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Polyelectrolytes layer-by-layer surface modification of PDMS microchips
for the production of simple O/W and double W/O/W emulsions:
From global to localized treatment

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Abstract

The fabrication of stable double emulsions remains a challenge in many industrial applications and appears as a hot topic in the microfluidics field. We apply a novel approach to selectively modify the wetting properties of the wall of the micro-emulsifier by depositing successive layers of polyelectrolytes on the targeted section of the microsystems. The PDMS surface of a microfluidic droplet generator is locally modified by a layer-by-layer polyelectrolytes assembly of poly(allylaminehydrochloride) and poly(acrylicacid). This surface functionalization is successfully performed on flat surfaces, the complete microchip constituted of two steps flow-focusing, or on localized sections of the microsystem, which led to the hydrophilization of hydrophobic PDMS surfaces. The long lasting treatment, over a month, is confirmed. Direct O/W emulsions are enabled, and more interestingly, double W/O/W emulsions of controlled size and shell thickness are formed in PDMS microchip. This work presents an interesting alternative to the other systems commonly used such as imbricated glass capillary microsystems and also other approaches of surface modifications of PDMS microchips. This layer-by-layer surface functionalization on a microsystem displays the advantage of the versatility and ease of use of the PDMS. Polycarbonate and poly(methyl methacrylate) based microfluidic devices are successfully functionalized by simply transposing the process.

Keywords: Microfluidics, PDMS, double emulsions, surface modification, flow-focusing, layer-by-layer deposition

1. Introduction

Double emulsions consist of droplets dispersed in a continuous phase which contain smaller dispersed droplets. One of the most common types of double emulsions is water-in-oil-in-water (W/O/W) double emulsions. They have received considerable attention as they are promising systems for drug delivery [1] and for industrial applications in the areas of medicine, cosmetics, food products or optical display technology [2]. However, the current ways of production of double emulsions by conventional apparatuses like high shear devices (Ultraturrax®, helical roll mills) or ultrasound devices are usually operated in two steps, and result in broad size distributions [2,3,4]. This large size distribution of the droplets accelerates significantly the ageing of the emulsions, improves drug delivery and leads to less positive controlled release of loaded active substance.

Microfluidics opens new perspectives for the continuous and controlled production of monodispersed simple, double or multiple emulsions [3,5] as well as morphology-controlled polymer core-polymer shell particles [2] thanks to its excellent control of experimental conditions like temperature, flow rates and residence time. Since the pioneer work of Utada et al. [3] and Okushima et al. [6], a considerable amount of work has been achieved to find simple and time-stable microfluidics devices for the production of double emulsions. In their study, double emulsions were produced in one step by using coaxial jets in cylindrical glass capillaries inside of a square tube [3]. However, the fabrication of these glass devices requires precise alignment of three microcapillary tubes to form a coaxial geometry, which is extensive time and energy consuming [7]. This technique has been applied and extended to the production of polymer core-polymer shell particles [2]. They used a capillary-based microfluidic device consisting of two-coaxial capillaries (hydrophilic fused silica tubing,

and/or hydrophobic PEEK or PTFE tubing) which relative tips position influences the type of particles formed (large double droplets, droplets with multiple core, rod-like particles...). The main advantage of this device is to keep the dispersed phase away from the outside walls, what prevents phase inversion [2]. The other approach consists in using 2 successive junctions (T-junction, flow-focusing, etc). As an example, Bai et al. [7] used a glass microchip with a T-junction followed by a flow-focusing junction to produce W/O/W double emulsions. They hydrophobized their glass channels with an octadecyltrichlorosilane layer, and, then locally photo-degraded this layer by deep UV light to recover the natural hydrophilicity of glass substrate. Nevertheless, PDMS remains the most widely used material in microfluidics owing to its flexibility and transparency in UV-Visible spectrum range. This material is hydrophobic by nature and the way to render it hydrophilic in long term is challenging, as the elastomeric property of the PDMS induces its surface recovery, hence, its hydrophobicity [8-25].

