Microfluidic device for alginate-based cell encapsulation

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Introduction

Cell encapsulation is used for a wide range of therapeutic treatments including diabetes, renal failure, etc. It is a promising alternative for the transplantation of cells (P. DeVos et Al, 2006 & R. Calafiore, 2006). Encapsulation allows to reduce the amount of immunosuppressive therapy since the polymer capsule isolates the transplanted cells from the immune system. At the same time, capsules must be permeable to nutrients, oxygen or other molecules that are essential to cell viability. For a rapid diffusion of these molecules, the size of the capsules must be adapted to the size of the cells or aggregates of cells (L. Canaple et Al, 2002). Major challenges for cell encapsulation are now to control accurately the size, polydispersity, shape or surface of the capsules (G. Orive et Al, 2003). Recently, several research groups have used microfluidic devices to produce monodisperse microcapsules (K. Liu et Al, 2006). But only few viability tests have been performed (VL. Workman et Al, 2008). For instance, S. Sugiura et Al. (2005) develop a microfluidic micro-nozzle array to produce 50-200µm 1.5% alginate IL-2 beads (CV between 8% and 28%). Human kidney cells viability was roughly estimated at 70%.

This study describes a microfluidic device for the encapsulation of cells or beads using Keltone HV alginate. Alginate droplets are produced using a Microfluidic Flow Focusing Device (MFFD). The emulsion formation is based on the focusing forces in a cross junction channel: two immiscible liquids, an oil phase (with surfactant) and an alginate phase, are respectively introduced in the side and central channels. The surfactant reduces the interfacial tension in order to produce controlled size droplets and avoid coalescence between alginate droplets. For biocompatibility concerns, surfactants are chosen immiscible in the alginate phase, so that capsules are surfactant free.

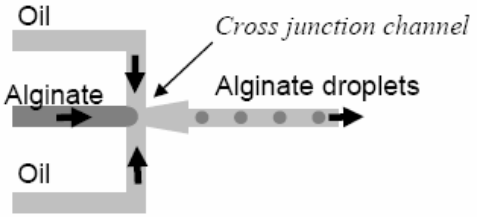


Figure 1: Experimental set-up: the geometrical particularity is the triangular enlargement where the alginate filament breaks up into droplets (droplet size in the range of 200 µm).

In this work, alginate droplets are produced using 1 to 1.75 wt% keltone HV alginates. Rheological measurements have shown that in this range of concentration, Keltone HV alginate solutions are highly viscous. Their viscosities vary from 300mPa.s to 2600mPa.s at low shear rate while water viscosity is only 1mPa.s. In droplet microfluidics, flow focusing devices are currently used for producing water emulsions in an oil phase. But the use of very viscous non Newtonian liquids, like alginate, is still not well known (CH. Choi et Al (2007)). We show that phase diagrams for water and alginate emulsions are very different. Size, frequency and polydispersity of alginate droplets are analysed. For a given viscosity, the size of the capsules can be adjusted by modulating the ratio of applied pressures, interfacial tension or geometry (width, depth) of the microfluidic device. Polydispersity of alginate droplets is very low (CV below 1%), independently of viscosities. Finally, biocompatibility of the materials used in the device has been studied.

Material and methods

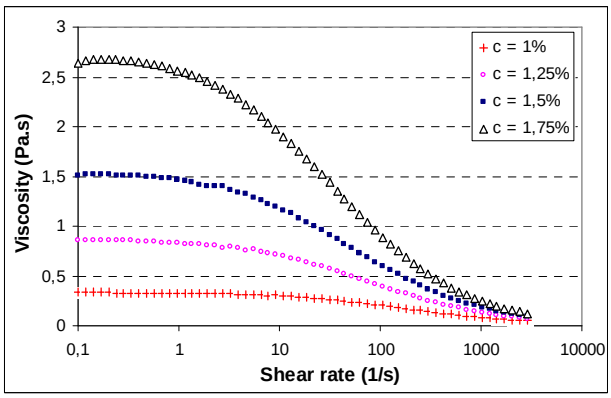
Alginate capsules are produced in a MFFD using keltone HV sodium alginate (purchased from ISP Provigen) and an immiscible oil phase - mineral oil light M5904 (Sigma Aldrich) with 1% sorbitan monooleate as surfactant (span 80 S6760, Sigma Aldrich). Span 80 is one the most commonly used surfactant in microfluidics. Aqueous alginate solutions are prepared in a buffer 150mM NaCl, 10mM Hepes (pH 7.4), with concentrations ranging from 1 to 1.75 wt%.
The experimental setup for cell encapsulation is shown in Figure 1: a commercially available thick resist film (ordyl) is patterned with standard photolithography process on a silicon substrate. A glass cover seals the channels that are silanized in order to achieve a hydrophobic coating. Oil and alginate solutions are motioned by regulated driving pressures furnished by micropumps (Fluigent).

Capsule diameters are determined with Image J software from the measure of the area of the droplet according to the following equation: $d = 2\sqrt{S/\pi}$, where S is the projection area of the droplet. Average diameter is determined from more than 30 capsules. Coefficient of variation (CV) is defined by the ratio between standard deviation and average diameter.

