# New microfluidic chip for the production of spherical gelled capsules for cell encapsulation

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## ABSTRACT

In this paper, we report a novel microfluidic device for cell encapsulation application. This device couples a monodisperse emulsion of alginate made with an MFFD geometry with a phase transfer device that extracts 100% of the alginate droplets from oil into an aqueous gelling phase. First the phase transfer device is described, followed by an optimization of the gelation process in order to retrieve spherical and monodisperse alginate droplets.

***Keywords***: alginate, encapsulation, pancreatic cells, microfluidic, biphasic flow, MFFD, transfer device.

## 1 INTRODUCTION

Implantation of Langerhans islet in alginate shell is an alternative for the treatment of diabetes type 1. Finding an adapted process for cell encapsulation is a challenge. The process must product spherical and monodisperse capsules in a limited time with a good reproducibility [1][2]. Up to now conventional systems are mainly manual which induces batch disparities and do not provide repeatable clinical results. This novel device is the first step towards a full automated and reproducible process for cell encapsulation.

The device—represented in figure 1—is divided in four sections. Section 1 is composed of a typical MFFD [3] device, which produces an alginate monodisperse emulsion. Two channels are added to inject calcium acetate crystals suspended in oil as proposed by Zhang et al. [4].

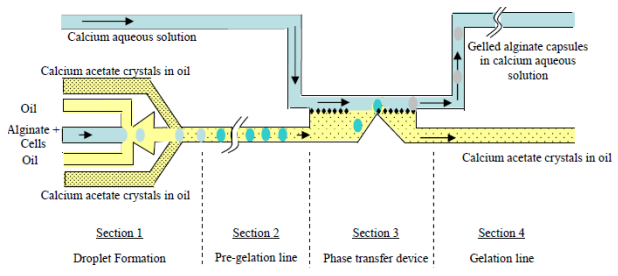


Figure 1: Schematic presentation of the device.

Acetate calcium is used to begin the gelation of the droplet before crossing the oil/aqueous phase interface; this process may be called pre-gelling step. Section 2 defines the pre-gelation stage which will be described later on. Basically, the length of the channel is fixed but the inlet pressure of oil determines the duration of the pre-gelation process. Section 3 depicts a phase transfer device in which the oil and the aqueous calcium flows formed an interface crossed by pre-gelled alginate droplets [5]. Complete gelation of the capsule is achieved in section 4.

In this paper, we will focus on one of the main constraints for cell encapsulation application which is the sphericity of the capsule. First, the phase transfer mechanism will be described and detailed. Critical steps to preserve the droplet sphericity are pointed out. Finally, an optimization of the pre gelation process will be assessed to guarantee the sphericity of the retrieved capsules.

## 2 MATERIAL AND METHOD

### 2.1 Microsystem

Microfluidic chips are made in silicon, channels are 200µm large and depth. The size of the droplets formed with the MFFD range from 130µm to 160µm. In the phase transfer chamber, upstream and downstream micropillar rows and a deflector (figure 2) are defined by deep dry etching technology. Finally, walls are coated with an hydrophobic coating (water contact angle: 108-110°), to produce aqueous alginate droplets without using additional surfactants.

### 2.2 Experimental Bench

Solutions are motioned by regulated pressures between 0 to 1bar, delivered by micropumps (Fluigent® micro-pumps MFCS-8C). For all the experiments, the same pressures have been used either for oil or oil supplemented with calcium crystals. Experiments are visualized under a x5 objective microscope and image sequences acquired with a high speed camera (Mikrotron GmbH, MotionBlitz Eosens) up to 5000fps.

## 2.3 Preparation of solutions

The alginate solution used for these experiments is a 3% (w/w) ultra-pure alginate (Pronova SLG100, Novamatrix), diluted in an aqueous solution of 150mM NaCl, 10mM Hepes. The pH is adjusted to 7.4. Viscosity of alginate solution is 5300mPa.s at zero shear. Hyper-refined soybean oil (CRODA EP-NP-LQ-(MH)) is used as continuous phase and calcium acetate organic salt (Macco Organiques INC) as pre-gelling agent. Crystals diameter is around 100µm, which is too large to flow correctly into the device. Crystals grinding is performed to reduce their size. Final calcium acetate crystals diameter ranges from 5µm to less than 1µm. Finally, the solution is diluted to obtain a concentration of 1% or 3% (w/w). The gelling phase is a solution of 100mM CaCl2, 80mM NaCl et 10mM Hepes adjusted to pH 7,4.

