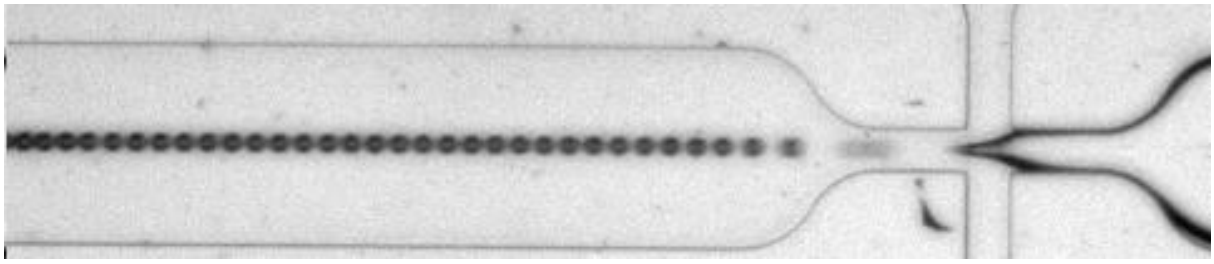
LUND UNIVERSITY
Division of Solid State Physics

Silk capsules in micro channels

Assessing the possibility to use a simple microfluidic system for the creation of self-assembled protein microcapsules



Master thesis in engineering nanoscience
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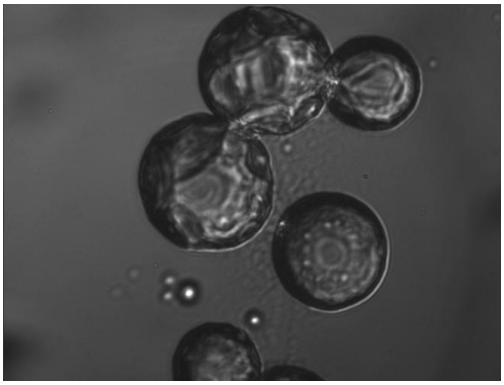

Abstract

Droplet microfluidics deals with the creation of emulsions from two or more immiscible fluids in a controlled, repeatable way. The method has several advantages over traditional emulsification techniques, including the possibility for detailed imaging of the process, precise control and the possibility to manipulate the formed droplets in any number of ways.

Silk is a fibrous, protein-based material generated by several insects and spiders. Traditionally associated with textiles it is today highly explored as an advanced biomaterial. Apart from its remarkable mechanical properties silk has a high degree of biocompatibility and in the last decade it has been gaining increasing use in biomedical applications. Silk is today produced and used in several forms, including fibers, films and solid microspheres. It has been shown that silk proteins will self-organize into thin films at an amphiphilic interface and this mechanism has been utilized to create hollow silk microcapsules in a water-oil or water-organic solvent emulsion.

Our work is intended as a proof of concept and aims to determine whether the conditions inside a flow focusing device (high shear forces and close proximity to channel walls) are compatible with the silk proteins and the capsule formation process. It also briefly explores the differences in film formation at a water-oil and water-organic solvent interface.

We successfully demonstrate that silk microcapsules can be formed within the chip and that sticking to the channel walls is a minor problem that can be managed without the need for surface treatments. There is however a problem with buildup of a silk membrane at the permanent water-oil interface which appears to interfere with the droplet generation. The formed capsules also suffer from poor stability once outside the channels. We suggest further experiments with refinement of the channel design and fine-tuning of the chemical conditions to overcome these problems. Overall droplet microfluidics is a promising method for further studies of silk microcapsule generation.

Acknowledgements:

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Contents

1 Introduction

1.1 Droplet Microfluidics

Microfluidics deals with flow confined in channels of micrometer proportions. On this small scale viscous and capillary forces come to dominate over inertial, leading to a flow free from turbulence. This means that particles can be expected to remain in their respective streamlines and that parallel streamlines will continue alongside with no mixing except for diffusion.

Droplet microfluidics is a subfield of microfluidics which deals with the creation of emulsions inside microfluidic channels. Using a variety of different techniques one fluid is caused to break up into droplets dispensed in a surrounding immiscible fluid. The fluid that forms the droplets is hereafter referred to as the dispersed phase while the surrounding fluid is referred to as the continuous phase.

There are two principal designs used for generating micro-droplets, the T-junction geometry and the flow focusing geometry. In a T-junction the dispersed phase is injected from the side, perpendicular to the continuous phase (Figure 1, left). The droplets being formed almost fill the width of the channel and have a length which is determined by the ratio of flow between the continuous phase and the dispersed phase. In a flow focusing geometry the dispersed phase is forced into a thin filament by two surrounding co-flowing streams of the continuous phase [1] (Figure 1, right).

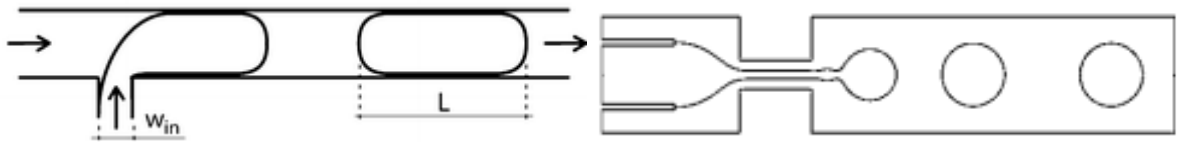


Figure 1 Left: A sketch of a T-junction geometry [2]. Right: A sketch of a flow focusing geometry [3].

As a result of the highly ordered flow inside the channels the breakup of the dispersed phase into droplets will take place in repeatable cycles, causing the formed droplets to be almost identical in size. Another advantage is that the confinement in microfluidic channels allows for easy manipulation of the droplets. By various microfluidic techniques droplets can by fused, split, sorted, stored and exposed to various chemical environments.

This high degree of control, monodispersity and versatility combined with the miniscule volumes makes micro-droplets excellent for micro-reactor applications [4]. Examples include enzyme rate experiments, creation of nanoparticles and protein crystallization experiments. Micro-droplets are also used as templates for microcapsules, for example alginate capsules [5] and solid silk microspheres [6]. A more thorough description of applications has been made by Huebner *et al.*[7] and a more thorough review of droplet microfluidics in general has been made by The *et al.*[8].

1.2 Silk

Silk is a collective name for a class of fibrous proteins produced by spiders and various insects. It is utilized by these animals for various purposes including capturing nets, safety lines and cocoons.

Silk produced by domesticated caterpillars has been used for several centuries for textile production. A few decades ago silk, especially spider dragline silk, began to receive attention for its remarkable mechanical properties, surpassing most other known biological and synthetic fibers. More recently

silk has also been investigated for its beneficial immunogenic properties which make it excellent for biomedical applications such as wound dressing and scaffolds for bone tissue engineering [9].

Silk has therefore been subject to intense research, both with regards to its protein structure and the process by which it is spun into fibers *in vivo*.

It has also been shown that silk proteins will spontaneously self-assemble into ordered films at the water-air [10] and water-oil or water-organic solvent [11] interfaces due to their amphiphilic nature. These films have a distinctly different structure from spun silk fibers.

This propensity to form films has been utilized to induce skin formation on the surface of water droplets in a water-organic solvent emulsion to form silk microcapsules [12].

Conventional emulsification techniques have proven to be sufficient to create stable silk microcapsules but suffer in that they tend to create a wide distribution of droplet sizes and that it is difficult to observe the silk formation process in bulk. By transferring the process into a microfluidic droplet generating chip a highly monodisperse emulsion can be generated which means that effects due to size variations can be avoided in experiments with the droplets. Another advantage is that the whole process, from droplet formation to skin formation, can easily be observed with optical microscopy and possibly by fluorescence microscopy. As an added bonus it is possible to include other functionalities on the chip, for example to transfer the droplets between flow streams of different composition.

2 Theory

2.1 Droplet generation

In a flow focusing geometry the dispersed phase is injected between two co-flowing streams of the continuous phase. The three streams are squeezed in a narrow part of the channel, the nozzle, and are then allowed to expand into the collection channel where the middle stream is broken up into droplets. As the droplets are broken off body and surface forces acting on the fluids must balance. This means that interfacial stress between the two phases and viscous stress created by the movement of the fluids can be assumed to be equal.

We can state the interfacial stress as:

$$\sigma_i \propto \frac{\gamma}{R}$$

Where γ is the surface tension and R is the radius of the fluid cylinder. The viscous stress can be stated as:

$$\sigma_v \propto \frac{\eta v_0}{h}$$

Where η is the viscosity, v_0 is a characteristic velocity and h is a characteristic length, typically the width or depth of the channels.

Balancing these stresses and introducing the dimensionless capillary number, $Ca = \frac{\eta v_0}{\gamma}$, we get

$$\frac{R}{h} \propto \frac{\eta v_0}{\gamma} = Ca$$

This tells us that the ratio between the droplet radius and the channel width will depend on the balance between interfacial and viscous stresses, given by the capillary number.

The above description does in no way capture the full dynamics of the droplet formation process, rather it is an attempt to capture the most important forces at work. The situation is complicated by the fact that viscosities and velocities of both fluids are highly relevant, making it difficult to clearly define the capillary number.

There is an ongoing effort to shed more light on the detailed mechanics of droplet generation in flow focusing. Both capillary pressure and the shear-induced drag by the outer fluid have been suggested as important factors.

Interfacial tension acts to increase the pressure inside a body of fluid. This pressure difference is known as capillary pressure and is given by the Young-Laplace equation — — where γ is the surface tension and R_1 and R_2 are the principal radii of curvature.

One result of capillary pressure is the Plateau–Rayleigh instability which accounts for the fact that a cylindrical fluid jet will become increasingly perturbed over time and eventually break up into a series of droplets. In a cylindrical fluid jet the cylinder radius, R_c, is the only relevant radius of curvature and the capillary pressure is then given by $\Delta p = \frac{\gamma}{R_c}$. We can see that the pressure increases as the radius decreases. This suggests that if a sinusoidal perturbation is imposed on the circumference of the cylinder fluid will be driven from the troughs and into the peaks increasing the amplitude of the perturbation. However, the radius of curvature of the perturbation, R_p, will add a second term to the Young-Laplace equation. This term will increase the pressure in the peaks and decrease it in the troughs, where P_p is negative, counteracting the growth of the perturbation (Figure 2).

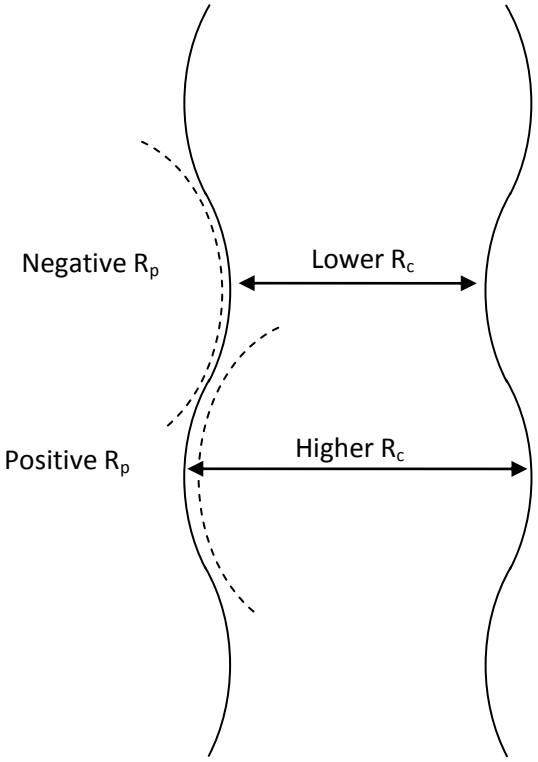


Figure 2: An exaggerated sketch of a perturbed fluid cylinder with the radius of the cylinder, R_c, and the radius of curvature of the perturbation, R_p, marked in a trough and at a peak.