Fabrication of double emulsions in 2 stages flow-focusing PDMS devices faces two main challenges: (i) selective modification of the PDMS surface on localized sections of the microsystem, and (ii) long-lasting stability of the coating layer with time while keeping the transparency of the system for the monitoring of the emulsion formation [25]. Until now, only few approaches are able to fulfill these requirements. For example, Barbier et al. [5] used a plasma (argon/acrylic acid) for the deposition of acrylic acid, and cross-linked the deposited polymer for increased cohesive strength. Double emulsions were obtained by two successive T-junctions where the first one had been masked during the plasma coating to keep the natural hydrophobicity of the PDMS [5]. Kim et al. [9] patterned the PDMS chips with spatially controlled plasma oxidation to create double emulsions. Other authors patterned the double flow-focusing PDMS chip with ink before exposition to oxygen plasma [14]. After removing the protective ink, the partly hydrophilic – partly hydrophobic chip could produce double emulsions. Nevertheless, the most recent attempt to create hydrophilic PDMS surfaces is to

build a layer-by-layer (LbL) assembly by consecutive adsorption of polyanions and polycations [26]. The main advantage of this process is the long-term stability of the polyelectrolytes multilayered film onto the PDMS surface [8,19,27,28,29]. However, the spatial control of the LbL deposition only on localized part of the microsystem remains a challenge. Bauer et al. [30] coated successfully the PDMS by a sequential layer-by-layer deposition of poly(allylamine hydrochloric)/poly(styrene sulfonate) (PAH/PSS) polyelectrolytes. This coating lasted for month and was efficient for producing double emulsions. Their process had the drawback of using the non-biocompatible Aquapel for coating the hydrophobic part of the channel. The most promising method was presented recently by Choi et al. [31], where LbL deposition of the polyelectrolytes was conducted using syringe-vacuum-induced segmented flow in the system to spatially control the surface modification.

In the present study, the focus is placed on the surface PDMS treatment based on the formation of a polyelectrolytes assembly of poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA). The process is successively applied on flat surfaces, on the complete PDMS microsystem, or on localized sections of the microdevice in order to form monodisperse oil-in-water (O/W) simple and water-in-oil-in-water (W/O/W) double emulsions. The method to selectively and spatially control the modification of the surface of the wall of the microfluidic device is inspired from the approach of Choi et al. [31]. Our approach and results corroborate and complement theirs, with differences pointed out in the discussion. In addition, the use of water-based solvents for PAH and PAA ensures the stability of the microfluidic channels by preventing any swelling of the PDMS as shown by Zhou et al. [21] when organic solvent are used.

2. Materials and methods

2.1. Chemicals and solutions preparation

The silicone elastomer (Sylgard[®] 184) and the curing agent were provided by Dow Corning (Auburn Mi. USA). The PDMS were obtained by mixing 90%w of silicone elastomer with 10%w of curing agent. The mixture was degassed under vacuum before use for the fabrication of plates or microsystems.

All the used glass beakers and the glass syringes were previously cleaned with piranha solution and rinsed with Milli-Q water. The piranha solution was a mixture of H₂SO₄/H₂O₂ 1/1 V/V (H₂SO₄ 98%_v from Sigma Aldrich and hydrogen peroxide 30%_v (from VWR Chemicals)).

Poly(allylamine hydrochloride), denoted as PAH, was provided by Sigma Aldrich. It possessed a molecular weight Mw of 450 000 g/mol. Poly(acrylic acid), *i.e.* PAA, was purchased from Sigma Aldrich. It had a Mw of 450 000 g/mol. The molecular weights of both polymers were chosen such as to favour the constitution of layer-by-layer assemblies. The structural formulas of PAH and PAA are depicted in the Fig. 1. Aqueous solutions of PAH and PAA of concentrations of 10⁻² mol/L of monomer unit were prepared. The molecular mass of PAH and PAA's monomer was respectively, 57.1 g/mol and 72 g/mol. This meant that PAH and PAA both of 450 000 g/mol were composed of 7895 and 6250 monomer units, respectively. Hence, solutions of PAH and PAA of concentrations of 0.571 g/L and 0.72 g/L, respectively were prepared. The pH was adjusted to 6.5 by adding small amounts of HCl or NaOH solutions (0.5 M) under magnetic stirring. The pH acted on the folding of the chains [32]. It was important to adjust the pH to 6.5 to obtain a good balance between the degree of ionization of the molecules and the thickness of the layers [32]. Before use, the polyelectrolytes solutions were filtrated by a 0.4 μm pore size filter.

