Droplet Generation in Flow-Focusing Microfluidic Devices for the Creation of Artificial Cells

Weilong Li[1,3], Teresa Petralli-Mallow[2], and Zhihong Nie[4]

Student[1], Teacher[2]: Poolesville High School, Poolesville, Maryland, 20837
Intern[3], Mentor/Assistant Professor[4]: University of Maryland, College Park, Maryland, 20742
*Correspondence: weilongli1@gmail.com

Abstract

This paper systematically investigated the effect of flow rate ratios between the water and oil phases, and concentrations of sodium alginate within the water phase, on the generation of droplets and capsules in a microfluidic reactor in order to facilitate artificial cell production within the water phase for biological experiments. Artificial cells, which simulate the processes that occur within organisms in vitro without the use of real tissues, can be made within flow focusing microfluidic chips. However, researchers must first fine tune the systems in which they are made in order for these cells to be generated. These tests will help make artificial cells more efficient to fabricate within microfluidic systems. Droplets were formed by the emulsification of water in a continuous oil phase. Capsules were formed through a reaction of sodium alginate, carried in the water phase, and calcium ions, carried in the oil phase, to create a cross-linked membrane of calcium alginate surrounding a droplet. An increase in the flow rate of water had a positive correlation on the size of the droplets created, and an increase in the flow rate of oil had a negative correlation. Changes to the flow rates of the continuous oil phase affected the system more than changes to the flow rates of the dispersive water phase. In addition, high concentrations of sodium alginate within the dispersive phase increases the stability of the droplet formation, as well as the stability of the capsules formed within the microfluidic device.

Introduction

Much of biological research involves describing systems and how each part functions in relation to each other and the whole. However, this approach may sometimes be very complicated on a cellular level to observe and interpret. An example of such a system is seen in biomineralization, where organisms have the ability to make minerals with both organic and inorganic properties[1]. These abilities allow the organisms to create tissues with many structural levels that build upon each other to create complex and stable structures[2]. Because of the complexities of the structural levels, it is difficult to study biomineralization processes in active, living, cells.

Through artificial cells, in vivo systems, such as those involved in biomineralization, can be studied in controlled circumstances. This process will allow researchers to isolate specific aspects which they wish to study, instead of only being able to look at the process as a whole.

One way to produce these artificial cells is through the use of microfluidic reactors. Microfluidics is a recent technology that creates micro-size environments in which chemical or biological experiments can take place[3]. Artificial cells can be created in a microfluidic reactor by manipulating the flow rates of oil and water inside the chip to create water in oil droplets that have characteristics of biological cells, such as DNA, RNA, and proteins, which are included in the water phase, and lipids, which are included in the oil phase[4].

Because microfluidics is a newer method of conducting research, less is known about how to conduct experiments using this technology than other, more conventional research methods. Therefore, investigation into how droplets form and behave within a chip is necessary for conducting experiments that involve microfluidics, in order to help researchers understand what to expect when running an experiment and how to run experiments more efficiently.

In this paper we will show experimentally the nature of droplet formation in relation to flow rates within a microfluidic reactor, as well as the relationships between the concentrations of reactants to form capsules with cross-linked polymer membranes.

