. It is generally accepted that the flow rate of EHD jetting and the size of resulting micro-objects show a positive correlation. However, it was observed that an increase of flow rates from 1.5 up to 4.5 ml/hr caused only a slight variation in the size of the resulting microgels, which agrees to a previous literature describing the EHD jetting of alginate solutions (Gryshkov et al., 2014). Even if the size of the microgel was not significantly changed by the flow rate, the polydispersities in size significantly increased as higher flow rates are employed (Figure 4).
Transition of jetting modes from dripping mode to cone-jet mode.

In the present study, we attempt to achieve jetting conditions to generate and attain stable jetting that can produce microgels with a near-uniform size distribution. As we could not achieve this goal with dripping mode, we tried to achieve a stable cone-jet mode. As a comparative approach to achieve a cone-jet mode, the viscosity of sodium alginate solution was compared to the viscosity of other jetting solutions that yield a stable cone-jet. For example, a solution of poly(acrylamide-co-acrylic acid) with 20.39 mPa⋅s yielded a very stable cone-jet (Roh, Yoshida and Lahann, 2007). In a viscosity measurement, 0.7 % (w/v) alginate solution was 11.36 mPa⋅s. In fact, with the alginate solutions below 1.0 % (w/v), we could achieve EHD jetting with a stable cone-jet mode when much higher voltage (above 20 kV) is applied compared to the dripping mode (around 12 kV). The microgels acquired from cone-jet mode were significantly smaller with much narrower size distributions, compared to the microgels acquired from dripping mode (Figure 5).

In addition, if the stable EHD jetting with cone-jet mode can be achieved using alginate solutions prepared with a physiological saline or phosphate buffered saline (PBS). The varying working parameters, the composition of jetting solutions, and the characteristics of resulting microgels from 6 different EHD jetting experiments shown in Figure 5 are summarized in Table 1. The representative microscope images of the resulting microgels are also shown in Figure 6. It is noteworthy that the morphology and size of the microgels were significantly varied by employing different jetting modes and varying parameters (solvent, voltage, flow rate and collecting height), even for the same concentration (1%, w/v) of sodium alginate (conditions 1, 5, and 6 in Table 1). Indeed, 1.0 % (w/v) alginate solution either in saline or PBS could generate a very stable cone-jet to yield spherical microgels with diameters of approximately 150 µm. This result is significant because the EHD jetting at the same condition now can be applied with living cells suspended in the jetting solution to create the microgels containing living cells. Such microgels containing living cells can be developed as a microcarrier or a microbioreactors for various applications.
Table 1. Different jetting parameters and conditions and the corresponding size and shape of the resulting microgels.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Alginate Concentration (w/v%)</th>
<th>Solvent</th>
<th>Voltage (kV)</th>
<th>Flow rate (ml/hr)</th>
<th>Collecting height (cm)</th>
<th>Diameter (µm, mean ± std)</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>Ultrapure water</td>
<td>12.3</td>
<td>1.5</td>
<td>8.0</td>
<td>864.3±113.2</td>
<td>Bowl</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>Ultrapure water</td>
<td>12.3</td>
<td>1.5</td>
<td>8.0</td>
<td>581.5±581.5</td>
<td>Mushroom</td>
</tr>
<tr>
<td>3</td>
<td>0.7</td>
<td>Ultrapure water</td>
<td>21.5</td>
<td>3.4</td>
<td>9.0</td>
<td>142.6±54.1</td>
<td>Bowl</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>Ultrapure water</td>
<td>24.0</td>
<td>3.8</td>
<td>9.0</td>
<td>149.77±52.3</td>
<td>Bowl</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
<td>Saline</td>
<td>24.7</td>
<td>3.8</td>
<td>9.0</td>
<td>143.4±39.6</td>
<td>Sphere</td>
</tr>
<tr>
<td>6</td>
<td>1.0</td>
<td>PBS</td>
<td>24.3</td>
<td>3.8</td>
<td>9.0</td>
<td>168.2±71</td>
<td>Sphere</td>
</tr>
</tbody>
</table>

Figure 6. Optical microscope images of microgels that are created by EHD jetting in cone-jet mode. (A) 0.5 % (w/v) alginate solution in ultrapure water, (B) 0.7 % (w/v) alginate solution in ultrapure water, (C) 1.0 % (w/v) alginate solution in PBS, and (D) 1 % (w/v) alginate solution in isotonic saline. The scale bar represents 1,000 µm.

IV. Conclusion

Here we demonstrated a series of EHD jetting conditions that can yield a production of small hydrogels (microgels) of well-defined shapes. By selecting and controlling the working parameters and jetting parameters, not only the size and size distribution but also the morphology of the resulting microgels could be varied. The microgels of mushroom-like, bowl-like, and spherical shape could be useful for various applications (Gao et al., 2015). Alginate microgels of uniform shape and size can be created using physiologically relevant jetting parameters demonstrates that the technology described here could potentially be further developed as microbioreactors for various cellular engineering applications such as manufacturing of therapeutic cells (Mendoza García, Izadifar and Chen, 2017).

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References