Several oils and surfactants were used for the fabrication of O/W and W/O/W emulsions. For the simple O/W emulsions, the oil phase consisted of castor oil containing 5 %_w of Span 20. The water phase was composed of water with 5 %_w of Tween 80. The non-ionic surfactants (Tween 80 and Span 20) were provided from Sigma Aldrich. For the double W/O/W emulsions, 2.5%_w of sodium dodecyl sulfate (SDS, from Sigma Aldrich) in distilled water was used as the aqueous phase. Tri(propylene glycol) diacrylate (TPGDA) was employed as the oil phase. It was provided by Sigma Aldrich.

2.2. Surface modifications of flat surfaces

Three flat surfaces were employed, namely polydimethylsiloxane (PDMS), poly(methyl methacrylate) (PMMA) and polycarbonate (PC). For the PMMA and PC surfaces, commercial plates were utilized. Conversely, the PDMS surfaces were fabricated. They were obtained by mixing of 90%w silicone elastomer Sylgard[®] 184 with 10%w of curing agent. The mixture was degassed under vacuum and poured on a microscope slide in a petri dish. The PDMS was degassed once again to eliminate the bubbles formed during pouring. The PDMS was then polymerized in an oven at 60 °C for 1h30 min. These three surfaces are hydrophobic. Consequently, as the layer-by-layer assembly is conducted in water, the homogeneous deposition of the LbL of PAH/PAH can be altered. It was then necessary to activate the surfaces in order to switch their properties from hydrophobic to hydrophilic and take advantages of the new active groups created at the surface to interact with the polyelectrolytes chains.

The PDMS surfaces were activated by three different methods: (i) oxygen plasma, (ii) piranha solution, and (iii) piranha solution followed by the plasma. For the activation by oxygen plasma (i), the PDMS plate was placed inside a Harrick Plasma Cleaner (Ithaca, NY, USA) during 2 min at full power under a vacuum of about 80 Pa. The oxygen plasma had the

effect of creating silanol, carboxylic and carbonyl groups at the surface of PDMS which are able to interact with the polycation backbone groups [20,23,24,33]. For the activation with a piranha solution (ii), the PDMS plate was dipped into the piranha solution for 30 min. According to Maji et al. [34], PDMS exposed to a piranha solution remove methyl groups from surface and replace them by polar silanol (Si-OH) groups. For the last activation (iii), the PDMS plate was first dipped in the piranha solution (30 min) and, secondly, activated by oxygen plasma (during 2 min at full power under a vacuum of about 80 Pa). For the PC and PMMA surfaces, the activation was always produced by oxygen plasma.

Layers of PAH and PAA were deposited on the surfaces by the following protocol that had been adapted from Alem et al. [35]. The PAH layer deposition on the slide was performed in a first step. Immediately after the activation, the flat slide was dipped in the PAH solution for 10 min. The plate was withdrawn and rinsed with distilled water. After that, it was dried by blowing compressed air. Then, the plate was immersed 3 times for 5 s into distilled water and dried by blowing compressed air. Finally, the plate was immersed 15 s into distilled water and dried by blowing compressed air. After these steps, the PAA layer deposition on the flat slide was conducted. It followed the similar sequence, *i.e.* 10 min of immersion inside the PAA solution followed by the successive rinsing and drying steps. At the end of all these steps, one monolayer of PAH and one monolayer of PAA were deposited onto the flat substrate. This counted for one layer, namely one PAH/PAA bilayer. In the present study, 5 bilayers of PAH/PAA were successfully deposited. The layer of PAA formed ionic bonds with the previously deposited PAH layer. The following deposited PAH and PAA layers were also linked by ionic bonds (Fig. 2). At the end of the LbL process, the modified plates were stored in the open-air.

To check the efficiency of the surface modification (long-term stability as well as affinity with oil and water), contact angles measurements were undertaken. Solid/drop and

solid/drop/liquid contact angles were measured with the OCA20 system (Dataphysics Instruments GmbH) before and after the surface treatment. For that purpose, a liquid drop ($V = 5 \mu\text{L}$) was deposited, at $20 \text{ }^\circ\text{C}$, on the surface. A camera connected to a computer captured the image of the drop on the surface and the contact angle between the liquid drop and the surface was determined by SCA software (Dataphysics Instruments GmbH). For the solid/drop/liquid measurements, a quartz cuvette was used to contain the continuous liquid phase (denoted as “liquid” in solid/drop/liquid). All measurements were repeated 5 times on different positions of the surface. The data presented were the averages of the repeated results.