The balance between these two terms and thus the growth rate of the perturbation is determined by the perturbation wavelength and the cylinder radius. This, together with the fact that even a perfectly smooth surface can be described as a sum of sinusoidal components tells us that a cylindrical fluid jet will always display perturbations of a certain wave length that will grow over time eventually growing large enough to break the cylinder into a series of droplets.

Another effect of capillary pressure which is likely to be relevant in the flow focusing geometry is that of end-pinching. This arises as the flow slows down as the channel expands downstream of the nozzle and the middle stream expands into a near-spherical bulb. Since the bulb has a higher radius of curvature than the jet feeding it there will be a drop in pressure. The pressure gradient will create an increase in the flow just upstream of the bulb leading to the creation of a neck in the jet (Figure 3).

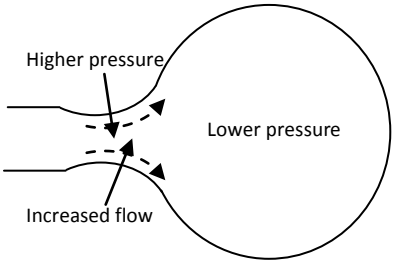


Figure 3: A sketch of the end-pinching mechanism.

In addition to these interfacial effects the outer fluid will exert a viscous drag force on the bulb leading to a stretching and thinning of the inner fluid jet which may also influence the droplet break-off process (Figure 4). This effect will be counteracted by the flow inside the jet supplying it with more fluid.

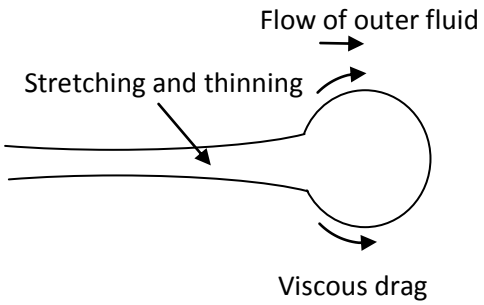


Figure 4: A sketch depicting the action of the viscous drag of the outer fluid to stretch and thin the inner fluid jet.

Though the above described mechanisms are likely involved in the droplet formation process their relative importance remains unclear. Zhou *et al.*[3] have carried out a numerical simulation in which they found that none of them alone can account for the dynamics of droplet formation, the conclusion being that the process is shaped by all of them to a degree.

When qualitatively describing the dynamics in the flow focusing geometry three distinct modes of droplet formation are often mentioned. They are briefly discussed below and are here referred to as jetting mode, dripping mode and tip streaming mode.

- **Jetting** occurs at high flow velocities when the rate of droplet formation cannot keep up with the advancing fluids. In this mode the inner fluid extends as a continuous jet in the collection channel downstream of the nozzle. Droplets may form at the tip of the jet but these are less regular in size than in dripping mode (Figure 5, top).
- **Dripping** is the mode that is normally of interest when using flow focusing. In this mode droplets are formed just downstream of the nozzle and each cycle of droplet break-off is identical to the previous creating a highly monodisperse emulsion (Figure 5, middle). As the droplets are pinched off one or several satellite droplet may form as well (Figure 6).
- **Tip streaming** occurs when the flow velocity of the inner fluid is very low. In this mode droplets are formed inside the nozzle before the inner fluid can expand into the collection channel. The droplets thus formed have a radius that is a fraction of the nozzle width (Figure 5, bottom).

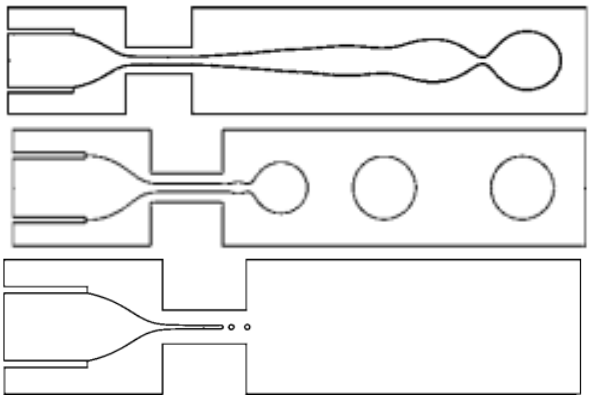


Figure 5 Top: Jetting. Middle: Dripping. Bottom: Tip streaming. Figures from reference [2].

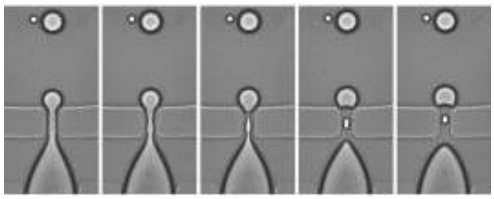


Figure 6 Formation of satellite droplets. Image from reference [1].

Since all of this takes place inside microfluidic channels in close proximity to the channel walls the surface properties of the device are also of great importance. For the droplet formation process to be unperturbed it is important that the middle fluid does not wet the channel walls. If, for example, the emulsion being formed is oil droplets in water the channel walls should be hydrophilic, while in the inverted case the walls should be hydrophobic.

2.2 Silk

The protein structure of silk

The remarkable mechanical properties of silk fibers have triggered an intense research into its molecular structure. Among the most studied types are the cocoons of the *Bombyx mori* silkworm and the spider dragline silk. The proteins from the insect silks such as *Bombyx mori* are referred to as fibroins and the one form spiders as spidroins.

The major protein components of a silk fiber are the fibroins, which forms the β-sheet rich complexes, and the sericin, a hydrophobic protein which mostly acts as adhesive cement between the fibers [13].

The fibroins that compose the core of a silk fiber consist of a heavy chain (H) with a molecular weight of approximately 390 kDa, a light (L) chain 25 kDa (Figure 7) and a linker protein P25 at 30kDa. The fibroins make a unit consisting of six heavy chains, six light chains and one linker unit with L and H joined by a single disulphide bond. The heavy chain has an amphiphilic structure, showing an alternance of hydrophobic and hydrophilic blocks. The hydrophobic blocks are mainly made up of repeats of the sequence (Gly–Ala–Gly–Ala–Gly–Ser) with slight variations which is the crystallizable domain responsible for the formation of β-sheets when the silk is spun [14]. In between the hydrophobic blocks there are small hydrophilic spacers, whose main role seems to be prevention of premature crystallization. At the N- and C-termini there are large hydrophilic end blocks and the intervening parts are mainly hydrophobic.

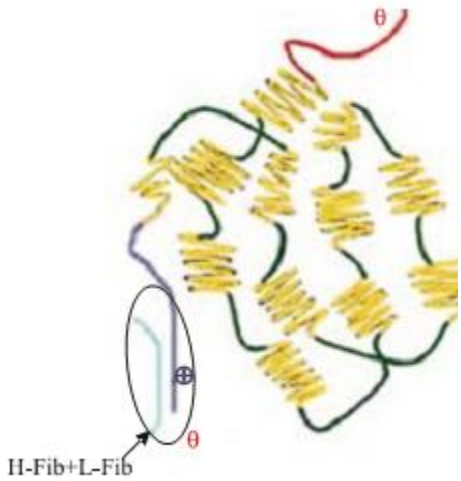


Figure 7: A sketch of the heavy and light fibroin chains. Image from reference [15]

Spinning

The silk is produced as a liquid crystalline dope (silk I) in the posterior part of the gland. As it moves through the lumen of the gland a number of factors combine to induce the structural change from the liquid crystalline silk I into the β-sheet rich water-insoluble fiber (silk II). The transition is a highly regulated process were interactions between the large hydrophobic blocks play a major role. Regulation of interactions between hydrophilic terminals and spacers by altering charge distribution through changes in pH and concentration of ions are also important [15].

Film formation

Fibroin proteins have been reported to self-assemble into membranes at the interface between water and air. The structure of these membranes, named silk III, has been studied [10] and was found to be different from the structure of silk I and II. The crystallizable domain $(Gly–Ala–Gly–Ala–Gly–Ser)_x$ formed in this case a helical structure rather than a β-sheet.

The structure has also been observed at the interface between water and oil or organic solvent [11]. The basic crystal structure appears to be the same but the packing is found to be different. The most

probable reason for this is differences in thickness of the various interfaces, the water-air interface is very sharp, while the water-organic interface is about 10 nm thick, and the water-oil interface 500 nm [11].

Encapsulation

The capacity to self-assemble into membranes at water/non polar interfaces has also been demonstrated in emulsions. Hermansson *et al.*has reported formation of skin on the surface of droplets in an emulsion of aqueous silk solution and toluene [12]. The protein, a recombinant spider fibroin analogue, diffuses out of the solution and assembles into a thin membrane at the interface between the droplet and the surrounding solvent. The result is a very thin (less than 50 nm) and resilient silk capsule of micrometer dimensions.

Collaboration with C. Dicko et al. on a similar method using a solution of regenerated *Bombyx mori* fibroin is the basis for the work presented in this thesis. The silk solution is emulsified in oil or organic solvent, and subjected to stirring in order to keep the emulsion stable while the silk membrane is formed. At this stage the capsules are not stable as the hydrogen bonds between the proteins are partially hydrated. The droplets are therefore transferred into an alcohol-containing phase using centrifugation. The alcohol is believed to dehydrate the membranes by inducing an irreversible β-sheet conversion within the capsules' skin [16].

2.3 Device manufacture

Soft lithography

Microfluidic channels can be fabricated in many different ways, including etching in glass or silicon or combination of capillary tubes. One of the simplest and most commonly used methods is however to make the channels out of polydimethylsiloxane (PDMS) using soft lithography.

PDMS has several properties that make it suitable as a material for microfluidic channels. Its transparency in the visible range allows for easy visualization of events inside the channels. It has a UV cutoff at 240 nm which allows for near to middle UV fluorescence imaging. It is impermeable to liquid water, permeable to gases, non polar or apolar solvents, non-toxic, and nonreactive to most reagents [17].

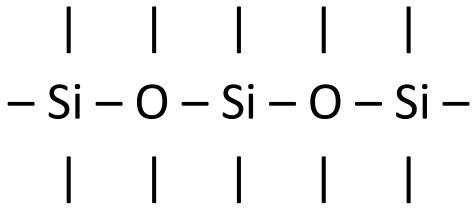


Figure 8: Sketch of PDMS polymer unit.

Soft lithography is a molding technique by which a very large number of devices can be produced from one previously defined master. The master contains a negative of the desired channel pattern, normally defined in silicon using UV-lithography and etching.

A liquid pre-polymer is mixed with a curing agent and poured over the master and is then allowed to cross-link. The solid polymer slab, now containing the channel pattern, can be then removed. After some post processing, such as stamping holes for in- and outlets, the slab is attached to a bottom plate and the device is ready for use.

Device assembly and surface properties

Before the device can be used the PDMS slab containing the channels must be attached to a bottom plate, usually a glass slide or another piece of PDMS depending on the application. Adhesion is often achieved by treating the surfaces with oxygen plasma. The oxygen plasma reacts with the Si-CH_3 groups in the surfaces creating Si-OH groups. Upon contact with another treated surface these will react to form Si-O-Si, covalently linking the two surfaces [18].