## 2.4 Parameter fo circularity

Capsule sphericity is assessed for 50 capsules in each condition via image analysis with ImageJ software [6] through two parameters : the circularity and the aspect ratio of the capsule's fitted ellipse. They are calculated as :

$$c = 4\pi \frac{Area}{Perimeter^2} \quad (1)$$

$$r = \frac{MajorAxis}{MinorAxis} \quad (2)$$

For a perfect circle the values of these both parameters is 1.

# 3 PHASE TRANSFER DEVICE

## 3.1 Phase transfer device description

The phase transfer device is composed of two parallel channels separated by upstream and downstream micropillar rows (figure 2). Calcium and oil flow respectively in the upper and lower channel. A deflector is defined in the oil channel. Micropillar rows and deflector are respectively involved in the stabilization of the oil/calcium interface and the deviation of droplet from oil to aqueous phase. This device continuously transfers the incoming pre-gelled droplets in the following sequence.

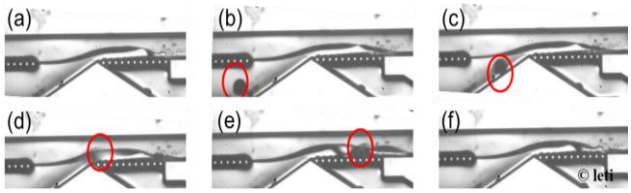


Figure 2: Phase transfer device functioning.

## 3.2 Interface position

The position and stabilization of the oil/calcium interface depend on the input pressures. Figure 3 depicts the position of the interface for different pressure of calcium. For given oil and alginate pressures respectively fixed at 500mbars and 470mbars, the interface position is shown for pressures of calcium ranging from 350mbars to 700mbars.
When $P_{calcium}$ is fixed at 350mbars, the interface does not grip the pillars row. A thin film of oil flows into the calcium channel, leading to channel pollution.
For $P_{calcium}$ = 450mbars, the interface starts gripping the pillars. For higher pressures, the interface grips pillars closer from the deflector, which reduces the oil passage. The interface remains stable for $P_{calcium}$ ranging from 450mbars to 700mbars. The $P_{oil}$ and $P_{calcium}$ couple has to be chosen properly as it can influence the transfer mechanism performance.

## 3.3 Phase transfer

The images sequence in figure 2 illustrates the transfer of a pre-gelled droplet. First, the interface is kept stable (a) then the continuously incoming capsules are forced to leave the oil phase to join the aqueous phase with a deflector (b, c). Oil is evacuated through micro pillars while the alginate capsule crosses into the aqueous phase (d-e) and finally flows in the gelling phase (f). The graph in figure 4 shows the evolution of the velocity of three pre-gelled droplets in function of their position in the phase transfer chamber. This position x on the abscissa corresponds to a position on the micro pillars row drawing in blue. Each picture illustres one step of the transfer mechanism which is described as follow :
1-The droplet entrance in the biphasic chamber with the same velocity as the oil, around 0.01m/s (x= 0 to 100µm).
2-The droplet is deviated by the defector and arrives in front of the calcium flow. In this step the droplet is accelerated up to 0.08m/s since the oil flow section is reduced (x= 100µm to 200µm) and keeps a high velocity in the micro pillars closed to the deflector (x=200 µm to 300µm).

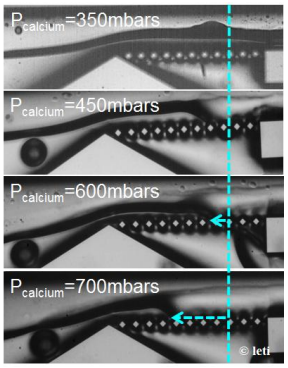


Figure 3: Interface position for different pressures of calcium.

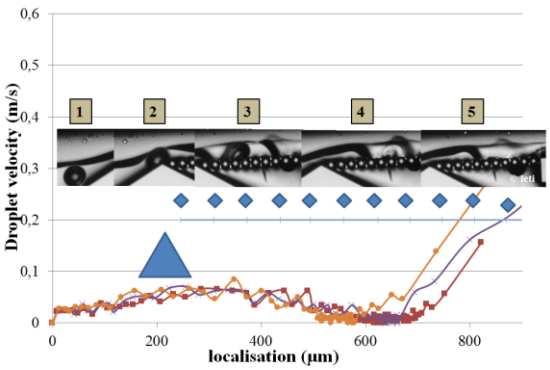


Figure 4: Evolution of the velocity of the droplet in function of the localisation in the chamber.