2.3. Global and localized surface modification of PDMS microchips

Microchips were manufactured with the soft technology which was based on the various classical steps: SU8 resin / PDMS reticulation / oxygen plasma bonding [36,37]. Our microsystems consisted of two successive flow-focusing junctions in order to produce size controlled and monodispersed double emulsions (Fig. 3). The channels were $200 \mu\text{m}$ large, $40 \mu\text{m}$ in the flow focusing restriction, and $64 \mu\text{m}$ deep. The PDMS was by nature hydrophobic. In order to have all walls with identical surface properties, the microscope glass slide commonly used for closing the microsystems was replaced by a homemade slide of polymerized PDMS deposited onto a microscope glass slide. The PAH/PAA layer-by-layer deposition procedure presented in the previous section was adapted to global and, mostly, localized treatment of the channel’s walls.

Four glass syringes were employed. The first syringe was filled with the PAH aqueous solution (concentration of 10^{-2} mol/L of monomer unit), the second contained the PAA aqueous solution (concentration of 10^{-2} mol/L of monomer unit), the third was filled with distilled water, while the last contained only air.

2.3.1. Microsystem completely covered by PAH/PAA LbL

In a first step, the LbL deposition process was adapted to cover completely the whole microsystem with five bilayers of PAH/PAA. This appeared as an intermediate step before developing the treatment on localized sections of the microchip. The LbL treatment was achieved immediately after the plasma treatment and bonding as the plasma treatment activated all the surfaces of the microdevice. The Teflon pipes were connected to the entries of the microchip (Fig. 3b). All the solutions were introduced by the outlet D. Consequently, the syringe was connected to the pipe from the outlet D. The PAH solution was first introduced inside the system through the outlet D. The injection was stopped as soon as the first drops of solution were detected at the outlets C, B, and A. The PAH solution remained in contact, in a stationary state, with all the channels of the microchip during 10 min. At the end of the 10 min of adsorption, the syringe containing the PAH solution was replaced by the syringe filled with air. The air flow was introduced inside the system until the microchip was completely dried. The aim of the air flow was to dry the channels by removing the liquid from the microchip. Then, the syringe filled with air was replaced by the syringe which contained the water. The rinsing took place by continuously introducing the water during 15 s. Another air flow step was performed. A new rinsing step was conducted three times for 5 s. It was followed by an air flow step. Then, the water was introduced in continuous flow during 15 s. Finally, the air flow step was conducted. The same sequence was used to adsorb the PAA onto the system (10 min of contact with PAA solution followed by air flow and rinsing steps). All these steps were carried out five times to deposit five PAH/PAA LbL bilayers. Note that all the solutions and air were injected with the syringe by hand (not with a syringe pump). The coated microsystems were stored with the channels filled with distilled water to enhance the stability of the coating.

2.3.2. Localized treatment by PAH/PAA LbL

The aim was to produce multiple water-in-oil-in-water emulsions (W/O/W). These types of emulsions are very interesting for the pharmaceutical industry because the active ingredients which are most of the time hydrophilic, could be encapsulated in an oil shell double emulsion. Therefore, a W/O emulsion must be produced in the first flow-focusing junction. Since water-in-oil emulsions are favoured by hydrophobic walls [6,38], walls at this junction have to be hydrophobic and stay hydrophobic until the next junction to avoid that the aqueous phase wets the walls and, thus, breaks down the W/O emulsion. At the next junction, an O/W emulsion must be produced. Therefore, the walls must be hydrophilic (Fig. 3a).