The plasma treated surface is known to be unstable and for bonding to take place the treated surfaces must normally be brought into contact within 30 min. The proposed reason for this instability is the presence of mobile oligomers in the polymer network. Since the exposure of a hydrophilic surface to a non polar environment (air) is unfavorable, diffusion of oligomers will act to remove OH-groups from the surface, thus increasing its hydrophobicity.

A side effect of the aforementioned oxygen plasma treatment is that the naturally hydrophobic PDMS is rendered hydrophilic. This means that oil-in-water type emulsions, which require hydrophilic channel surfaces, can be created within a device sealed with oxygen plasma. Water-in-oil type emulsions, however, require the channel walls to be hydrophobic which means that the fabrication method needs to be altered in some manner. We tried two approaches: sealing the device without using plasma, avoiding the creation of hydrophilic surfaces altogether, and sealing the device with oxygen plasma and allowing the surfaces time to recover their original hydrophobicity.

Plasma free bonding

In order to find a suitable method approach to sealing the device without using oxygen plasma three different methods were evaluated: curing agent diffusion, partial curing and stamp-and-stick.

- Curing agent diffusion - In curing agent diffusion the ratio between pre-polymer and curing agent is altered, one layer is made with a surplus of curing agent and one with a surplus of pre-polymer. The theory is that there should be enough unreacted groups at the surfaces for the polymerization to continue across the interface, bonding the two surfaces together.
- Partial Curing - Partial curing relies on shorter baking time or lower baking temperature to make sure that there are unreacted groups at the surfaces, similar to curing agent diffusion.
- Stamp-and-stick - The stamp-and-stick method relies on an adhesive to bond the two surfaces together. The key to success is getting the adhesive layer thin enough to not fill the channels. This is achieved in stamp-and-stick by spin-coating the adhesive onto a cover slide, then “stamping” the slab containing the channels onto the slide, transferring adhesive only to the base of the slab and not to the channel walls. The channels and the bottom plate can then be brought together and cured as per the requirements of the adhesive in question.

These methods have been described and investigated in a comparative study by Eddings *et al.*[19].

Plasma bonding and recovery

The hydrophilic surface generated by plasma treatment is not stable and plasma treated PDMS is known to recover its hydrophobicity within a few days. If this recovery period can be made short enough the strong adhesion and high reproducibility of plasma bonding may outweigh the increase in fabrication time. In order to establish a relevant benchmark for the other bonding methods the rate of recovery after plasma treatment was investigated.

3 Materials & Methods

3.1 Device Manufacture

Creation of channels with hydrophilic surfaces by soft lithography

PDMS pre-polymer and curing agent (Sylgard 184, Dow Corning) was mixed at a ratio 10:1 and degassed for 25 minutes. The mixture was poured over the master and was baked for 1 hour at 80 ˚C. The cured PDMS layer was peeled off and holes were stamped at the in- and outlets. The PDMS surface was treated with oxygen plasma for 60 seconds and immediately attached to a similarly treated glass slide. Reservoirs were glued to the in- and outlets, the glue was allowed to dry for one hour.

Variation: In order to better preserve the hydrophilic surface the channels may be filled with water prior to attachment of reservoirs.

Creation of channels with hydrophobic surfaces by soft lithography

PDMS pre-polymer and curing agent (Sylgard 184, Dow Corning) was mixed at a ratio 10:1 and degassed for 25 minutes. Part of the mixture was poured over the master; part was spread over a glass slide to form the bottom plate. The mixture was baked for 1 hour at 80 ˚C. The cured PDMS layers were peeled off and holes were stamped at the in- and outlets. The PDMS surfaces were treated with oxygen plasma for 60 seconds and immediately assembled. Reservoirs were glued to the in- and outlets, the glue was allowed to dry for one hour.

The channels were filled with oil and stored for at least 24 hours at room temperature.

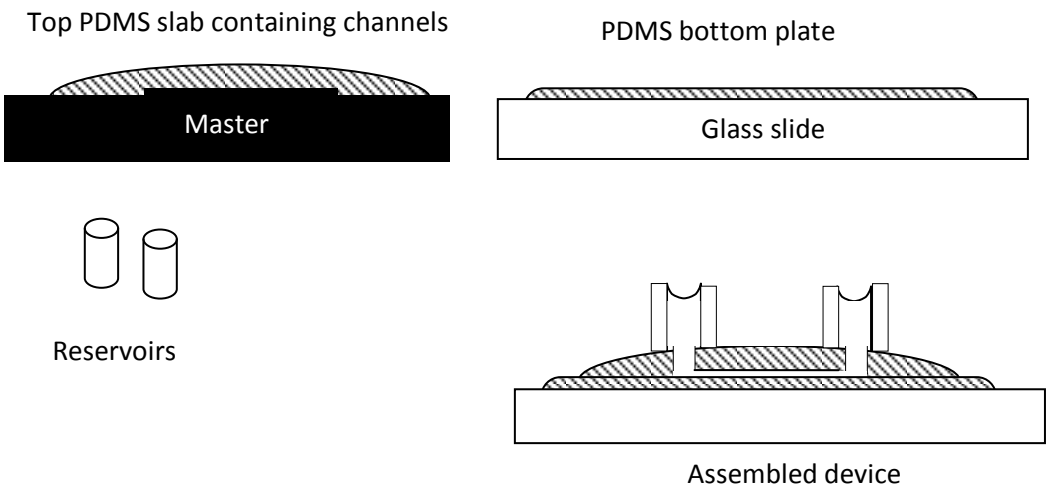


Figure 9: A sketch of the cured PDMS components and the assembled device.

Plasma free bonding

We tested three different methods of sealing two pieces of PSMS together without using oxygen plasma treatment. The achieved bond strength was evaluated by simply trying to pull the two pieces apart. As a benchmark we used the bond strength which is achieved with plasma bonding, where the attached pieces cannot be separated without tearing.

Apart from bond strength the risk for detrimental effects on channel geometry was also considered to be an important factor.

- **Curing agent diffusion** - Two batches of PDMS pre-polymer and curing agent were mixed, one with a mixing ratio of 15:1 and one with 6.7:1. The mixtures were degassed for 25 minutes. Each mixture was poured onto a glass slide and baked for one hour at 80 ˚C. The cured PDMS layers were peeled off and brought together with the flat surfaces facing inward. The assembled device was baked overnight at 80 ˚C.
- **Partial curing** - PDMS pre-polymer and curing agent was mixed at a ratio 10:1 and degassed for 25 minutes. The mixture was poured onto two glass slides and baked for 10 minutes at 80˚C. The cured PDMS layers were peeled off and brought together with the flat surfaces facing inward. The assembled was baked for one hour at 80 ˚C.
- **Stamp-and-stick** - PDMS pre-polymer and curing agent was mixed at a ratio 10:1 and degassed for 25 minutes. Part of the mixture was poured over the master; part was spread over a glass slide to form the bottom plate. The mixture was baked for one hour at 80 ˚C. The cured PDMS layers were peeled off and holes were stamped at the in- and outlets. PDMS pre-polymer and curing agent were mixed 10:1, degassed and then spun to a glass cover slide at 6000 RPM for one minute. The channel part was stamped onto the coated slide. The device was then assembled and baked for one hour at 80 ˚C.

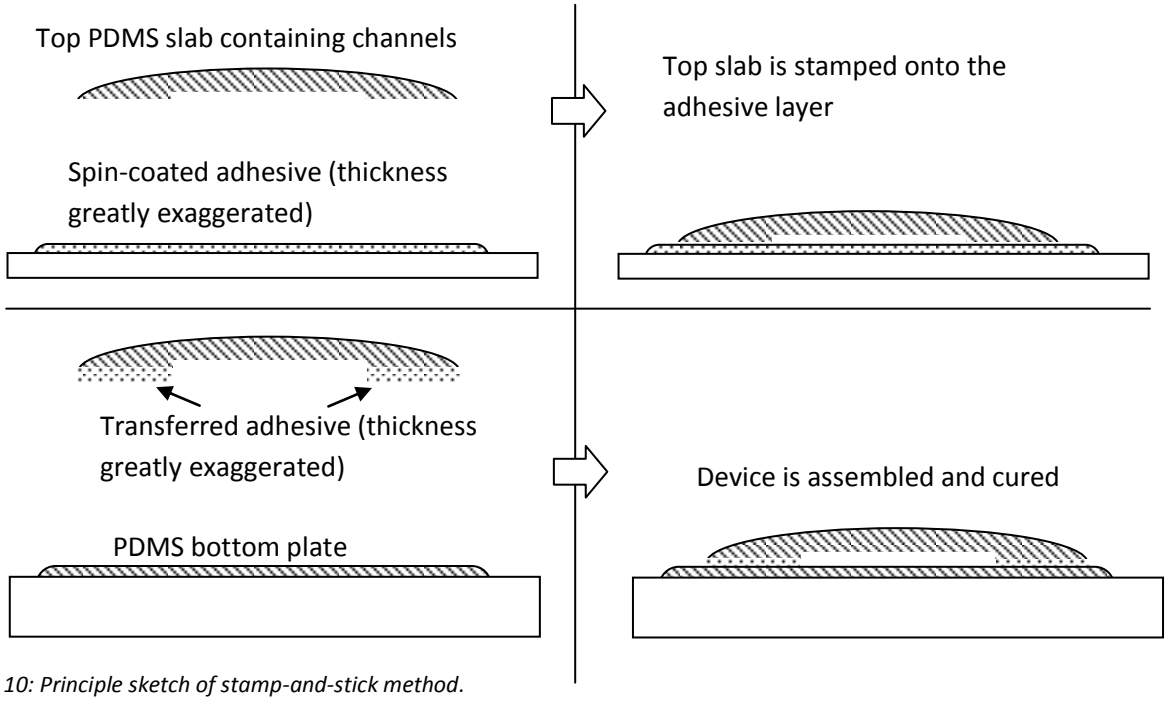
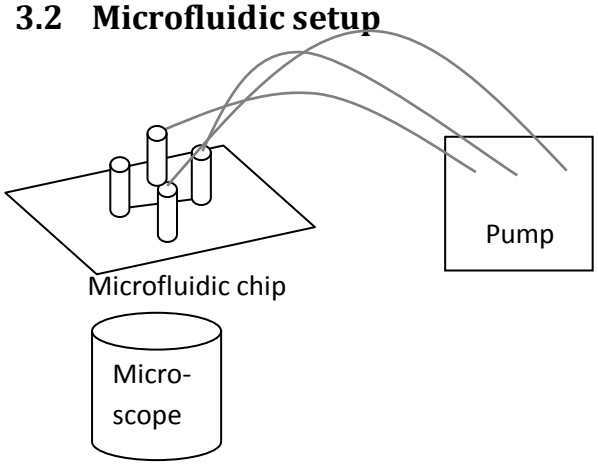


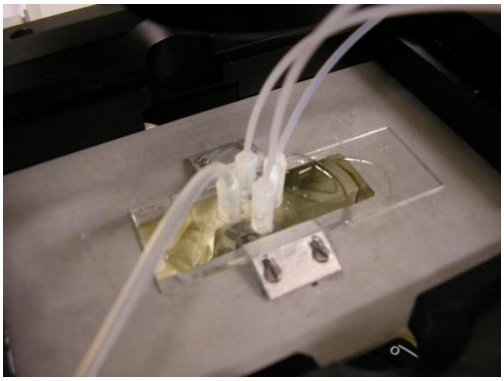
Figure 10: Principle sketch of stamp-and-stick method.