Biocompatibility of the microfluidic device is tested on Jurkat cells (maintained in RPMI 1640 supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin). Cell viability test is carried out with trypan blue exclusion method.

Results and Discussion

Droplet formation in a MFFD results from the balance between interfacial tension force and drag force which depends on viscosity. For an alginate concentration ranging from 1 to 1.75 wt%, interfacial tension (between alginate solution and mineral light oil, 1% span 80) was found to be approximately constant: around 2.9mN/m. Rheology of alginates is quite complicated due to the fact that alginates are long polymers that can be entangled or stretched depending on flow conditions. Figure 2 shows that viscosity of Keltone HV alginate solutions drastically increases with the concentration. Viscosity decreases when the applied shear rate increases. In our device, shear rates are ranging from 10 to 100s^{-1}.



	η (mPa.s) $\dot{\gamma}$=10s^{-1} *	η (mPa.s) $\dot{\gamma}$=100s^{-1} **
Keltone HV 1%	305	210
Keltone HV 1.25%	700	410
Keltone HV 1.5%	1175	625
Keltone HV 1.75%	1950	925

* 10s^{-1} is an order of shear rate inside microchannels.
** 100s^{-1} is an estimation of the shear rate applied on the elastic filament linking the incoming alginate to detaching droplet (Figure 5).

<u>Figure 2:</u> Dynamic viscosity η as a function of the shear rate $\dot{\gamma}$ for various concentrations of Keltone HV alginate (measurements at room temperature, 20°C ± 2,5°C)

Different flow patterns can be observed depending on the applied driving pressures, interfacial tension, viscosity ratio, non Newtonian characteristics, geometry of the device (width, depth).
When the discontinuous phase (i.e. water) has a small viscosity compared to that of the continuous phase (i.e. oil), droplet regime is difficult to obtain: for de-ionized water (η=1mPa.s) in a mineral oil phase-1% span 80 (η=33mPa.s), the droplet regime is obtained for a ratio P_{water}/P_{oil} precisely

comprised between 0.53 and 0.56. The situation is much more comfortable in the case of 1 wt% alginate solution: the viscosity of the alginate is then more than 10 times that of the mineral oil-1% span 80, and droplet regime covers a much larger domain in the [$P_{alginate}$, P_{oil}] diagram (Figure 3).

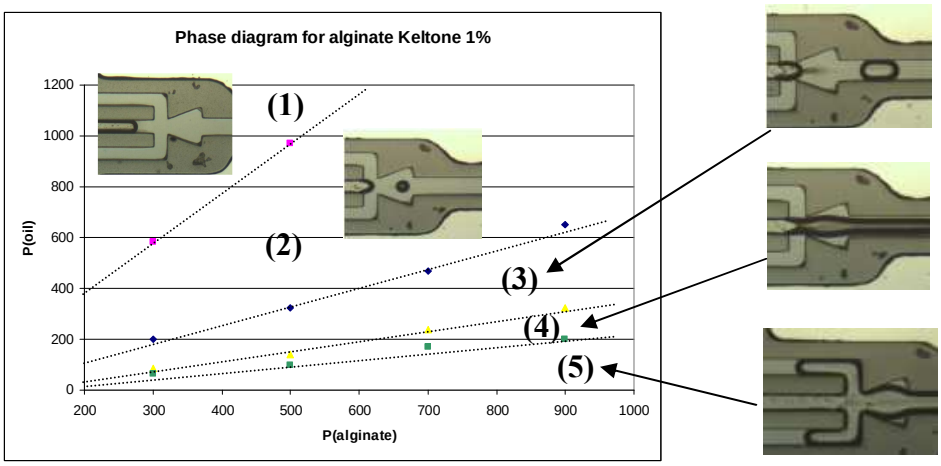


Figure 3: Phase diagram for 1% alginate Keltone HV, mineral oil with Span80: (1) oil invades the alginate channel, (2) droplet regime, (3) plug regime, (4) annular flow regime, (5) alginate invades oil channel. Droplet size is tuned by adjusting the pressure ratio ($P_{alginate}/P_{oil}$).
Increasing the alginate concentration (or viscosity) widens the droplet regime domain.

Droplet size essentially depends on the pressure ratio $P_{alginate}/P_{oil}$: capsule diameter increases with this ratio. Moreover, at a fixed alginate driving pressure, an increase of the oil driving pressure results in smaller capsules (Figure 4). The frequency is essentially controlled by the applied driving pressures: it increases with increasing $P_{alginate}$, and decreases with increasing P_{oil}). Droplet size and frequency are also extremely dependent on the viscosity of the alginate solution, since an increase in alginate viscosity (or concentration) results in smaller capsules, with lower frequency. So a compromise between viscosity, droplet size and frequency has to be found in order to optimize the encapsulation performances of the device. A major advantage of our device is the low polydispersity: the CV is less than 1%, independently of viscosities or applied pressures.