The procedure for layer-by-layer deposition of PAH/PAA on the localized part of the channel, *i.e.* the exit channel, was the following. The aim was to render hydrophilic only the exit of the last flow-focusing junction up to the outlet of the system (Fig. 3). Directly after plasma activation and sealing of the microsystem, the LbL deposition was conducted. The entry C was blocked by using a plug (Fig. 3a). A constant air flow of 500 $\mu\text{L}/\text{min}$ was introduced through the entry B. This airflow was promoted thanks to a NEMESYS syringe pump (Cetoni GmbH, Korbuse, Germany). The air flow was maintained during the whole LbL deposition process. The idea was to protect the channels C, B, and A from the polyelectrolyte solutions by using air at counter flow. The entry A served as the exit channel for the air. The aqueous solution of PAH, of concentration of 10^{-2} mol/L of monomer unit, was introduced from entry D at a flow rate of 3 $\mu\text{L}/\text{min}$ for 15 min by a syringe pump. The whole deposition process was monitored with a microscope focused on the second flow-focusing junction to ensure that the polyelectrolyte solutions remained at the outlet of the 2nd flow-focusing junction and did not penetrate inside the system (entries A, B, C and 1st flow-focusing junction). After 15 min, the air flow rate blowing from entry B was increased to 3 000 $\mu\text{L}/\text{min}$ while the syringe introducing the PAH solution in entry D was removed. The

increase of the air flow rate aimed to remove the PAH solution by flowing through the outlet D. The air flow was introduced inside the system until the microchip was completely dried. Then, the syringe containing the water was plugged to the entry D. The water was introduced from the entry D at a flow rate of 3 $\mu\text{L}/\text{min}$ for 15 s by a syringe pump while the air flow rate through entry B was decreased to 500 $\mu\text{L}/\text{min}$. At the end of the 15 s, the air flow rate blowing from entry B was increased to 3 000 $\mu\text{L}/\text{min}$ to remove the water while the water syringe was displaced from outlet D. Two new rinsing steps (15 s at a flow rate of water of 3 $\mu\text{L}/\text{min}$ (entry D) under air flow rate of 500 $\mu\text{L}/\text{min}$ (entry B)) followed by drying steps (air flow rate of 3 000 $\mu\text{L}/\text{min}$ (entry B)) were conducted. At the end of the last air flow step, the syringe introducing the PAA solution was placed in entry D. The aqueous solution of PAA (of concentration of 10^{-2} mol/L of monomer unit) was introduced at a flow rate of 3 $\mu\text{L}/\text{min}$ for 15 min by a syringe pump while the air flow rate was decreased to 500 $\mu\text{L}/\text{min}$. At the end of the 15 min, the same sequences of rinsing and drying were carried out. To summarize, this overall process was applied 5 times to produce 5 bilayers of PAH and PAA. Once the channel was dried, it was used immediately to produce multiple emulsions.

3. Results and discussions

3.1. Flat modified surfaces: long-term stability and affinity with oil and water

First, the stability and lifetime of the PAH/PAA multi-layered coatings are tested on flat PDMS surfaces for the different activation processes. For this purpose, we follow the temporal evolution of the contact angle of a 5.0 μL water droplet deposited on a PDMS surface modified by the PAH/PAA assembly (Fig. 4). The water contact angle (θ) onto untreated PDMS is around 100° indicating hydrophobic properties of the surface (dotted line in Fig. 5). After PAH/PAA treatment, the initial contact angle drops significantly to $33\text{-}40^\circ$. This confirms the presence of the polyelectrolyte layers onto the PDMS surface. A slight

difference in the contact angles after one day can be noticed depending on the activation process prior to the LbL deposition. The lowest θ (33°) is obtained after plasma followed by piranha activation while larger contact angles are recorded with piranha or plasma activation alone ($\theta = 39^\circ$). However, the values of contact angles between 1 and 17-20 days of ageing remain similar regardless of the activation process used. This points out that the mode of activation of the PDMS surface does not affect the LbL deposition and the contact angles. Regarding in details the temporal evolution of the contact angle, the 3 curves follow the same trend regardless of the activation process. After a period of increase of the contact angle that last 17 days, a stabilization is reached. It is also striking that all the contact angles after 60 days remain significantly lower than the value obtained with untreated PDMS, given as 100° . This confirms the stability of the coating onto the PDMS surface since no appreciable degradation of the coating could be observed for several weeks with the presented procedure of deposition of poly(allylamine hydrochloride) and poly(acrylic acid).

Tests of the previously presented surface treatment have been conducted on polycarbonate (PC) and polymethyl methacrylate (PMMA) plates (Fig. 5). The low initial contact angles confirm the success of the polyelectrolytes depositions onto the two substrates. Five bilayers of PAH/PAA produce a significant diminution of the contact angle from 65° (untreated PC) and 77° (untreated PMMA) to $15-25^\circ$. In terms of temporal stability, no influence of the surface composition was witnessed. An increase of θ with ageing time followed by a plateau obtained after 15-20 days is highlighted in Fig. 5, which confirms the stability of the coatings on all the studied surfaces. Moreover, after 60 days, the contact angles is of 40° on PC and 42° on PMMA. These values remain significantly lower than those of the untreated materials. This confirms the long-term stability of the three coatings. It is then believed that this surface treatment may be extended to other polymer materials.

