

# On-line production of polyelectrolyte coating on monodisperse alginate microbeads in an integrated microfluidic cartridge

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## ABSTRACT

Here we introduce a new automated continuous flow microfluidic platform dedicated to the production of hydrogel microbeads followed by an *in situ* polymeric shell coating. The whole process is implemented into a disposable microfluidic cartridge based on a hybrid technology composed of silicon chips and COC plates.

Monodisperse and spherical alginate microbeads of 150  $\mu\text{m}$  diameter covered with one layer of Polyallylamine hydrochloride (PAH) have been obtained by using a micro-flow-focusing module associated to micropillars technologies and a specific fluorosilane “based” functionalization. These results are the first step towards the complete automation of polymeric multilayers coating on hydrogel microbeads within a microfluidic cartridge.

**Keywords:** Alginate microbeads, polyelectrolytes coating, Microfluidics, on-line production

## 1 INTRODUCTION

The field of polymeric microbeads has been thoroughly studied in the last decade for applications such as drug delivery [1] and cell therapy [2]. More often, the microbeads are composed of a biocompatible core material in which molecules of interest can be loaded or cells encapsulated. Various studies have demonstrated the interest in covering the microbeads with a mono- or multilayer of polyelectrolytes (PEMs). For instance the coating can be used to increase both the mechanical strength and the biocompatibility of microbeads for cell encapsulation purpose. PEMs have also been used for drug delivery applications by adjusting the overall permeability of microbeads [3].

The Layer-By-Layer (LbL) coating is the most common method used for PEMs deposition [4]. It consists of alternately coating oppositely charged polyelectrolytes on a template substrate. Centrifugation steps are also necessary to properly wash the microbeads between two consecutive layers deposition to remove the excess of unadsorbed polymers. However, this process is time consuming and requires careful handling.

Continuous flow microfluidic devices have appeared as a promising tool to achieve the LbL coating on microbeads in a repeatable and reliable fashion. Previous works have reported PEMs coating on oil droplets using multilaminar flow pillars channels [5] and PEMs coating on liquid crystal droplets flowing consecutively in different fluxes [6]. However, no microfluidic devices have demonstrated polyelectrolyte mono- or multilayer coating on hydrogel microbeads yet.

Here we introduce a new automated continuous flow microfluidic device devoted to the production of alginate microbeads followed by the coating of one polyelectrolyte covering shell. The whole process is implemented into a disposable microfluidic cartridge based on a hybrid technology composed of silicon chips bonded onto COC plates integrating specific modules dedicated for each fabrication step. Before experiments, microchannels are covered by a hydrophobic Perfluorodecyltrichlorosilane (FDTS) monolayer in order to avoid beads sticking or chip clogging. This FDTS layer has been characterized in terms of wettability and surface charge before and after PAH coating during solution flowing through the microchannels.

As a result, we present 150  $\mu\text{m}$  in diameter alginate microbeads, spherical in shape ( $CV=2\%$ ), monodispersed in size ( $CV=3\%$ ) and covered by one layer of Polyallylamine hydrochloride (PAH).

## 2 MATERIALS AND METHODS

### 2.1 Preparation of solutions

Dispersed phase used for microbeads core fabrication is composed of 3 wt% alginate (Pronova SLG100, Novamatrix), diluted in deionized water containing 150 mM NaCl and 25 mM HEPES. Hyper-refined soybean oil (CRODA EP-NP-LQ-(MH)) is used as a continuous phase and calcium acetate organic salt (Macco Organiques INC) as a pre-gelling agent. For the coating of the shell, a mixture of non labelled PAH (Sigma-Aldrich, 243051) and labelled PAH-FITC (Sigma-Aldrich, 83223) solutions (ratio 1/20) was prepared at a concentration of  $1\text{mg}\cdot\text{ml}^{-1}$ , in 0.5 M NaCl and 0.1 M  $\text{CaCl}_2$  buffer. We used a 0.5 M NaCl and 0.1 M  $\text{CaCl}_2$  solution to rinse and to ultimately collect the microbeads.