Plasma treatment and recovery

PDMS pre-polymer and curing agent was mixed at a ratio 10:1 and degassed for 25 minutes. The mixture was poured onto a glass slide and baked for one hour at 80 ˚C. The cured PDMS layer was peeled off and then cut into several squares, 1x1 cm^2. The squares were exposed to oxygen plasma for 60 seconds and then incubated in three different conditions, at room temperature, in an 80˚C oven and submerged in sunflower oil. After 24 hours the squares were submerged into sunflower oil and surface angle measurements were made using 2 µl water droplets.

3.2 Microfluidic setup





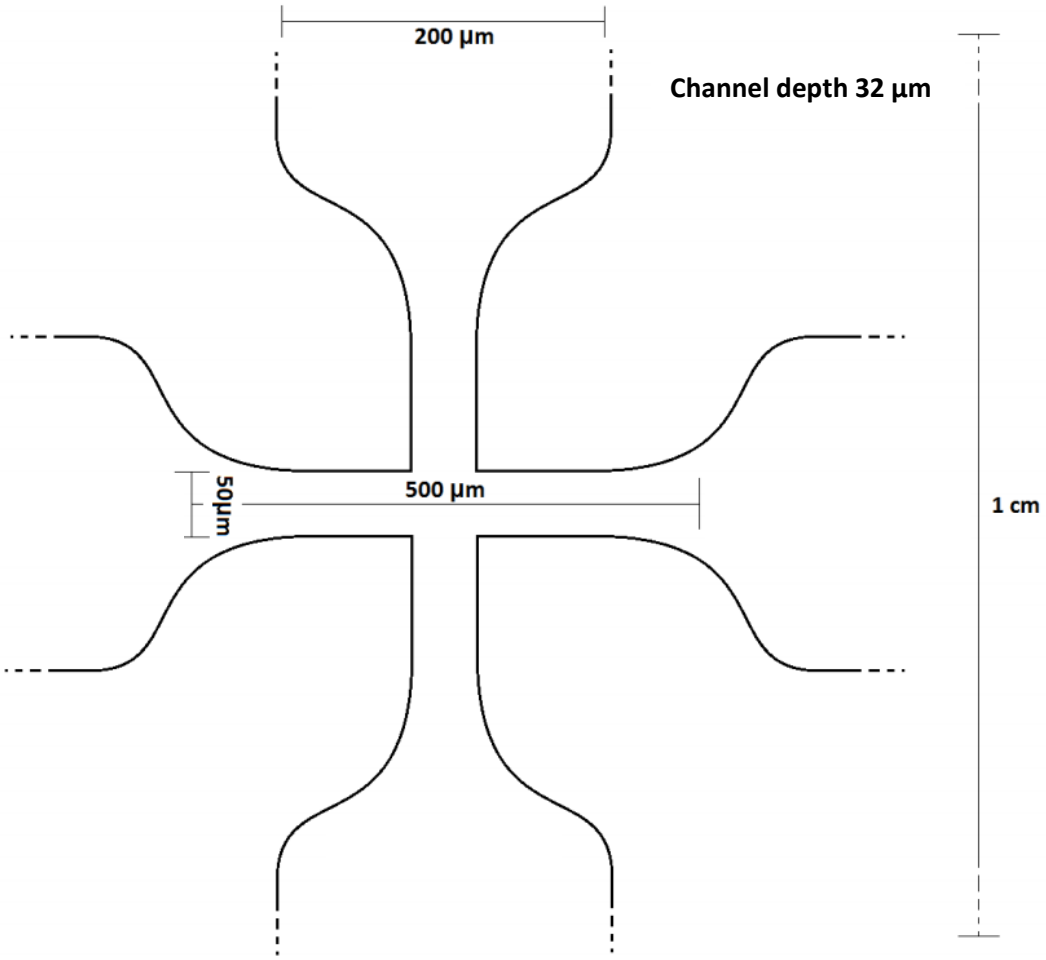


Figure 11 Top left: A general sketch of the setup Top right: An example photograph of the chip mounted on top of the microscope. Bottom: Outline of the channel design used for droplet generation.

Two methods for generating flow through the device were used, syringe pumps (Aladdin 1000 and SP210IWZ) and MFCS Fluigent. A syringe pump generates a constant volumetric flow rate by pushing the piston of a syringe with a given speed. The MFCS Fluigent delivers a constant gas pressure which is regulated with high precision through computer software.

3.3 Silk

Regenerated silk fibroin (RSF) solutions

Regenerated silk fibroin solution was prepared by C. Dicko.

Degumming (sericin removal): Cocoons of *Bombyx mori* were used as starting material. The cocoons were cut in small pieces and heated in an alkaline solution of Na_2CO_3 0.5% for 1 hour at 70 °C. Thorough rinsing in distilled water was performed to remove the dissolved sericin. The fibers were then left to dry in air overnight.

Regeneration: To produce soluble silk proteins, 2g of degummed silk were dissolved in 5mL of 9M LiBr solution at 70°C. The solution was dialyzed against distillated water for 24 hours at 4°C. Regular change of water were performed with the last one being milliQ water. After dialysis, the solution was transferred into a Falcon tube and centrifuged for 30 min at 3500 rpm and the supernatant transferred to a new tube. The protein concentration was determined using the dry weight method. The final solution concentration was 75mg/ml [16].

This solution was diluted 100:1 before being used in the droplet experiments.

4 Results

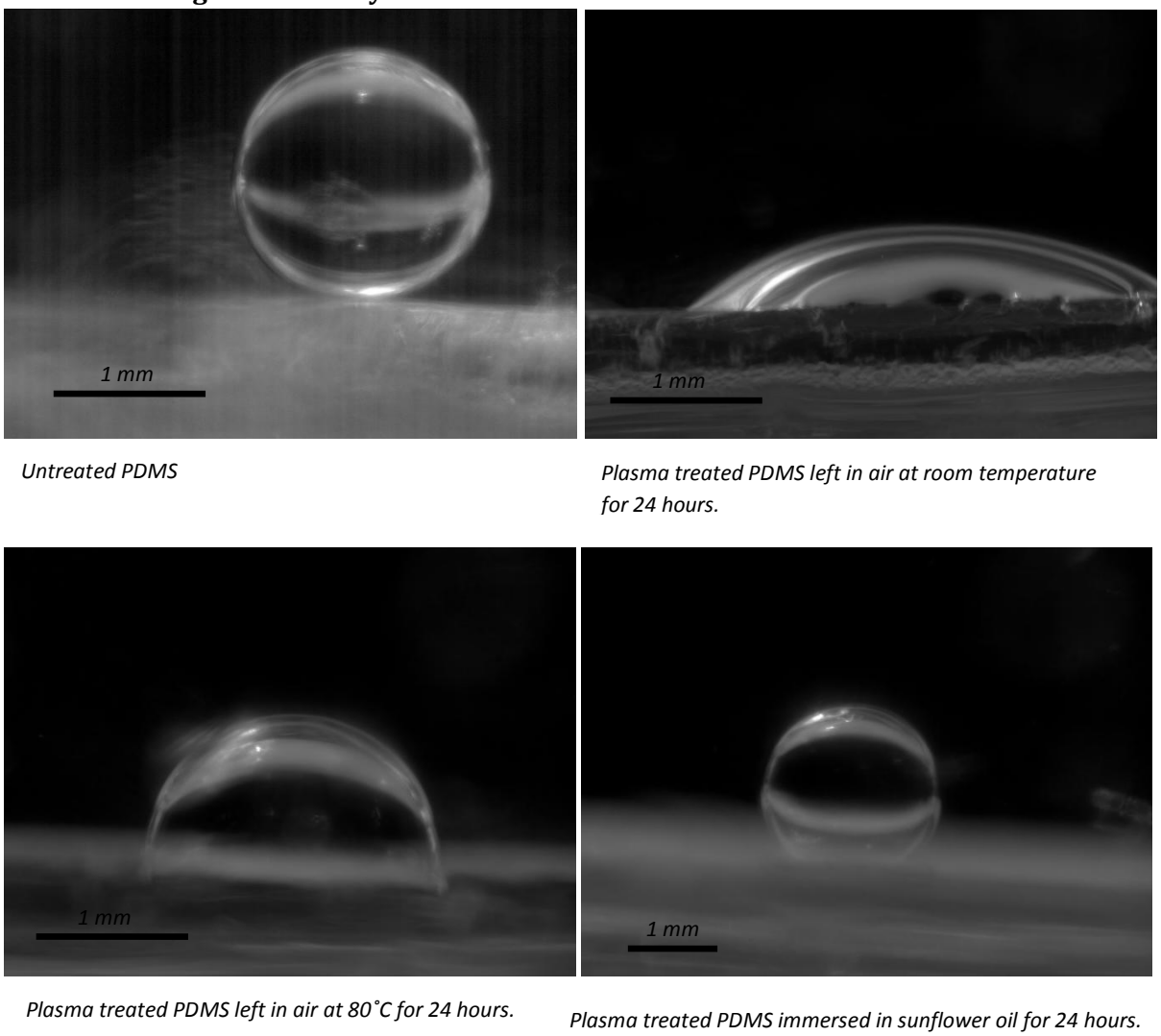
4.1 Evaluation of bonding methods

Plasma free bonding

All of the three methods displayed some flaws in performance in the first round of experiments. Out of the three the partial curing method seemed to hold most promise. Since the plasma bonding and recovery method performed well the plasma free bonding techniques were not pursued further.

- **Curing agent diffusion** - Altering the mixing ratios of PDMS resulted in undesirable material properties, increasing the amount of curing agent led the PDMS to become stiff and brittle, decreasing it made it sticky. The two surfaces could be pulled apart with relative ease even after overnight baking, indicating poor bond strength.
- **Partial curing** - With a baking time of approximately 10 minutes good bonding could be achieved but there were issues with reproducibility. The process was very sensitive to small variations in baking time, if the PDMS was left in the oven for too long no bonding at all would result, if it was taken out too early it would still be sticky, leading to risk for PDMS residues to stick to the master causing defects in the channel geometry.
- **Stamp-and-stick** - Using the curing agent as an adhesive gave no significant bonding whatsoever. Using the PDMS mixture gave moderate bond strength but we were not able to get the adhesive layer thin enough to prevent it from completely filling the channels. The results could probably be improved by altering the spinning conditions or decreasing the viscosity of the PDMS by mixing it with a solvent, but even a very slight filling of the channels would introduce a random alteration of the geometry which would be very difficult to control and account for.

Plasma bonding and recovery



Untreated PDMS

Plasma treated PDMS left in air at room temperature for 24 hours.

Plasma treated PDMS left in air at 80˚C for 24 hours.

Plasma treated PDMS immersed in sunflower oil for 24 hours.

Figure 12: Contact angle measurements. All images show 2µl water droplet resting on a PDMS surface immersed in sunflower oil.

The plasma treated surfaces displayed clearly different wetting properties after having been aged in the different environments (

Figure 12). The surface aged in air at room temperature showed moderate recovery, the one aged at 80 ˚C clearly more and the one aged immersed in oil had completely recovered the original hydrophobicity of untreated PDMS.

4.2 Droplet Generation

Device performance

The crossed-channel geometry proved to be quite sufficient for flow focusing droplet generation. The main drawback of the design was the shortness of the collection channel which severely limited observation of droplets once generated.

The devices created with the plasma bonding and recovery technique performed well for most of the time but occasionally a batch would prove completely incapable of generating droplets. The result

would instead be a jet of fluid, clearly sticking to one of the device surfaces. The most probable cause of this is that the channel walls are still hydrophilic to a degree, this will be addressed in more detail in the discussion section.