3- The droplet slows down as it moves to the further micro pillars (x=300µm to 500µm) and may stop (V=0 m/s for x= 500µm to 650µm).
4-The pre-gelled droplet coalesces with the gelling phase (x= 650µm to 800µm)
5-Capsule formed flows in the calcium. A streak of oil in the calcium channel testifies the droplet transfer.
Steps 3 and 4 correspond to the longest steps of the transfer. These steps take around 22ms whereas the global transfer lasts approximatively 30ms. This long process corresponds to the draining time of the oil layer surrounding the pre-gelled droplet. Until the droplet coalesces with the gelling phase, the interface pushes the pre-gelled droplet toward the pillars to progressively reduce the oil layer.
Depending on the pre-gelation state of the droplet, these steps can be critical for the sphericity of the droplet.

# 4 SPHERICITY OPTIMISATION

## 4.1 Monodisperse droplet regim

Different flow regimes are observed in a classical MFFD. A map of the flow regimes has been previously determined for high viscosity alginate [7]. For cell encapsulation application, the monodisperse droplet regime is required. In figure 5, the blue area represents the phase diagram of a five channels MFFD corresponding to the monodisperse droplet regime (pressure of oil and pressure of oil supplemented with calcium crystal are equal). The red area corresponds to the monodisperse droplet regim of a five branches MFFD coupled to the phase transfer device. $P_{calcium}$ is fixed at 450 mbars. The formation of monodisperse droplet is restricted to a thinner area of pressures. This is mainly due to the addition of fluidic connections and the restriction of oil flow inside the phase transfer device. Both result in an increase of the global hydraulic resistance of the device. We observe that the lower limit (red dotted line) of the monodisperse formation for the transfer device is not a flow reversal limit. Increasing Palginate pressure reduces the inter spacing between droplets [8] which induces coalescence in the section 2 and finally blocks the system.

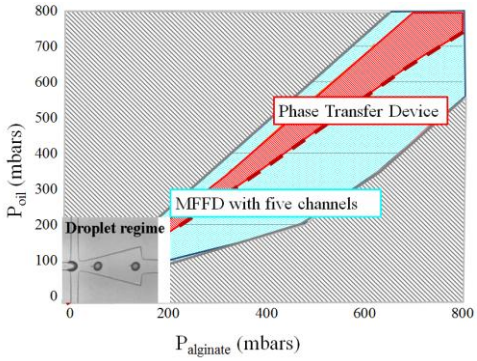


Figure 5: Phase diagram of the phase transfer device.

## 4.2 Pre-gelation optimization

We previously demonstrated [9] that the pre-gelation step is necessary to transfer the alginate droplet from the oil to the calcium phase. The concentration of the pre-gelling agent and the contact time between the droplet and the pre-gelling agent are the main parameters to optimize to preserve the droplet sphericity.
First, the influence of the pre-gelling agent concentration on the sphericity is evaluated. The pre-gelation time is fixed to 15s correponding to a pressure of oil of 500mbars. Two concentrations of calcium acetate crystals are tested : 1% and 3%.
Figure 6-a shows an images sequence obtained for a 1% concentration of pre-gelling agent which confirms the impact of the interface on the pre-gelled droplet during the oil draining step. The droplet is flatten and its minimum radius is 30% reduced. It results in ellipsoïdal capsules (figure 7-a). Figure 6-b shows an images sequence obtained for a 3% concentration of pre-gelling agent, no deformation of the pre-gelled droplet is measurable and droplets are spherical (figure 7-b).
The circularity parameters are evaluated for both concentrations (figure 8). 3% pre-gelling agent concentration provides both the higher circularity and the lower aspect ratio. Both parameters are closed from the perfect circle value. The aspect ratio measurement seems to be more significant as it better shows the shape changes. A 3% concentration is appropriate for this process.