Materials and Methods

Polydimethylsiloxane (PDMS) (Sylgard 184) was obtained as a two-part base and curing agent set from Dow Corning. Sodium alginate, octadecyltrichlorosilane, calcium acetate, sorbitane monooleate (Span 80), soybean oil and mineral oil were obtained from Sigma Aldrich. Oxygen plasma was generated using a plasma cleaner (PDC-32G) from Harrick Plasma. Syringe pumps (NE-1000) were from New Era Pump Systems. SU-8, an epoxy-based photoresist polymer) master silicon wafer templates were provided pre-made by the laboratory. Images were monitored using NIS-Elements software and taken using a Nikon optical microscope equipped with a CCD camera.The PDMS polymer base and curing agent were mixed in a 10:1 ratio by weight. The mixture was then degassed in a vacuum chamber at room temperature until the mixture was clear of bubbles. The degassed mixture was then poured into a SU-8 master template and put into an oven at 75° C and left to solidify for 4 hours. A separate PDMS substrate was produced in the same way, using a sterile petri dish instead of a template. The solidified PDMS chip was cut out of the master, and the substrate cut out of the petri dish. Holes were then punched through the inlets and outlets of the chip. The chip and substrate were then subjected to an oxygen plasma treatment and pressed together to bond as described by Duffy et al.[5]. The edges of the substrate were adjusted to limit the chance of the substrate from peeling off from the chip. The chip was then placed back into the oven at 75ºC until needed (Figure 1).