Inside the microsystems, surface tension forces become predominant over inertial, gravity and viscous forces. Therefore, the wetting or non-wetting properties of the walls determine the type of emulsion produced. If the walls are hydrophilic, oil droplets are created in a continuous aqueous phase. Inversely, if the walls are hydrophobic, water droplets in a continuous oil phase are produced [6,38,39]. The affinity of the oil with the surface in a water environment or, inversely, the water with the surface surrounded by oil, is also an important parameter. The contact angle measurements are conducted on flat PDMS, PC and PMMA surfaces coated with five bilayers of PAH/PAA (Fig. 6). For the three surfaces, the water contact angles in the presence of oil remain lower than 70° . Conversely, the oil contact angles in the aqueous environment are larger than 142° . These results confirm that the three hydrophilic modified surfaces interact preferentially with water (in the presence of oil) rather than with oil (in the presence of water). This indicates also that the oil drops do not adhere or stick to the modified surfaces. As a consequence, direct oil-in-water emulsions can be envisioned with these three surfaces. It is also interesting to compare the results obtained with the three surfaces. The lowest water contact angle and the largest oil contact angle are reported with the PAH/PAA/PDMS surface. This can be attributed to the properties of the modified surface as well as the presence of surfactants in both the oil and water phases. For the two other surfaces, the oil and the water do not contain surfactant.

Based on the data of stability combined to those of affinity with oil and water, it can be deduced that the PAH/PAA LbL system is very appropriate for the surface modification of the microsystems. As far as the nature of the microchip is concerned, the three materials lead to similar behaviour when modified with five bilayers of PAH/PAA. However, for practical reasons, the PAH/PAA/PDMS microsystems are used in the following to prepare simple and double emulsions. The PDMS microchips are selected because of the easy fabrication and

also due to the lower size of the channels with PDMS as compared to those obtained with PC and PMMA with our fabrication processes [40].

3.2. Production of simple and double emulsions

Preliminary experiments are conducted with the untreated PDMS microsystems. The aim of these experiments is to assess that the performances of the coated microchip are due to the polymer layers, and not to the presence of surfactants and/or to the geometry of the channels. To this purpose, some results without surface treatment are briefly presented. Simple emulsions are prepared at the second flow-focusing junction of the microchip (Fig. 3). When the water phase is introduced through the entry C while the oil phase comes by the entry A (Fig. 7a,b), inverse water-in-oil (W/O) emulsions are produced since the walls of the microsystem are hydrophobic. In cases where the water phase enters through entry A while the oil phase passes through the entry C, inverse W/O emulsions (drops of water) are still produced (Fig. 7c). This confirms that the untreated PDMS walls of the microsystem are preferentially wetted by the oil.

3.2.1. Microsystem completely covered by PAH/PAA LbL

To test the efficiency of the LbL coating inside the microchip, simple emulsions are prepared. In this particular case, the microsystem is fully coated with the LbL PAH/PAA assembly in order to have hydrophilic walls. In this configuration, direct oil-in-water (O/W) emulsions are expected to be produced inside the microchip. To this aim, only a single flow-focusing junction of the microchip is used, namely the second junction (Fig. 3b). To assess that O/W emulsions are produced in the system, the aqueous phase is coloured by methylene blue dye. The oil phase constituted of castor oil and 5%_w of Span 20 is introduced through the central channel (entry C in Fig. 3) while the aqueous phase (distilled water containing 5%_w of Tween 80 and methylene blue) is injected through the side channel (entry A). The pictures of

the droplets formed in the microchip are given in the Fig. 8. It can be clearly seen that the continuous blue solutions surround the drops indicating that the continuous phase is constituted of water. In other words, water coloured phase is the continuous phase while the transparent drops are constituted of castor oil. Castor oil droplets are then formed. This result clearly establishes the successful integration and deposition of the 5 bilayers of PAH/PAA onto the PDMS walls of the microchip.