## 2.2 Cartridge fabrication

The microfluidic cartridge is a home-made hybrid device made of two silicon chips bonded onto a COC microfluidic plate by an UV curable adhesive. The two silicon chips have been dry etched with standard microelectronics equipments set to obtain 200  $\mu\text{m}$  large and deep microfluidic channels. All microfluidic channels are covered by a 40 nm  $\text{SiO}_2$  layer which is functionalized through covalent Perfluorodecyltrichlorosilane (FDTS) grafting by molecular vapor deposition (MVD<sup>®</sup> from Applied MST). This fluorosilane “based” monolayer was chosen for its neutral charge and good hydrophobicity behavior.

Precisely, the first silicon microchip contains a Micro Flow Focusing module (MFFD) for the generation of alginate droplets in a continuous oil phase (see Fig.1-a). Two additional channels are used to inject calcium acetate crystals in oil as pre-gelling agent. Then, the droplets are transferred in a 30 cm long serpentine microchannel (red channel on Fig. 1 and Fig. 2) milled in the COC plate (see Fig.1-b). Channel is 400 $\mu\text{m}$  large and 500 $\mu\text{m}$  deep.

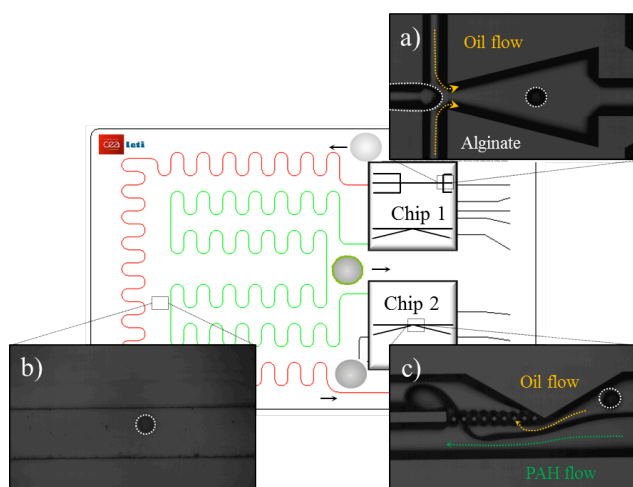


Figure 1: Schematic representation of the Silicon/COC hybrid cartridge including several microfluidic functions. a) MFFD device to produce alginate droplets. b) Serpentine microchannel for microbeads pre-gelation in oil phase. c) Transfer phase module to deviate microbeads in the PAH aqueous solution.

In the second silicon chip, the microbeads are deviated within a transfer phase module made of two parallel channels with a stabilized interface between oil and PAH aqueous solution phases (see Fig.1-c). This interface is maintained and allows oil evacuation through a network of pillars. Then microbeads flow into the PAH solution through a second 30 cm long serpentine microchannel (green channel on fig. 1 and fig. 2). Channel is 1mm large and 1mm deep. Microbeads are then extracted from the PAH solution to a rinsing solution through a second phase transfer device.

## 2.3 Experimental setup

The cartridge has standard credit-card footprint (Flow-Pad<sup>®</sup> Platform) and can easily be plugged into a home-made metallic holder for connection to PTFE fluidic tubings (see Fig. 2).

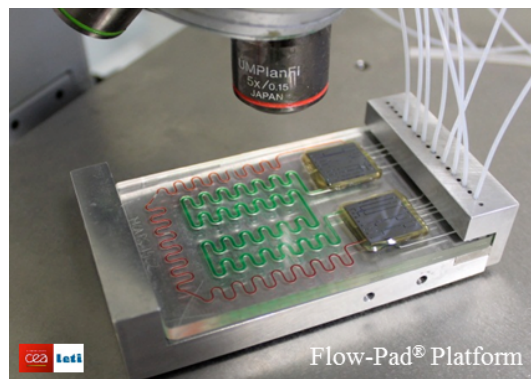


Figure 2: Image of the microfluidic hybrid silicon/COC cartridge integrated within the metallic holder (Flow-Pad<sup>®</sup>).