Octadecyltrichlorosilane (OTS) vapor was introduced into the PDMS device through a vapor-phase deposition system as shown in Figure 2. The PDMS device was placed on a hot plate at 70º C for 45 minutes while hydrolysis of OTS vapor created a thin film around the inner surface the microchannels within the chip. The device was then removed from the system and placed back into the oven. A solution of mineral oil and 0.4 (wt./wt.)% Span 80, a surfactant, was introduced from two side channels as the continuous phase (CP), while deionized water (DIW) was injected into the central channel as the dispersive phase (DP). The two liquids were forced through a narrow orifice of the microfluidic device using syringe pumps. Under the shear force of CP, the DP elongated and broke up into monodispersed droplets. The CP carried the droplets flowing in the downstream channel of the microfluidic device. The flow rates were adjusted accordingly to tune the size and size distribution of droplets (Figure 3). Flow rates of 1.8 ml/hr and 1.5 µl/min were used for the CP and DP respectively. The continuous flow rate (CFR) was reduced to 0.18 mL/hr and images were taken at 0.178 mL/hr increments up to a maximum of 3.56 mL/hr, with a constant dispersive flow rate (DFR) of 1.5 µL/min. The same was done with the DFR, from 0.5 µL/min to 2.5 µL/min with 0.1 µL/min increments at a constant CFR of 1.78 mL/hr. The four droplets from each change in phase were measured for diameter, and the average diameter and variance for each flow rate were calculated. The average diameters were plotted on a flow rate vs. diameter plot, and the average variance was calculated. Two monomer mixtures, 0.6 (wt./wt.)% and 1 (wt./wt.)% sodium alginate in DIW were prepared and used as the DP, with oil as the CP. The DP rates for the 0.6% mixture were increased to between 3 µL/min to 5 µL/min to compensate for the increase in viscosity. However, the increments were kept at 0.1 µL/min. Similarly, the rates for the 1% mixture increased to between 5 µL/min and 7 µL/min, again with 0.1 µL/min increments. Images were taken again for every flow rate, and average diameter was plotted against DIW droplets. Average variance was also calculated for each concentration of sodium alginate. A mixture of 1.5% Span 80 and 3% calcium acetate ($C_4H_6O_4Ca$) in soybean oil by weight was prepared and used as the CP. In addition, three solutions of sodium alginate ($NaC_6H_7O_6$) in DIW were prepared, at 0.5%, 1%, and 1.5% by weight. Each mixture of sodium alginate was used as the DP, and the soybean oil mixture was used as the continuous phase. Capsules were created in the same way as droplets, with adjustments to stabilize droplet formation. The 0.5% sodium alginate mixture stabilized around a CFR of 1.78 mL/hr, and a dispersive rate of 2 µL/min. The 1% solution stabilized around a CFR of 4.27 mL/hr and a dispersive of 1.5 µL/min, and the 1.5% solution around a CFR of 3.91 mL/hr and a dispersive of 4 µL/min. For each level of sodium alginate, images of the formation of capsules with calcium alginate surfaces were taken, and the capsules were then collected in a collection vial. The capsules within the vial were then viewed under a microscope and imaged once immediately after creation and once five minutes later.
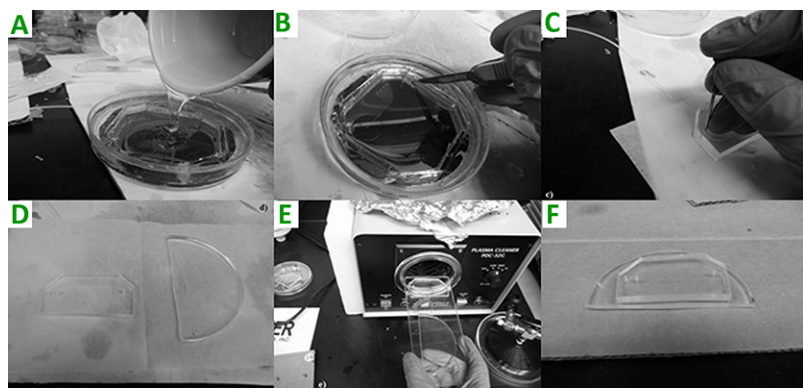
Figure 1. The fabrication of a microfluidic reactor: a) Pouring PDMS mixture into SU-8 master. b) Cutting out PDMS chip from master. c) Inlet holes being punched into the chip. d) PDMS chip on right, substrate on left. e) Chip and substrate placed into the plasma cleaner to be treated with oxygen plasma. f) The final completely bonded chip.
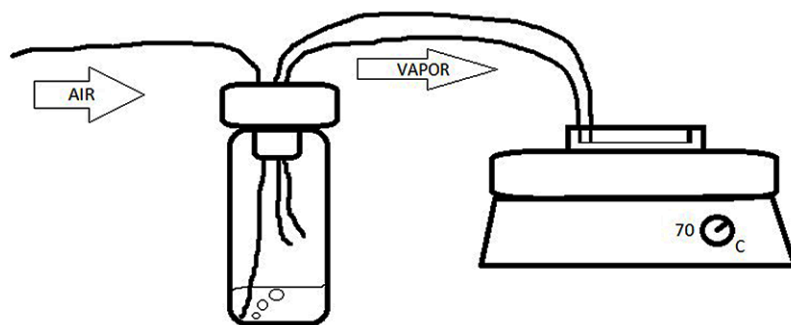
Figure 2. A vapor phase deposition system. Air moves in from the right through tubing to create bubbles containing octadecyltrichlorosilane vapor. The pressure in the vial pushes the vapor through the two outlet tubes into the inlets of the microfluidic chip.
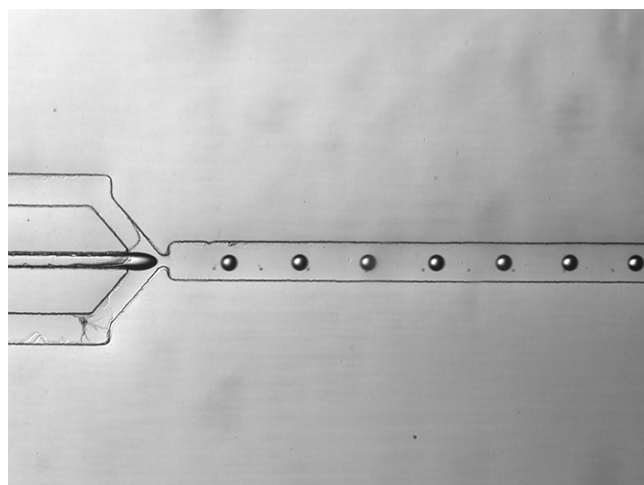
Figure 3. Optical microscope image of continuous droplet generation from a microfluidic device.