Flow delivery

Both the MFCS Fluigent and the syringe pumps proved useful for droplet generation. The MFCS Fluigent proved unstable at pressures below approximately 100 mbar and had a maximum output pressure of 1 bar. The response time of the MFCS Fluigent was very short, the system reached steady-state within seconds of a change in requested pressure.

The syringe pumps, using 3 ml syringes, provided a stable flow down to approximately 1 µl/min. The response time was very sensitive to the setup of tubes and connectors and in bad cases it could take several minutes for the system to stabilize after the flow rate had been changed.

Droplet experiments

We tried generating droplets using three fluid combinations: water-in-oil, oil-in-water and water-in-ethyl acetate. The water-in-oil system was used to study the general behavior of the droplet generating system and the majority of the droplet generation experiments were carried out using this combination. Oil-in-water was tested to assess the possibility of inverting the system. Water-in-ethyl acetate was tested to assess the compatibility of the device with a mild organic solvent. Ethyl acetate-in-water was not tested due to time constraints.

Water-in-oil

Water-in-oil droplet generation was tested with syringe pumps and fluigent. Three distinct modes could be observed, jetting, dripping and tip streaming. Figure 13 shows how jetting typically looked in this system. The shape of jet suggests that the water phase at least partially wets the channel surface and trails along it. Due to the laminar flow conditions a free flowing jet should have been perfectly straight.

Figure 13: Jetting in a water-in-oil system.

Figure 14 shows dripping mode, in which highly monodisperse droplets were generated. The generation frequency ranged between approximately 0.1-1 kHz and the droplet diameter between 20-40 µm. It is worth noting that the larger of the droplets thus had a diameter larger than the depth of the channel, which may be part of the explanation for the irregular device performance.

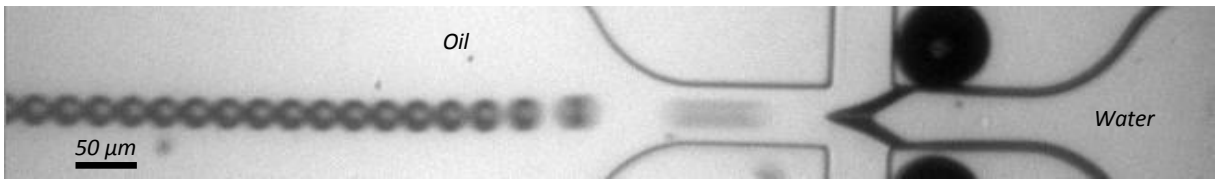


Figure 14 Water droplets being generated in oil using syringe pumps

Tip streaming was achieved using the fluigent and balancing the pressures so that the flow rate of the water phase was almost zero (Figure 15). This could not be done using syringe pumps, as these were not stable enough at low flow rate.

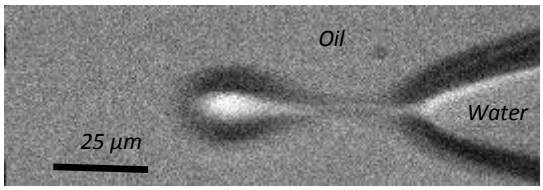


Figure 15 Tip streaming

The formation of satellite droplets could be observed in dripping mode (Figure 16, left). It was also seen that satellite droplets can easily be separated from the primary droplets by applying an uneven oil flow (Figure 16, right). If the two oil streams are balanced both primary and satellite droplets travel along the middle of the collection channel. If, as in Figure 16, the flow in the lower oil stream is much higher than in the upper the interface between the two streams will shift towards the upper wall of the collection channel carrying the satellite droplets with it. The primary droplets are however too large to make this shift and are pushed into more central stream lines effectively separating the two droplet types. This phenomenon is known as pinched flow [20].

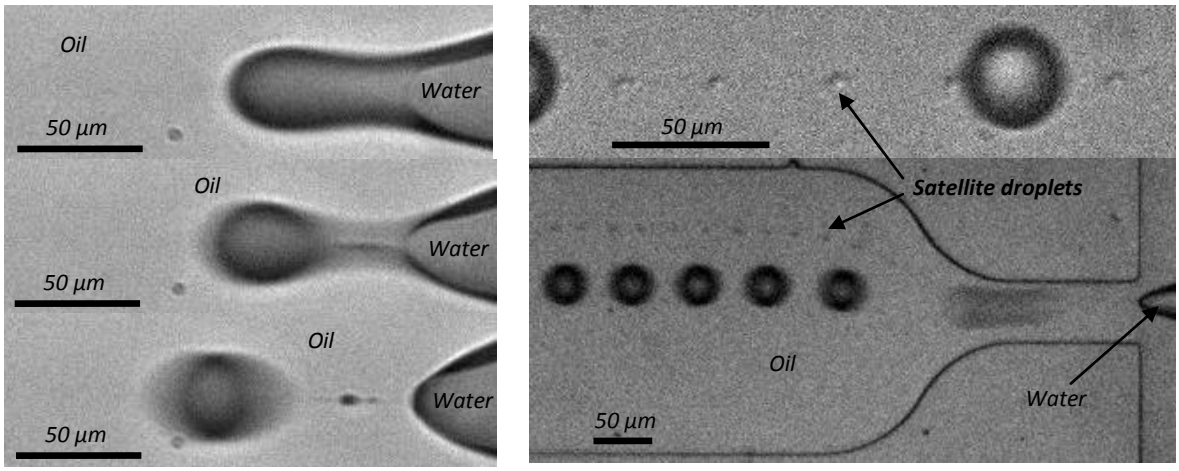


Figure 16 Left: Formation of satellite droplets. Top right: stream of satellite droplets within the stream of primary droplets. Lower right: Stream of satellite droplets being separated from stream of primary droplets by asymmetric oil flow.

Oil-in-water

Only one attempt was made to create oil-in-water droplets. It was quite obvious that this is not optimal for droplet generation as the low viscosity water has trouble exerting enough shear force to focus the high viscosity oil into a thread which can be broken up into droplets. Therefore a very high flow rate (several hundred µl/min) of the continuous phase was required, which in turn proved impractical for imaging.

Water-in-ethyl acetate

Filling the channels with ethyl acetate induced a moderate local swelling of the PDMS. The increased volume was accommodated partially by a narrowing of the channels (Figure 17) but mostly by upwards swelling of the device. The swelling stabilized after a few minutes and appeared to be fully

reversible.
Droplets could be generated in this system much like in the water-in-oil system (Figure 18).

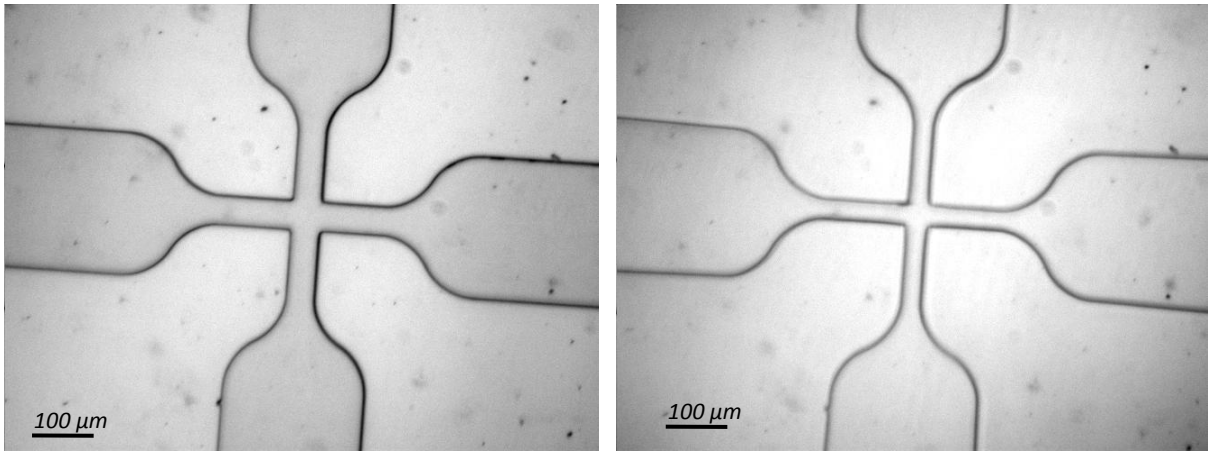


Figure 17 PDMS device before and after being exposed to ethyl acetate

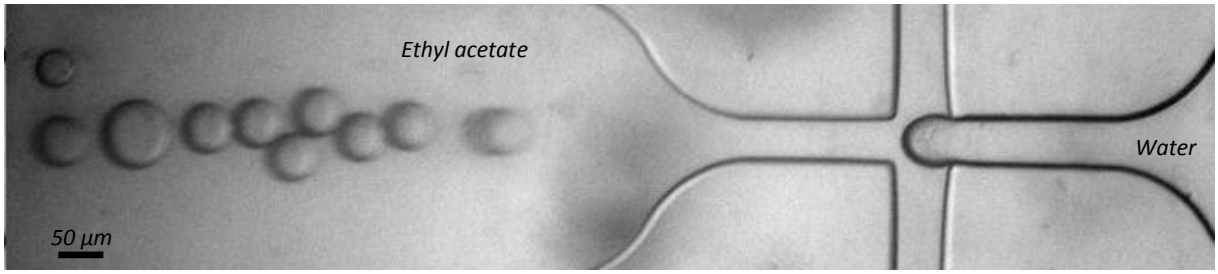


Figure 18 Water droplets being generated in ethyl acetate. The disorder in the droplet flow is caused by a defect in the channel wall that perturbs some of the droplets. Due to limited time the experiment was not repeated with a device free from defects.

4.3 Silk Capsules

Droplet generation

We tried to generate droplets using three different fluid combinations, silk-in-oil, silk-in-ethyl acetate and ethyl acetate-in-silk. Analogous to the pure water system, most experiments were carried out on silk-in-oil. Oil-in-silk was not tried due to the problems experienced with the oil-in-water system. Silk-in-ethyl acetate was tried in order to investigate the difference between water-oil and water-organic solvent interfaces. Ethyl acetate-in-silk was tested in order to assess the effects that inverting the system would have on membrane formation.

Silk-in-oil

Droplets were formed in the silk-in-oil system in a way quite similar to the water-in-oil system (Figure 19, top). The most marked difference was a greater variation in droplet size and frequency (compare Figure 19, top and bottom). As can be seen in the two pictures the droplets were monodisperse on a short time scale (tens of ms) but displayed a variation in size and frequency on a time scale of seconds. This seemed to be caused by the silk membrane temporarily obstructing the flow of the central stream.