Solutions are set in motion by a regulated pressure-driven apparatus, working between 0 to 1bar, delivered by micropumps (Fluigent<sup>®</sup> micro-pumps MFCS-8C). Experiments are visualized under a X5 objective microscope (UMPlan FI, Olympus) and image sequences acquired with a high speed camera (Mikrotron GmbH, MotionBlitz Eosens) up to 5000fps.

## 2.4 PAH coating characterizations

PAH shell coating on microbeads were observed on a Leica DM IRE2 confocal microscope equipped with a x50 0,85 D objective, an excitation light of 488nm (Ar/ArKr laser source) and 510–540 nm (green) emission band pass filter.

## 2.5 Surfaces coating characterizations

To monitor wettability and surface charge variation during PAH flowing through the silicon microchips, 10x20 mm flat <100> oriented silicon were plunged 30 min in the PAH containing solution and analysed by both zeta potential and contact angle measurements. These samples were covered by a thermally growth silicon dioxide ( $\text{SiO}_2$ ) layer (Tempress<sup>®</sup> furnace from AMTECH). At the end, silanized samples with FDTS were compared to non silanized samples.

Zeta potential characterizations were realized with an eletrokinetic analyser for solids surface analysis (Surpass<sup>®</sup> from Anton paar) in streaming current mode (pH=5.8; [KCl]=1mM). Contact angle characterizations were realized by dispensing a droplet of deionized water (1.5 $\mu\text{l}$ ) with a Digidrop<sup>®</sup> contact angle meter (from GBX).

### 3 RESULTS

#### 3.1 Microfluidic performances

The whole process for alginate microbeads fabrication and shell coating is integrated in the cartridge, meaning that the liquid alginate and PAH solutions are separately flowing in the inlets of the cartridge and gelled alginate covered with a PAH shell layer are collected at the outlets.

Once the microbeads formation is stabilized within the MFFD module by a perfect adjustment of applied pressures in both alginate and oil inlets, the speed velocity of the droplets was optically measured to be  $10,6 \pm 0,3 \text{ mm} \cdot \text{s}^{-1}$  in the first serpentine microchannel. In this way, a residence time of 30s is long enough to completely cross-link the alginate microbeads through  $\text{Ca}^{2+}$  ions diffusion. This condition is necessary to avoid microbeads deformation and penetration through the micropillars present in the transfert phase module. Similarly, the second serpentine microchannel was designed with a larger section in order to increase the residence time of the microbeads above 30s (i.e decreasing liquid flow velocity).

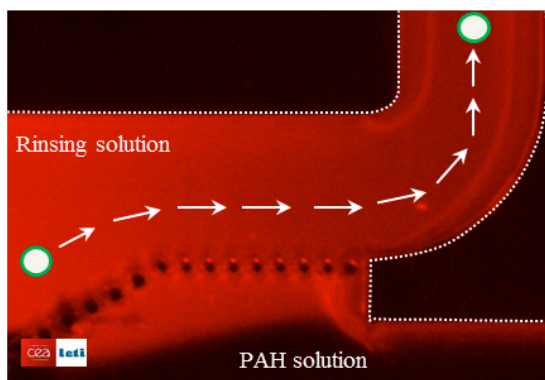


Figure 3: Optical view in fluorescence of the last micropillars transfer module showing evacuation of the rinsing solution stained with Rhodamine in the waste outlet.

A specific attention was taken to avoid cross contamination of PAH inside the rinsing solution in order to prove the efficient coating within the cartridge. In the last micropillars module, a higher pressure was applied to the rinsing solution inlet compared to the entering PAH solution containing microbeads. In this way, a part of the rinsing solution is evacuated into the PAH waste outlet and no contamination occurs.

The flux stabilization in the micropillars module is illustrated on Figure 3. Here, the rinsing solution was stained with Rhodamine in order to follow the interface behavior. As observed in fluorescence images, a part of the rinsing solution is evacuated at the waste outlet.