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Results

At each flow rate increment, four droplets were measured for diameter. The change in diameter between flow rates, average diameter at each flow rate, and variance at each flow rate were calculated. Average diameters were plotted against each other on a scatterplot (Figure 4). The average diameters of the droplets increased as DFR increased, and decreased as CFR increased. This was evidenced by the negative average change in diameter when increasing the CFR, while when increasing the DFR, the average change was positive on all occasions. An increment in CFR influenced the change in diameter more than an increment in the DFR did. For example, an increase in the CFR decreased water droplet diameter on average by 3.24 µm, while an increase in DFR increased water droplet diameter on average by only 2.1 µm. Furthermore, the addition of sodium alginate within the dispersive phase decreased the average variance by over 70% of the average variance when the dispersive phase was plain DIW. For example, when changing the DFR, the variance dropped from 7.64, with plain DIW only, to 1.71, with 0.6% sodium alginate, a 77.6% decrease over the average variance with the DIW. A measurement representative of the inverse pressure needed to create a droplet was devised as the average diameter of a droplet divided by the median flow rate tested (Equation 1). This measurement was used for the changes in DFR, and it showed a sharp decrease from 65.28 µm/µL/min to 24.12 µm/µL/min in the p-1 value, from the water to the .6% sodium alginate mixture respectively, and another decrease to 16.97 µm/µL/min when the concentration of sodium alginate increased to 1% (Table 1).

For each concentration of sodium alginate, a series of images were taken (Figure 5). For the .5% sodium alginate capsules, the formation of the droplets was not stable, with the capsules that were made fluctuating between diameters above 150 µm and below 70 µm, without changing the rate of flow. The stability of capsule formation increased directly with the amount of sodium alginate

$$p^{-1} = \frac{\overline{d}}{\overline{r}}$$

Equation 1. A measure of inverse pressure, where d is the mean diameter, and r is the average flow rate. It is important to note that p^{-1} is not an exact statistic and is only used as a guideline.

Table 1. A side-by-side summary comparison of DIW, 0.6%, and 0.8% sodium alginate droplet diameter data.

Change in Continuous Flow Rate	Water	0.60% Sodium Alginate	1% Sodium Alginate
Average Diameter (um)	100.6	108.24	118.64
Average Change in Diameter (um)	-3.24	-4.12	-3.11
Average Variance	9.475	1.89	2.67
Change in Dispersive Flow Rate	Water	0.60% Sodium Alginate	1% Sodium Alginate
Average Diameter (um)	97.92	96.48	101.84
Median Flow Rate (uL/min)	1.5	4	6
Average Diameter/Median Flow Rate (um/uL/min)	65.28	24.12	16.97333
Average Change in Diameter (um)	2.1	0.54	1.32
Average Variance	7.64	1.71	1.62

present, which allowed for the continuous, non-interrupted creation of capsules of roughly the same size.

However, none of the droplets created with any of the three concentrations of sodium alginate were visibly monodisperse, with some capsules having circular cross sections, while others having more elliptical cross sections, despite being created one after another in the same environment.

The created capsules were collected in a collection vial and imaged once after the experiment was completed, and once five minutes later (Figure 6). During the five minutes, the capsules that were constructed within the device broke down from their rigid state. There was a correlation between the amount of sodium alginate in the dispersive phase and the rigidity of the droplets after five minutes, with the 0.5% concentration having almost no visible wall around the droplet, while the 1.5% concentration still had rigid walls on nearly all of the droplets.

In addition, at the 3% concentration of calcium acetate, the calcium was still able to be seen under the microscope during the droplet formation, even after sonicating the mixture (Figure 7). These small particles often clogged up the chip, and rendered the chip unusable.

Discussion

As stated before, the value of average droplet diameter divided by the median flow rate has an inverse relationship with the pressure needed to create droplets within a microfluidic reactor. The decrease in the p^{-1} value, from 64.28 to 24.12 to 16.97 µm/µL/min, as the concentration of sodium alginate increased shows the increase in pressure needed to form a droplet, and is consistent with the increased viscosity of the mixtures with sodium alginate.