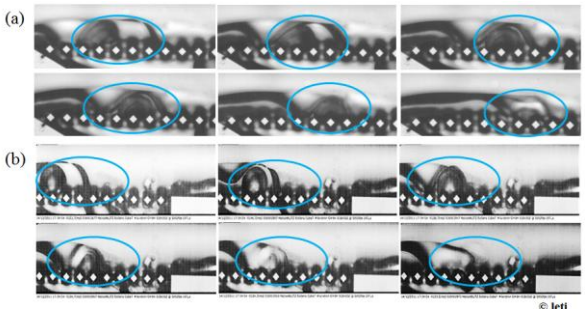


Figure 6: (a) Deformation of a 1% pre-gelled droplet (b) Deformation for a 3% pre-gelled droplet.

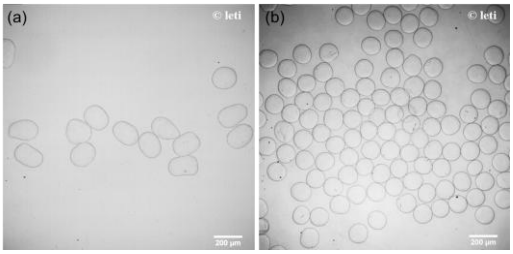


Figure 7: Capsules for 1% (a) and 3% (b)

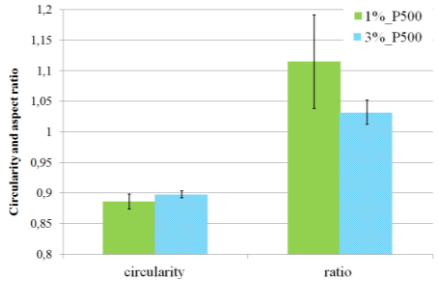


Figure 8: Evolution of the capsule shape for different concentrations of the pre-gelling agent.

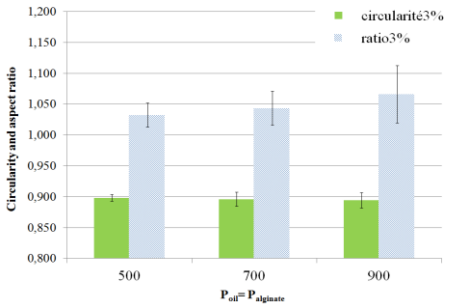


Figure 9: Evolution of the shape of the capsules in function of the time of pre gelation.

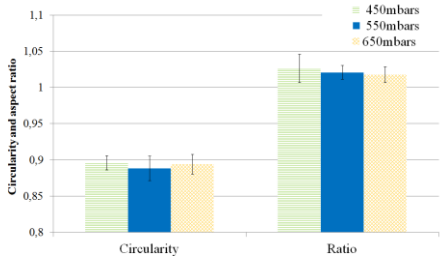


Figure 10: Evolution of the shape of the capsules in function of the pressure of calcium

Then, the influence of the pre gelation time is assessed by varying the pressure of oil for a fixed 3% concentration of pre-gelling agent and a fixed channel length. Different pre-gelation time of 15s, 10s and less than 5s corresponding respectively to pressures of 500mbars, 700mbars and 900mbars are tested. The calcium pressure is adapted to have a stable interface. The circularity and the aspect ratio are reported in figure 9. Both parameters indicates that the optimal parameters are : a pressure of oil of 500mbars with a pre-gelation time of 15s. Results clearly show the importance of pre-gelation on capsule sphericity.

Finally, for these optimized parameters ($P_{oil}$ = 500mbars, and 3% concentration of pre-gelling agent), the influence of the interface position on the capsule sphericity was assessed. As shown earlier, the position of the interface depends on the couple of pressures of oil and calcium. For a fixed oil pressure of 500mbars, calcium pressure varied from 450mbars to 650mbars. Results are shown on figure 10. Not many variation is measurable either on circularity or aspect ratio meaning that previous optimized parameters provide sufficient pre-gelled droplet to cross the interface in a large range of calcium pressure. The impact on the capsule shape is negligeable.

## 5 CONCLUSION

This novel microsystem allows the automated production of alginate capsules. We have optimized the pre gelation process and the flow parameters to keep the initial monodispersity and sphericity of the alginate droplets produced with the MFFD junction. This novel device works continuously for 2h at a frequence up to 200 droplets per minute. The process has been already tested for Jurkat cell encapsulation showing good result of viability [9].

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