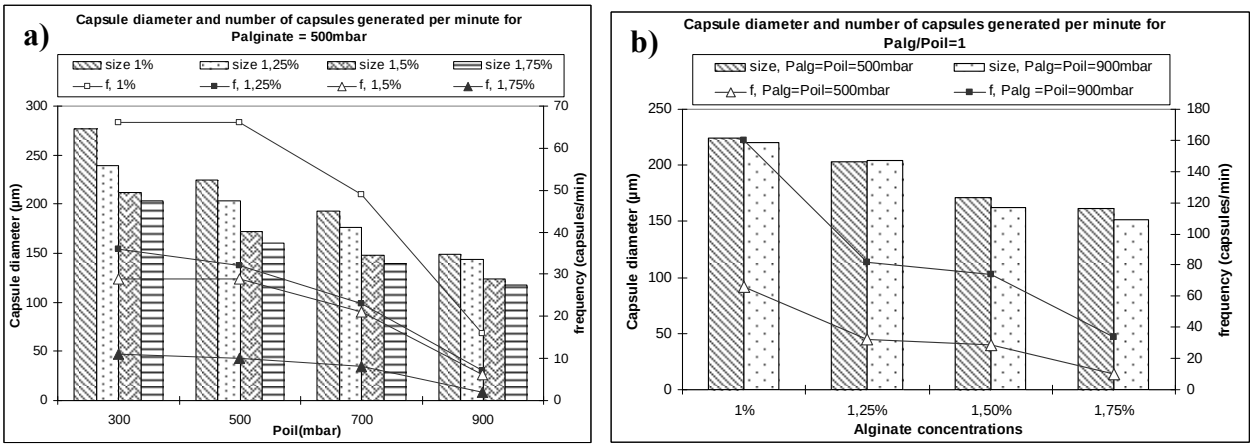
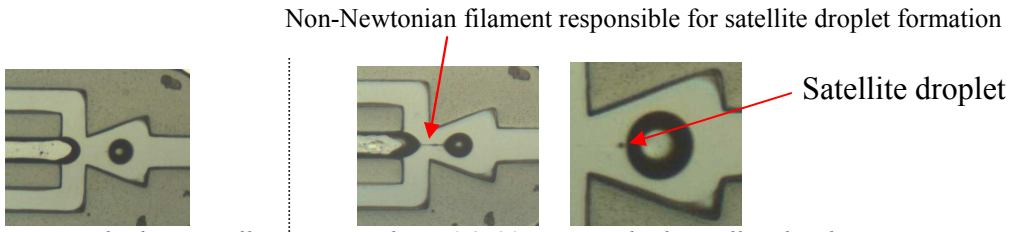


Figure 4: Influence of Poil (a) or Pressure ratio (b) on capsule diameter and number of capsules generated per minute for 1%, 1,25%, 1,5% and 1,75% Keltone HV concentrations

A drawback of MFFDs (when using viscoelastic fluids, such as alginate) is the formation of satellite droplets: they result from the breaking of the non-Newtonian filament linking the incoming alginate to detaching droplet (Figure 5, b). Satellites seem unavoidable when using viscous, non-Newtonian fluids. However they can easily be eliminated with hydrodynamic effects (M. Chabert et Al, 2008).



Water in mineral oil: no satellite *Keltone 1.25% in mineral oil: satellite droplets*

Figure 5: satellite droplets that are observed with alginate solutions

Cell and bead encapsulations have been performed in RPMI medium or Keltone HV alginates. Biocompatibility is depending on the alginate purity but also on the biocompatibility of the device. To test the biocompatibility of our chip, Jurkat cell encapsulations have been performed in RPMI medium using a mineral light oil phase-1% span 80. Immediately after encapsulation, viability was estimated at 70%. To analyse the toxicity of the mineral oil phase, with or without surfactant, RPMI medium containing cells has been emulsified in the oil mineral phase by stirring. Emulsions were incubated during one hour at 37°C. For cells in RPMI medium without oil (control), for cells emulsified in mineral oil, and for cells emulsified in mineral oil-1% span 80, viability was respectively estimated at 96%, 93% and less than 76%. Hence further work has to be done to find another biocompatible oil/surfactant phase but also to purify Keltone HV alginate: physico-chemical characterizations and biocompatibility tests on the purified alginate are in progress.

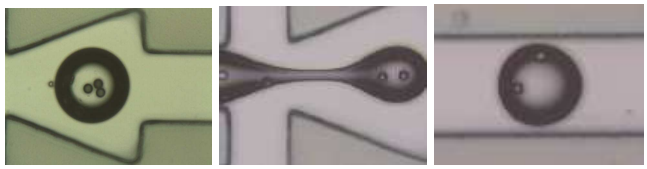


Figure 6: Keltone 1.75% capsules containing 30µm polystyrene beads.

Conclusions

Microfluidic devices are appealing for cell encapsulation. Indeed polydispersity is very low (CV≤1%). Shape and diameter of capsules can be controlled. It is possible to use highly viscous alginate solutions. So far, capsules production rate in our microfluidic device is still low. But, geometry optimization and parallelization of several MFFDs on the same biochip are expected to increase capsules production rate to a level close to that of conventional methods.

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