The drop in variance noted when changing the flow rates of either the continuous or dispersive phases after introducing sodium alginate shows that the sodium alginate increases the monodispersity of droplets generated. This could be a result of the increase in pressure needed to form a droplet, without increasing the threshold at which the droplet shears, therefore creating droplets of similar sizes.

The increase in average change in droplet diameter per flow rate increment increased from 2.1 µm for a change in dispersive rates

to one of 3.24 µm for a change in continuous rates, indicating that an increment in continuous flow rate has a greater effect on droplet size than an increment in dispersive flow rate. This is consistent with the increments at which the flow rates changed, as the increment of the continuous rate, 0.1 mL/hr, is equal to 1.66 µL/min, which is greater than the 0.1 µL/min increment for the dispersive rate.

The stability of capsule formation increased directly with sodium alginate concentration in the dispersive phase. The 0.5% sodium alginate mixture created fluctuations in the capsule size, while adding more sodium alginate decreased the frequency of these fluctuations. The capsules were noticeably not monodisperse, with no evident improvement from increasing the level of sodium alginate. The reduction in the fluctuations is similar to the increase in monodispersity in the water in oil droplets, in that it might be caused by an increase in the pressure needed to create a capsule, without increasing the maximum size of a capsule. However, this effect is much less evident here in terms of monodispersity, as there appears to be no visible increase in monodispersity with an increase in sodium alginate concentration.
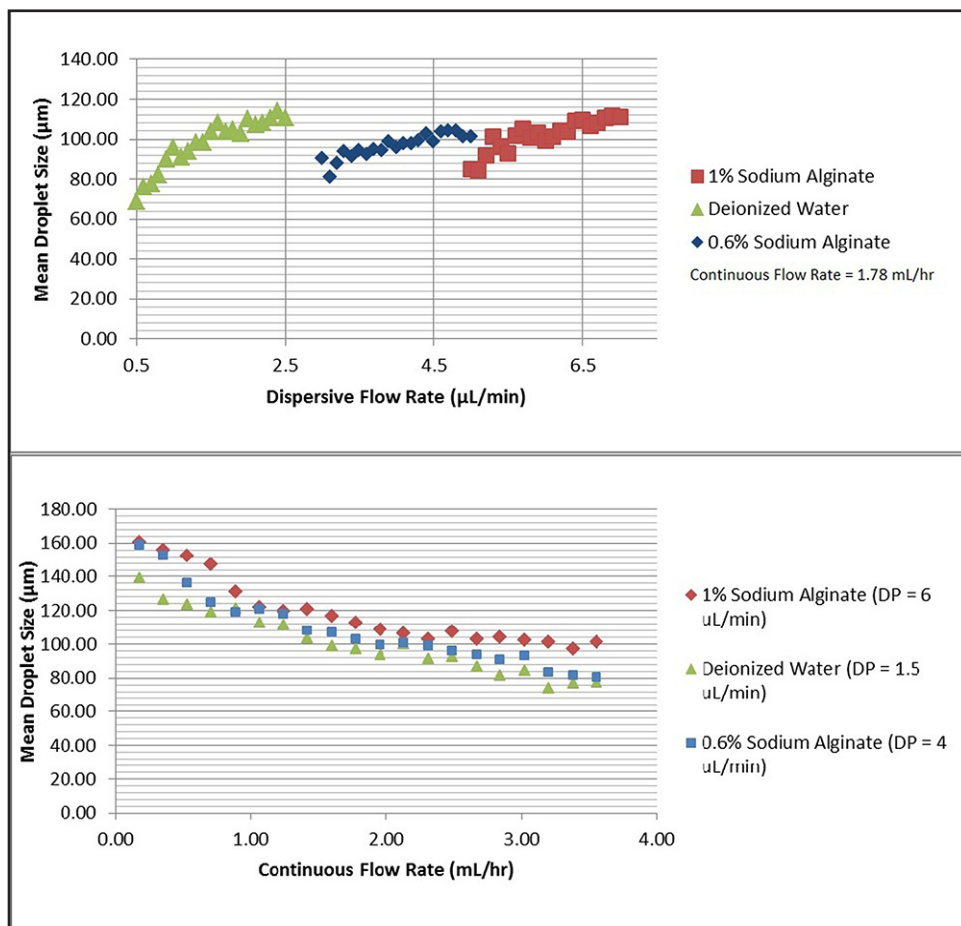
Figure 4. Scatterplots of flow rate vs. droplet diameter data. On top is the plot when changing dispersive flow rate, with a continuous rate of 1.78 mL/hr. On the bottom is the plot when changing continuous flow rate, with variable dispersive flow rates, listed next to the legend.
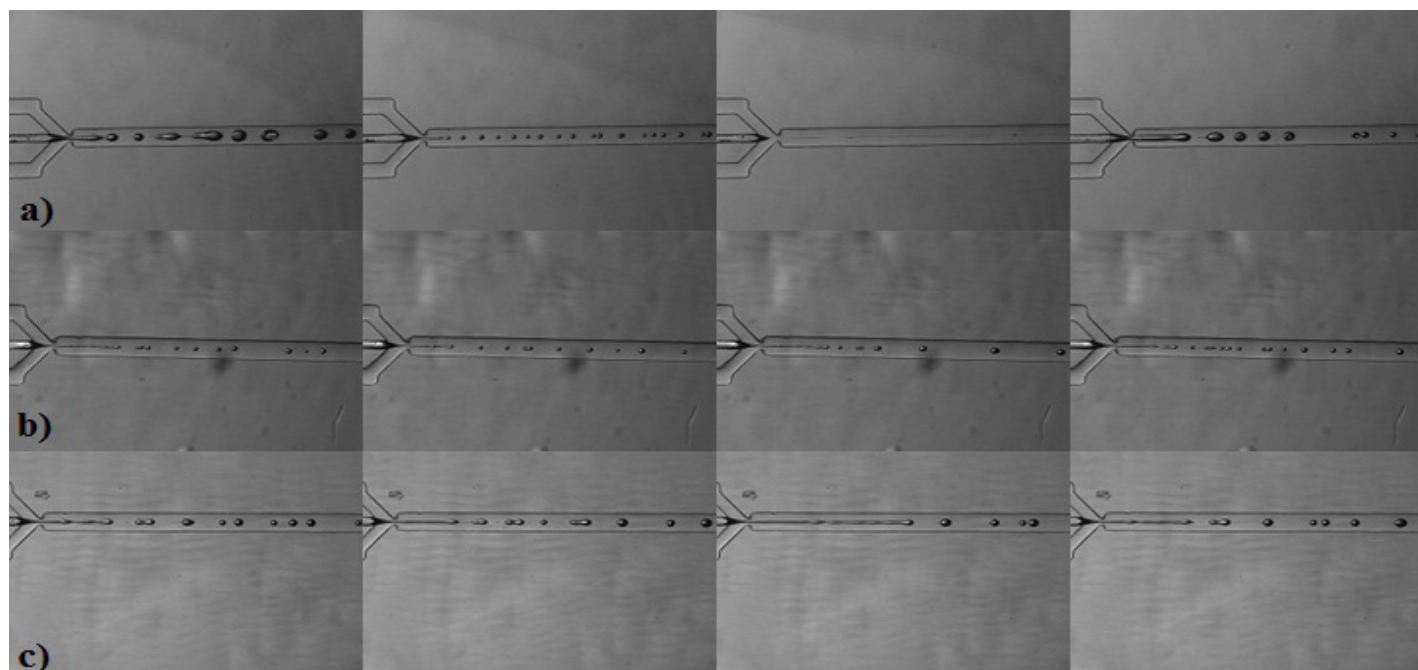
Figure 5. A comparison of the capsule formation for (a) 0.5% sodium alginate, (b) 1% sodium alginate, and (c) 1.5% sodium alginate. Images taken in sequence shown, from left to right.