#### 3.2 FDTS and PAH surface coating study

Contact angle measurements on flat  $\text{Si}/\text{SiO}_2$  substrate have exhibited a hydrophilic behavior with a contact angle of  $46,7 \pm 4,9^\circ$  (see table 1). After PAH solution immersing, the surface stay slightly hydrophilic with a value of  $60,5 \pm 4,4^\circ$ . Moreover, zeta potential characterizations have also demonstrated a surface charge inversion after PAH coating ( $\zeta = +38,5 \pm 1,2 \text{ mV}$ ) compared to initial value ( $\zeta = -77,2 \pm 1,1 \text{ mV}$ ). These considerations are problematic and can lead to increase microbeads sticking in the whole cartridge. These phenomenons are related to hydrogel microbeads affinity with the hydrophilic surface and also electrostatic attractions of anionic alginate with the positive surface charge of the walls[7].

On flat FDTS grafted  $\text{Si}/\text{SiO}_2$  substrate, experimental contact angle of  $110 \pm 3,5^\circ$  and zeta potential of  $-114,6 \pm 5,8 \text{ mV}$  were measured. This surface treatment is quite adapted to produce alginate microbeads while limiting sticking until the second serpentine microchannel. After PAH solution immersing, contact angle decreased to  $93,2 \pm 2,4^\circ$  and zeta potential decreased to  $-27,4 \pm 1,4 \text{ mV}$ . The surface still remains slightly hydrophobic and negatively charged as desired and tend to limit beads sticking on the last micropillars module.

	DI water contact angle ( $^\circ$ )		Zeta potential (mV) in 1mM KCl	
	No coating	PAH coating	No coating	PAH coating
$\text{Si}/\text{SiO}_2$	$46,7 \pm 4,9$	$60,5 \pm 4,4$	$-77,2 \pm 1,1$	$+38,5 \pm 1,2$
$\text{Si}/\text{SiO}_2$ -FDTS	$110,0 \pm 3,5$	$93,2 \pm 2,4$	$-114,6 \pm 5,8$	$-27,4 \pm 1,4$

Table 1: Comparison of both contact angle and zeta potential measurements obtained before and after PAH coating on  $\text{Si}/\text{SiO}_2$  surface with and without FDTS grafting.

#### 3.3 Microbeads characterizations

Collected microbeads at the end of the whole process were characterized by both optical and confocal microscopes.

Images on Figure 4-a show that microbeads produced within the cartridge are spherical in shape ( $\text{CV}=2\%$ ) and monodispersed in size ( $\text{CV} 3\%$ ) with an average diameter ranging from  $130\mu\text{m}$  to  $160\mu\text{m}$  depending on the applied pressure. Confocal images on Figure 4-b let display a fluorescent and uniformly distributed shell that confirm an efficient PAH-FITC coating in the second serpentine.

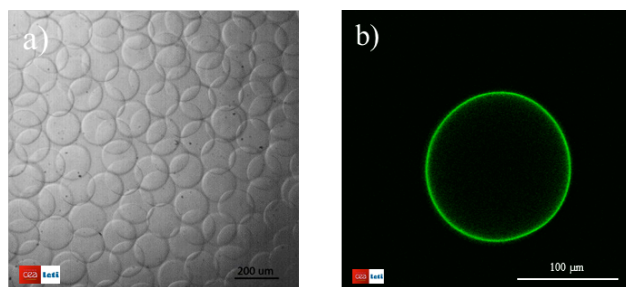


Figure 4: Optical (a) and confocal (b) microscopic views of perfectly 150µm spherical and monodispersed 3wt% alginate beads covered with one layer of PAH-FITC produced by the cartridges.

## 4 CONCLUSION

We have presented an efficient continuous flow microfluidic device dedicated to the on-line production of 150µm in diameter monodispersed and spherical alginate microbeads covered with one Polyallylamine hydrochloride (PAH) layer.

For that purpose, a hybrid cartridge integrating several microfluidic modules was designed. Silicon microchips containing a MFFD module and phase transfer modules were used to generate microbeads and transfer them towards PAH or rinsing solutions. Contact time between alginate capsule and PAH was adjusted by pressure and microfluidic channel length controls, to deposit uniform layer of polyelectrolyte (PAH) over each alginate microbeads. Special attention was paid to surface chemistry, in order to limit microbeads sticking due to hydrogel affinity with microchannels walls.

Results presented here open new perspectives for a full integrated LbL coating process on polymer beads for cell encapsulation [8] or drug delivery applications.

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