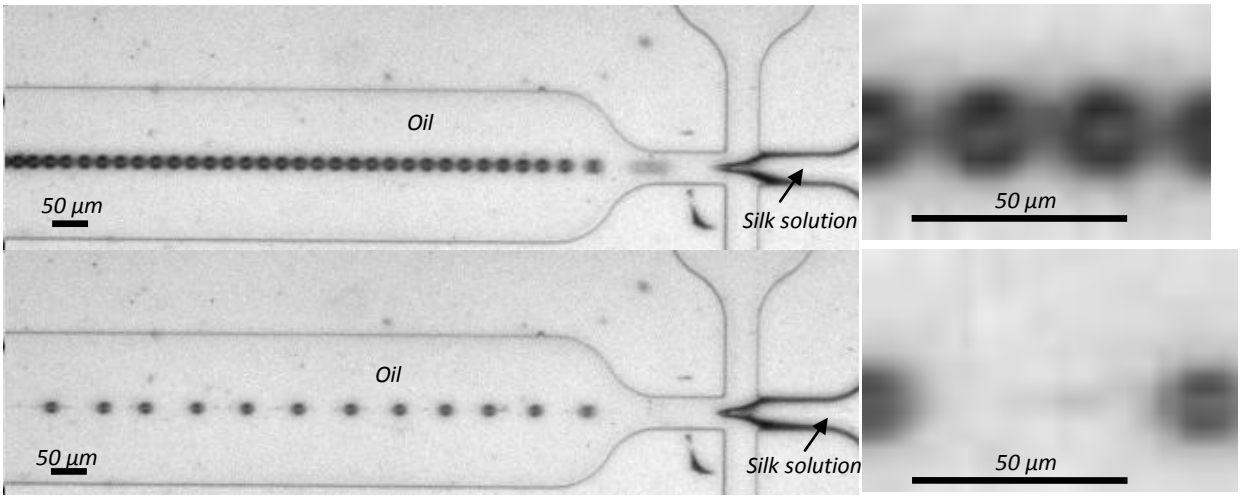


Figure 19 Top: Silk droplets being generated using syringe pump. Bottom: Droplets in the same device a few seconds later. Note the difference in droplet size and frequency.

At the interface between the oil phase and the silk solution a gel-like membrane would form (Figure 20). The edges of this membrane appeared to be anchored to the channel walls while the rest of it would move with the interface. The presence of the membrane seemed to interfere with the otherwise regular flow leading to a more erratic behavior. This is a probable cause for the variations in droplet generation conditions discussed above.

In some cases bits of the membrane would break loose, forming bag-like structures in the collection channel (Figure 21). Such membrane structures were stable on a time-scale of minutes but were eventually washed away by the oil flow.

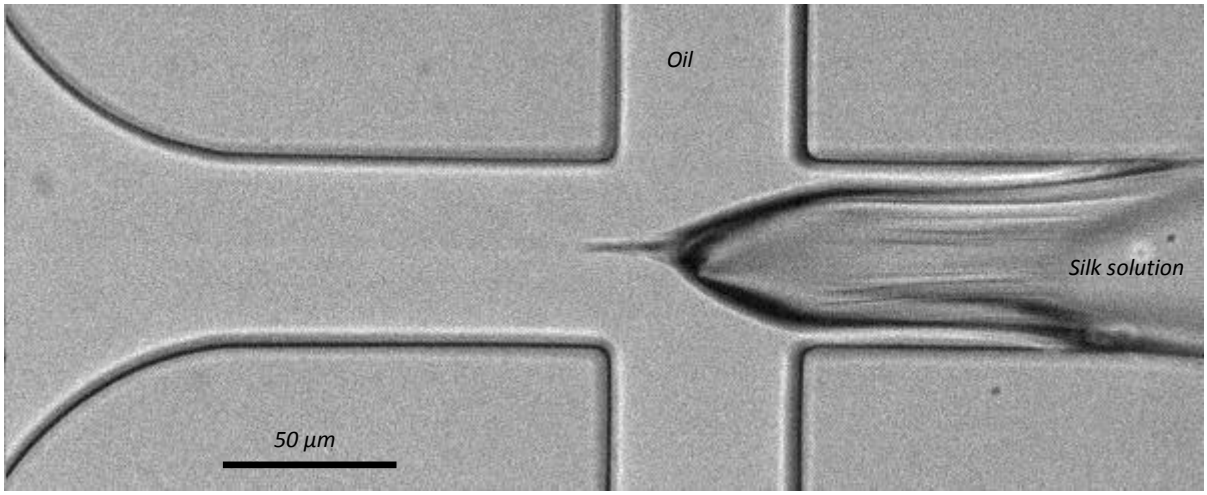


Figure 20 Membrane at the droplet forming interface in a silk-in-oil system

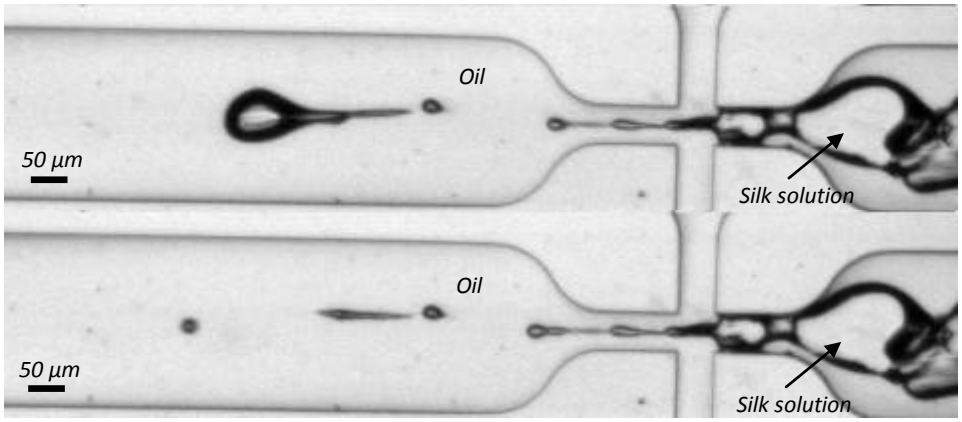


Figure 21 Top: Water filled silk bag stuck in the collection channel. Bottom: The same silk bag, now deflated.

Silk-in-ethyl acetate

Only brief observations were made on the silk-in-ethyl acetate system. Droplets formed much like in the water-in-ethyl acetate system. No visible membrane formation could be observed (Figure 22).

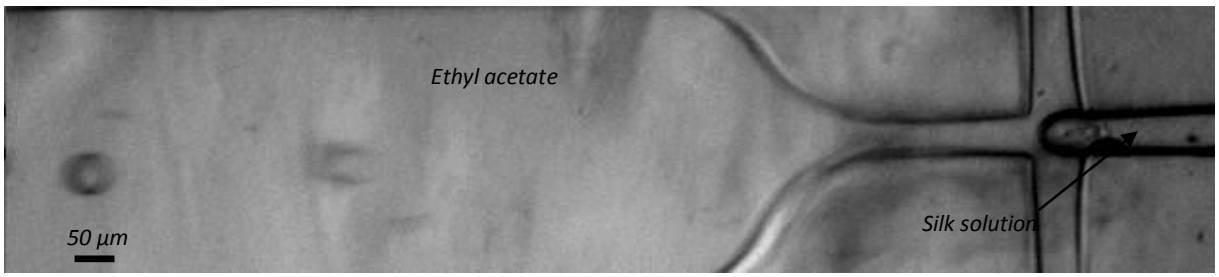


Figure 22 Formation of silk droplets in ethyl acetate.

Ethyl acetate-in-silk

Droplet formation in the ethyl acetate-in-silk system appeared quite similar to that in the silk-in-ethyl acetate system. Some membrane-like structures could be observed at the interface (Figure 23), but did not appear to perturb droplet formation. No immediate membrane formation appeared to take place on the droplet surfaces.

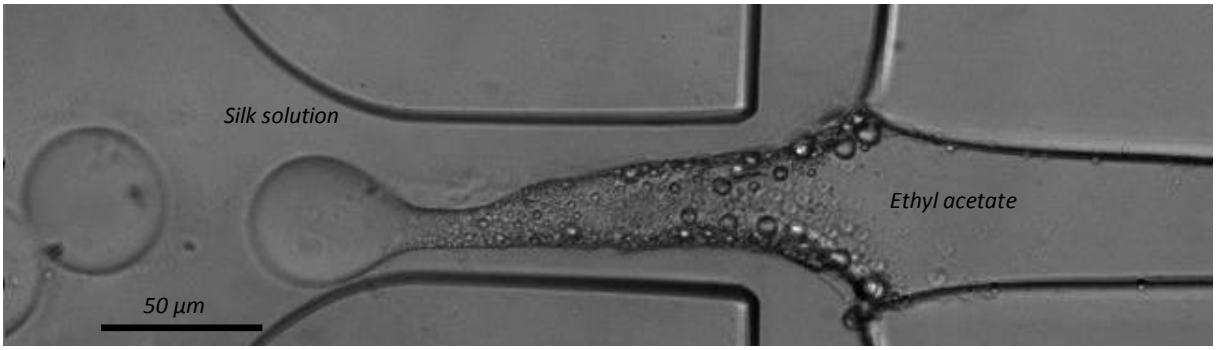


Figure 23 Formation of ethyl acetate droplets in silk solution. A membrane-like structure can be seen at the interface between the fluids. The droplets appear to be membrane free.

Droplet extraction and collection

In order to study the formed droplets and determine whether they seemed to be actual silk capsules we attempted to collect samples at the device outlet.

It proved difficult to extract intact silk droplets because of the high frequency with which they were generated. As soon as they entered the outlet tubes they were slowed down, leading to crowding. The droplets would then tend to stick together or partially coalesce. The samples that were collected from the outlet tubes consisted of large (several mm) aggregated lumps of silk (Figure 24, right).

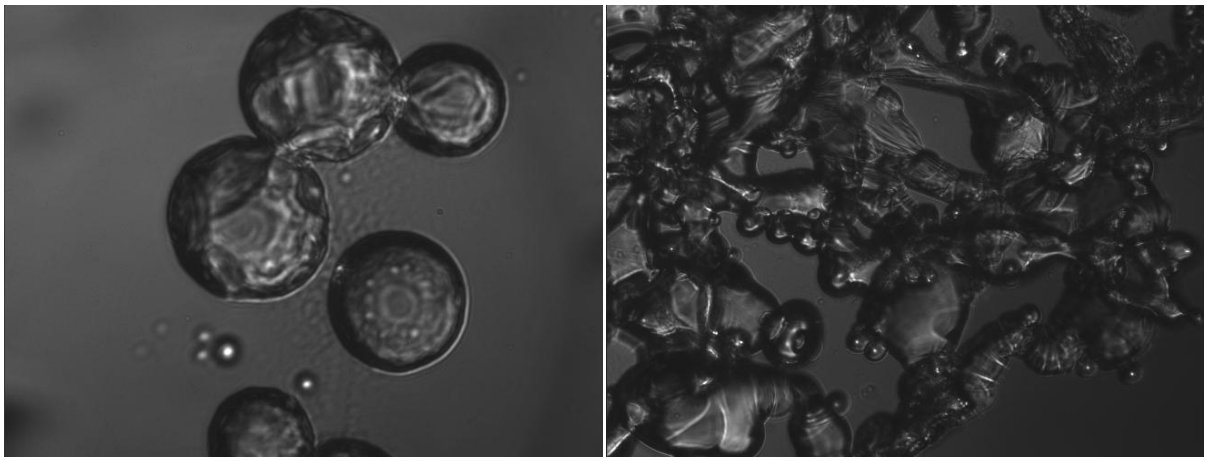


Figure 24 Left: Successfully collected silk droplets. Right: Partially merged silk droplets.

In order to successfully collect separate silk droplets the device was cut across the collection channel, allowing the droplet to pour directly onto the cover slide (Figure 25). By tilting the device and adding extra fluid downstream of the cut, the emulsion could be kept dilute enough to prevent coalescence. This new setup allowed us to collect the droplets for further study.

We tried the same method for collection of ethyl acetate-in- silk droplets, but collection proved difficult as the ethyl acetate, having lower density than water, would just float to the surface and evaporate once outside the channels.