The stability of the capsules within the collection container increased with an increase in concentration of sodium alginate, with the 0.5% sodium alginate capsules with obvious deformation within the 5 minutes, and the 1.5% capsules with little evidence of deformation. This is most likely the result of the increased viscosity of the liquid within the capsule, as well as the increased amount of time for reactions to form a stronger cross-linked membrane for the capsule.

The problem of particles of calcium in the continuous phase clogging the chip could be caused by the fast gelation of the polymer precursors due to more calcium added to an already saturated oil mixture. This result indicates that less calcium should be added in the future to reduce the likelihood of blocking the channels in the chip.
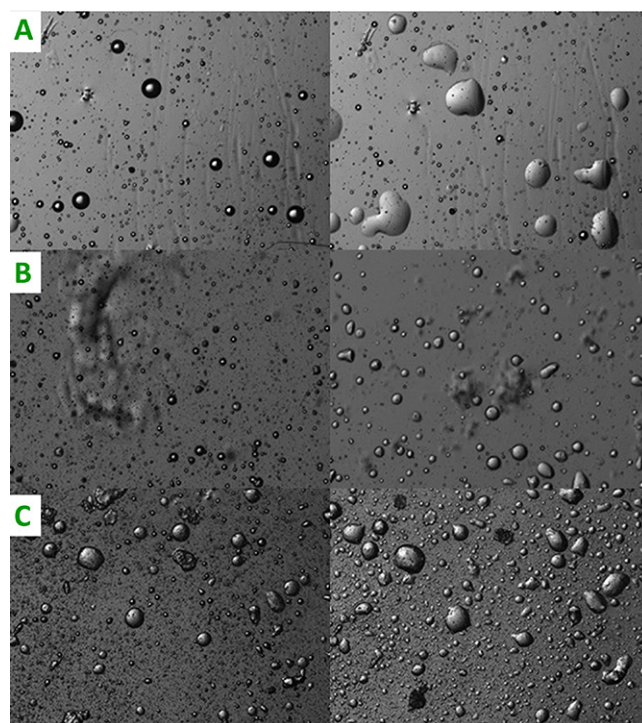
The data pertaining to the water in oil droplets align very well with expectations, such as the idea that solutions that have a higher viscosity would need a higher pressure to allow the formation of droplets. It is beneficial to note that a CFR increment would affect the droplet size more than a DFR increment. It is very obvious that the CFR will be greater than the DFR, but the implications of this when trying to create droplets of a specific size will make controlling droplet size easier.

It has been observed that an increase in the concentration of sodium alginate in the DP creates a more stable droplet formation process, and can create more stable capsules. However, it should not be forgotten that a very high sodium alginate concentration could raise the viscosity of the dispersive phase so much that the dispersive phase will not flow at all. A balance must be found to maximize the stability of the droplets in the future in order to increase the efficiency of capsule formation.
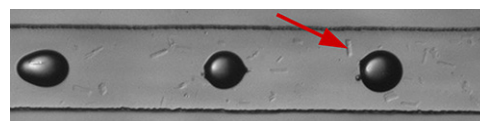
In addition to finding the correct concentration of sodium alginate in the dispersive phase, more research must be done into the effects of differing concentrations of calcium ions during the continuous phase on the formation and stability of the capsules, as well as investigating a way to minimize the excess particles of calcium acetate within the continuous phase. Lastly, experiments should be done in changing the flow rates during capsule formation, to determine if the observed droplet size changes are consistent with any changes in the size of capsules.

Figure 6. A comparison of the before (left) and after (right) images, taken 5 minutes apart, of capsules in the collection container for (a) 0.5%, (b) 1%, and (c) 1.5% concentration of sodium alginate.

Figure 7. Droplets surrounded by particles of calcium alginate (arrow).

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