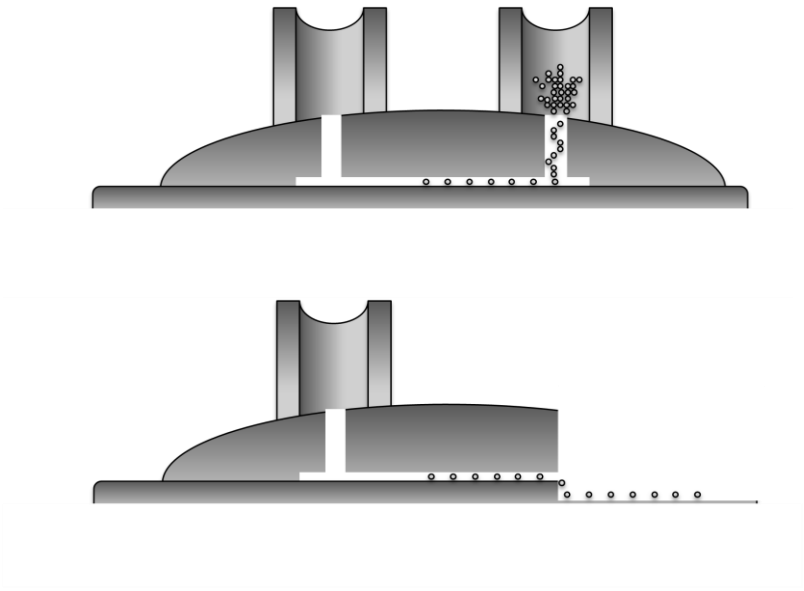


Figure 25 Top: Sketch of droplet clogging in normal device. Bottom: Sketch of cut device with droplets pouring out on the glass slide.

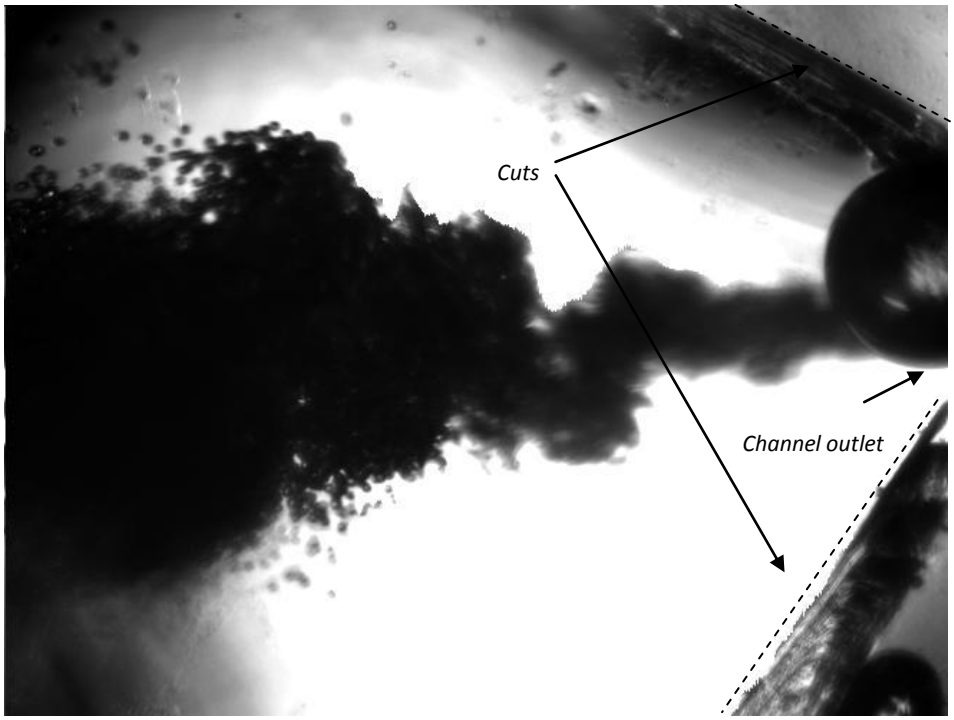


Figure 26 Silk droplets flowing out from cut channel.

Experiments on collected droplets

Collected droplets were transferred to a glass slide for observation. For several droplets the water rapidly leaked out onto the glass surface (Figure 27).

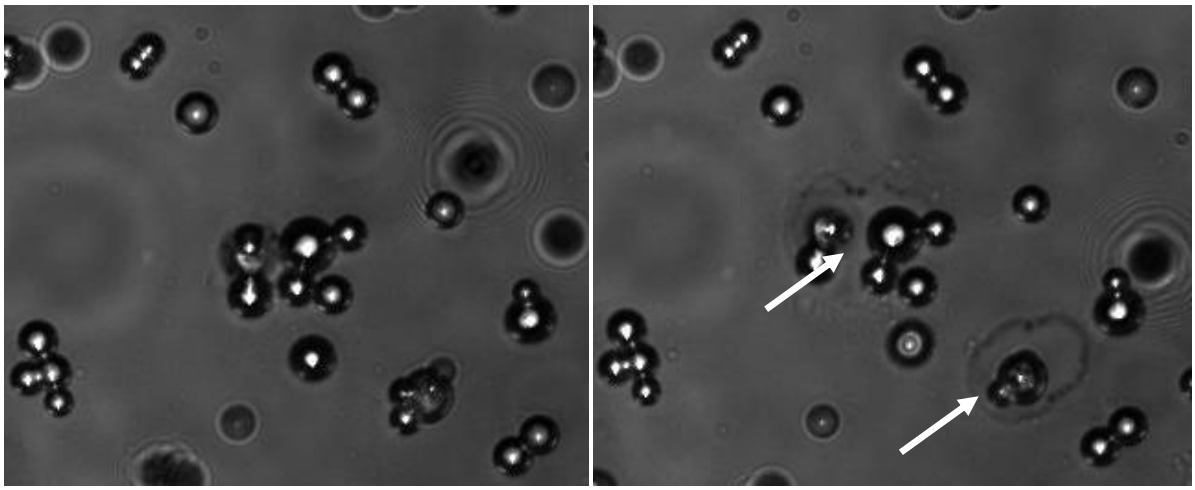


Figure 27 Water leaking from silk bags on a glass cover slide. Left: before. Right: after.

More stabled droplets could be deformed by placing a cover slide over them and applying pressure (Figure 28). Enough distortion would cause the droplet to burst, spilling the contained water.

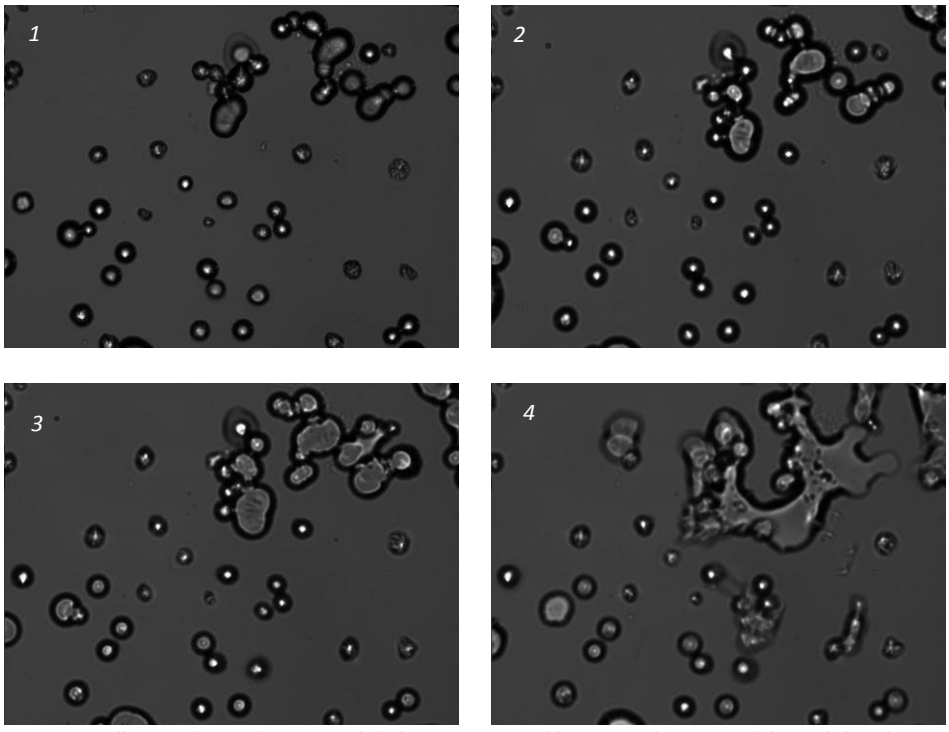


Figure 28 Silk capsules under cover slide being squeezed between the cover slide and the object glass and eventually caused to burst. The pressure increases from 1 to 4.

Collected droplets were left in a bath of oil overnight. A time lapse movie was made by taking one image/minute. It was seen that the droplets shriveled over time and then vanished (Figure 29).

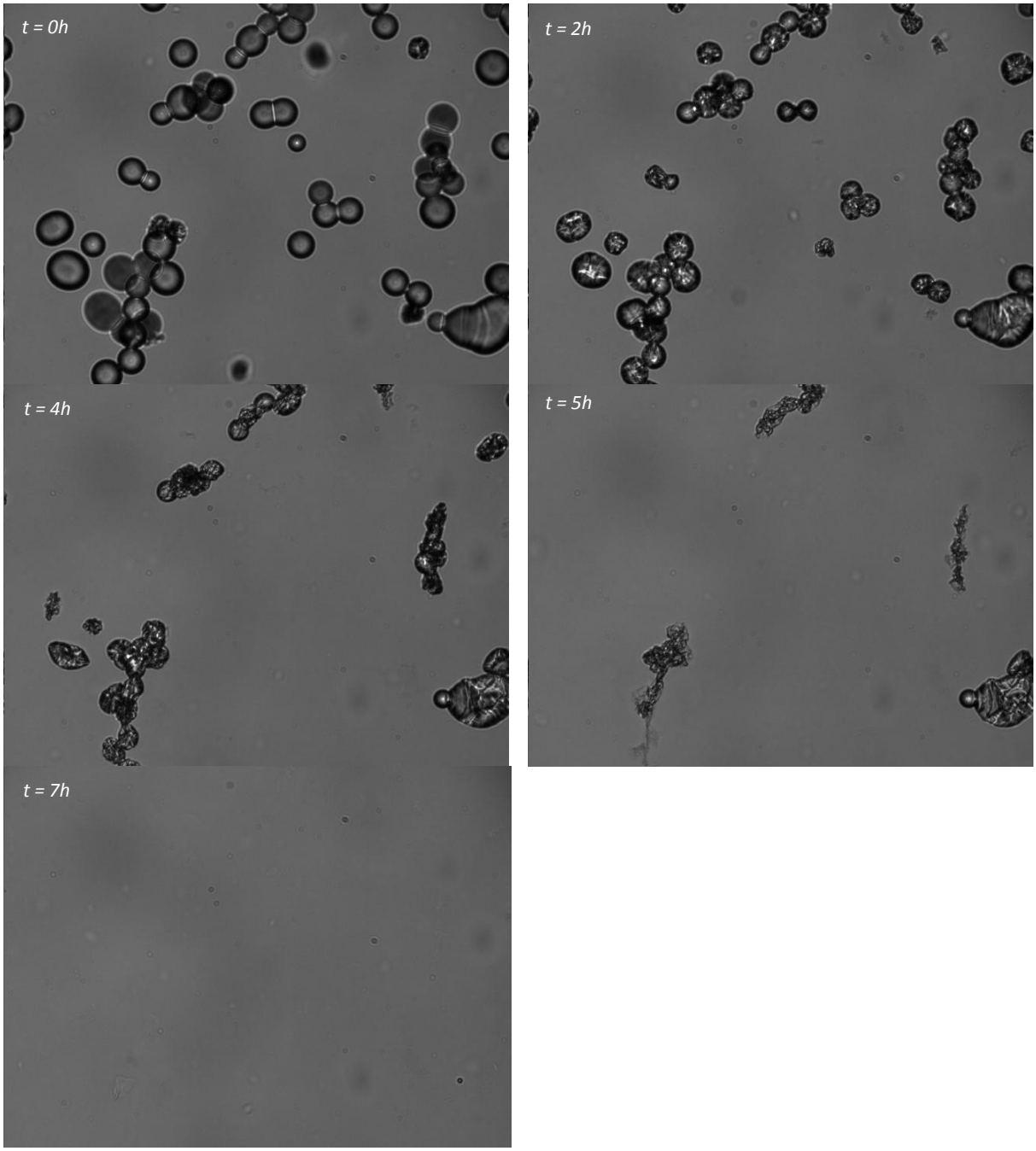


Figure 29 Silk droplets in oil bath overnight. Images taken at 0, 2, 4, 5, and 7 hours.

5 Discussion

5.1 Device Manufacture

Bonding technique evaluation

The three plasma free techniques and the plasma bonding and recovery method were tested in an exploratory attempt to decide which method could give an acceptable result without spending a large amount of time with optimization.

The plasma bonding and recovery performed well during the first round of experiments while all of the plasma free techniques had some issues that remain to be resolved. For this reason the latter were not pursued further. They have been reported to perform well elsewhere however and a more systematic evaluation can be found in the work of Eddings *et al.* [19].

Practical performance

In the second part of the project the plasma treatment and recovery method did not always result in good surface properties. The behavior of the fluid system in these cases suggested that a degree of hydrophilicity still remained. The prevailing hypothesis accounting for the ability of PDMS to regenerate its hydrophobic surface is that it is a result of diffusion of partially crosslinked mobile elements. Lee *et al.*has showed that it is possible to increase the stability of the hydrophilic surface by bathing the cured PDMS in an organic solvent, removing partially crosslinked elements [21].

It can thus be hypothesized that some variation in the fabrication procedure had altered the content of partially crosslinked elements in the PDMS, increasing the stability of the hydrophilic surface. The most probable cause is the conditioning of the bottom plates. They are typically prepared in large quantities and left in the oven until needed, sometimes for a few weeks. This dramatic increase in baking time could very well have an effect on the degree of crosslinking and hence surface stability.

Attempts were made to test this by comparing the performance of devices made from components that were baked for one hour and devices made from components baked for five days. The results were inconclusive but suggest that very long baking time may at least play some role in stability of hydrophilic surface.

Another possibility that needs to be considered is that the current method of coating the glass slide with PDMS does sometimes create an irregular surface which might disrupt the droplet formation. Switching to spin-coating would resolve this.

5.2 Droplet Generation

Both of the flow delivery systems, syringe pumps and MFCS Fluigent, performed well, but the syringe pumps were preferred for reasons of reproducibility. The rationale is that in flow focusing flow rate is a determining factor for droplet generation while pressure is not. In the case of the MFCS Fluigent the pressure is always known but the flow rate will need to be measured or calculated. Since the resistance may vary between different devices and setups it cannot be said with certainty that a given pressure will result in a certain flow rate. In the case of the syringe pumps on the other hand, the flow rate is the regulated variable and, assuming steady state, is always known.

A rigorous investigation into the effects of flow rate on droplet size and frequency was planned. As a follow up on this it was also planned to make a comparison of droplet size in the different system in

order to shed some light on the effects of surface tension and viscosity on droplet formation dynamics. These experiments were delayed by a series of technical misfortunes and had to be abandoned due to time constraints.

5.3 Silk Capsules

The results show that silk capsules can indeed be formed in a simple PDMS microfluidic device without the use of surfactants or advanced surface treatments.

The silk-in-oil system was the only one that successfully produced capsules during this project. The question is, however, whether the mode of capsule formation is really the same as in previous studies performed in bulk emulsions. Since there is a membrane present prior to droplet formation it is likely that the capsules are formed by pinch-off of this membrane rather than by silk proteins diffusing to the interface following droplet formation or at least a combination of the two. If this is the case it can be expected to have a detrimental effect on capsule quality, as the membrane is not built up in a uniform fashion. In any case the early membrane formation is a clearly unwanted effect of this system as it interferes with the droplet formation process. The seemingly random movements of the membrane lead to the formation of a wide distribution of droplet sizes rather than the desired uniform population.

The formed capsules also lacked in stability, but this can be attributed to the absence of a cross-linking step, something to be addressed by future experiments.

The silk-in-ethyl acetate and ethyl acetate-in-silk systems did not display any unwanted membrane formation, at least not to any degree that influenced droplet formation. There was also no observable skin formation on the surface of the droplets. These results are not unexpected as previous experience with the behavior of fibroin at the water-organic solvent interface shows that the skin formation is much slower than at the water-oil interface and requires several hours of incubation [16].

There is however good reason to believe that capsule formation is possible if only the droplets can be incubated in the channels for long enough. Capsules formed in this way can be expected to be better defined than the droplets that can currently be made in the silk-in-oil system.

It is possible that a membrane will build up over time eventually leading to irregular droplet generation, but even so the system will be able to produce a finite number of monodisperse droplets.

6 Further Work

In this project we demonstrate the general compatibility of PDMS-based microfluidics with the process of generating hollow silk microcapsules. In order to further the goal of creating a microfluidic system that successfully produces high quality capsules there are a few options that should be pursued.

6.1 PDMS surface issues

The issues with wetting properties of the PDMS channel walls need to be resolved. A thorough investigation into the effects of baking time on stability of the post-plasma treatment hydrophilic surface of PDMS should be performed; the effects of immersion in oil should also be investigated more rigorously. The partial curing bonding method described previously should be further pursued as an alternative procedure.

Another potential variation in the design is making the channels significantly deeper than the typical diameter of the droplets which should make the wetting properties of the channel walls less critical.

6.2 Streamline transfer

The possibility to transfer droplets between different flow streams has not yet been investigated. This technique has the potential to greatly increase the versatility of the system so a concept test should be made a high priority. If such a test would be successful the next step would be designing a device where streamline transfer is integrated.

There are at least two methods that have been demonstrated to selectively transfer particles across streamlines in a microfluidic device. One is through pinched flow, in which the stream carrying the particles is squeezed so thinly that they are pushed into neighboring streamlines [20], much like how the satellite droplets were separated from the primary droplets in this project. The other is by using asymmetrical rows of posts in a so-called bumper array, where particles above a certain size threshold are displaced in a deterministic way [22].

Several options can be considered, perhaps the most obvious would be to transfer all steps of the present bulk process into one microfluidic chip. This would include droplets of silk solution being formed in a pure oil/organic phase, to later be transferred into a crosslinking (alcohol-containing) phase (Figure 30, top). The times required for full skin formation and crosslinking would be critical factors in designing such a chip.

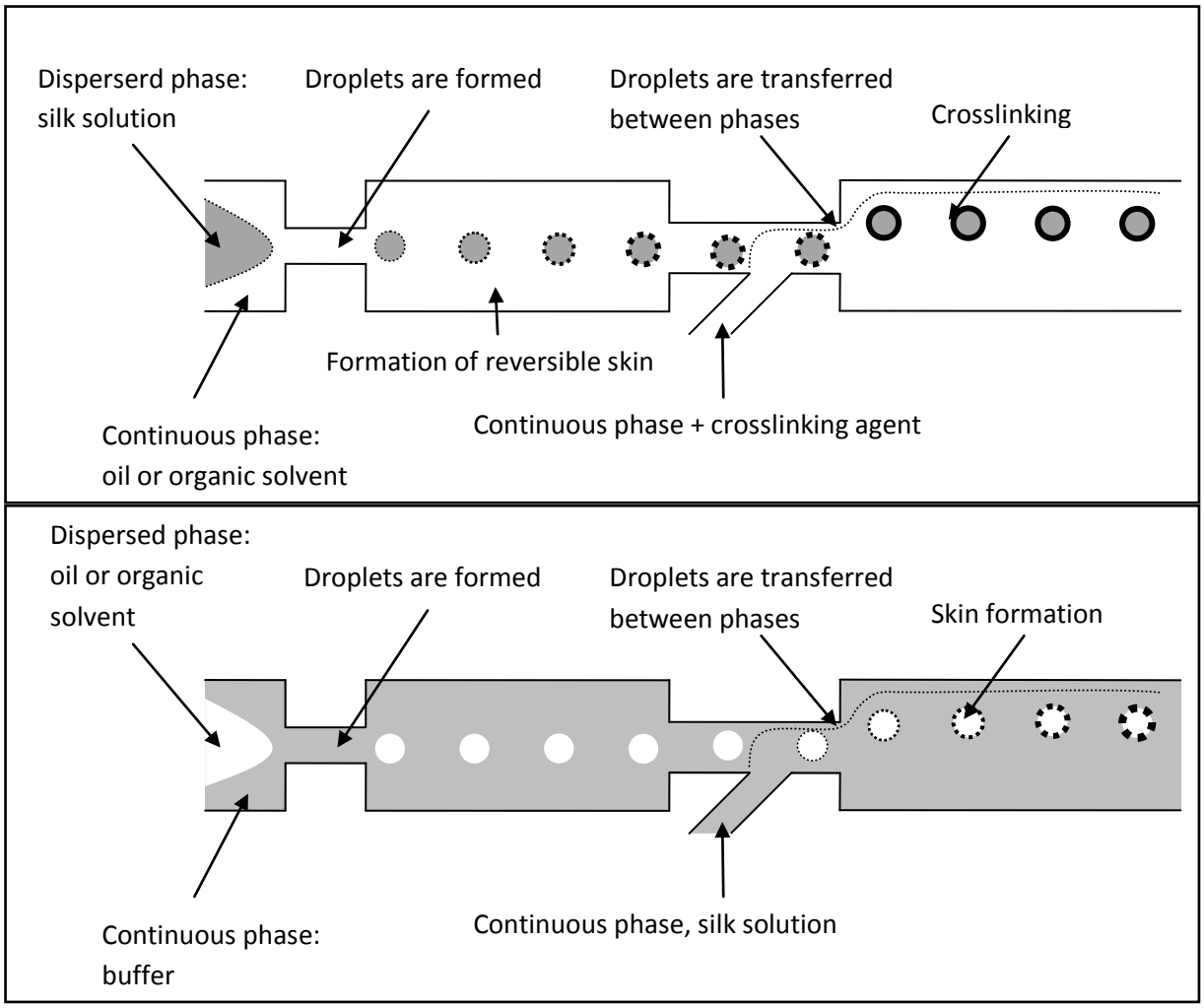


Figure 30 Top: Droplets of silk are generated and transferred into a crosslinking environment on-chip. Bottom: Droplets of oil or organic solvent are generated in a silk free environment and transferred into silk solution on-chip. Displacement is achieved through pinched flow in both cases

Another possibility which would be suitable for performing standardized experiments with silk capsules made under different conditions would be to implement flow lane transfer in the oil-in-water type geometry (Figure 30, bottom). This way oil/organic droplets could be generated in pure water, and later be transferred into silk solution. Generating the droplets in the complete absence of silk would eliminate the problem of membrane buildup prior to droplet generation. This setup would also offer the possibility to control the skin formation by tuning the amount of time the droplets spend in the silk solution.

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