Fig. 9 depicts the influence of the continuous water flow rate (Q_w) on the castor oil drops diameter for various oil flow rates (dispersed phase, Q_o). The drop size decreases with increasing flow rates of the continuous phase, which can be attributed to the enhancement of the shear rate or shear strength onto the dispersed phase. The drop sizes are not substantially affected by the flow rate of the dispersed phase. In our conditions, drops of castor oil with size ranging from 23 to 40 μm are obtained. These trends and results are very similar to those reported in the literature [40-43].

In addition, the long-term stability of the PAH/PAA coating inside the microchip is evaluated. After completing the initial cycle of drops fabrication, the microsystem is washed with water and, finally, stored by ensuring that all the channels are filled with distilled water. Then, the days after, the same microchip is employed for the generation of O/W emulsions using fresh oil and water under the same conditions of flow rates for another cycle of droplets production. For the exploitation of the results, only the pictures of the oil drops are reported in the Fig. 10. The properties of the drop formed is found to slightly vary with the ageing time during 30 days. This confirms that the PAH/PAA coating is inherently stable during prolonged time and also prolonged utilization. The PAH/PAA/PDMS microchip maintains a high activity and good stability for the production of oil droplets in water for one month. It is also interesting to note that after three months, it was impossible to use the microchip for the formation of direct O/W emulsions. However, no intermediate data is available between one

month and three months to estimate accurately the exact ageing time for which the microchip, and also the coating, remains stable.

To confirm the long-term stability of the LbL coating inside the microchips, it appears necessary to report on the effect of injecting a continuous flow of oil and water for several cycles of various hours on the performances of the microsystem. In this context, we measure the oil drop sizes for several hours during the continuous flow of oil and water. The experiments are conducted in between the previous stability tests. The first experiment is performed after 2 days of ageing. The continuous flow of oil and water is maintained during 10 hours. The oil drop size is measured after 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 7 and 10 hours. The sizes reported in the Fig. 11 are the average of the sizes of 20 consecutive oil drops. At the end of the cycle of continuous experiments, the microchip is washed with water and maintained filled with water. In ageing day 8, the second continuous flow test is carried out during 10 hours. The results, *i.e.* drop size versus duration of the experiment, are also reported in Fig. 11 (empty squares in the figure). The last continuous flow test is undertaken after 13 days of ageing. After 2 days of ageing, no substantial variation in the flow behavior and also in the oil drop shape and size can be distinguished during 10 h of continuous flow. The same conclusion can be reached after 8 days of ageing. A slight variation in the oil drop size with the time of experiment can be outlined after 13 days of ageing. However, the difference between the diameters remains weak and can be safely attributed to uncertainties in the evaluation of the diameters of the drops. At the same time, the flow behavior of oil and water, as well as the shape of the drops, are not affected by the time of experiments. Therefore, it appears that the continuous injection of oil and water does not affect the stability of the polyelectrolytes coating and the properties of the microsystem. It is interesting to note that the multilayered film stability remained unchanged compared to the previously described

ones, which unambiguously confirms the long-term stability of the LbL coating inside the microchips.

3.2.2. Localized treatment by PAH/PAA LbL

In a first step, it appears necessary to confirm that the LbL deposition is only performed on the exit channel of the microsystem (Fig. 3a). To this aim, simple emulsions are prepared separately in the first and second junctions. On the one hand, it is expected that the first junction remains hydrophobic because it is uncoated by the polyelectrolytes. Consequently, an inverse water-in-oil W/O emulsion might be obtained when the water phase is introduced through the entry C and the oil phase is injected by the inlet B. To assess the nature of the drops formed at this 1st junction, the aqueous phase is colored by methylene blue dye. The pictures reported in Fig. 12A display blue water drops. This confirms that the outlet of the first junction, *i.e.* the channel between the first and the second junction is not covered by the polyelectrolytes layers. This might also indicate that the channels from the first junction to the inlets B and C are not covered by the LbL layers. On the other hand, the channel from the outlet of the second junction up to the outlet (exit D) is expected to be treated by the PAH/PAA bilayers. When the oil phase is introduced through the entry C while the water phase is injected by the inlet A, the spontaneously formed O/W emulsion at the second junction, highlights the hydrophilic behavior of the exit channel of the second junction (Fig. 12B). These data confirm the efficiency of the LbL process for the localized functionalization of the PDMS surfaces. However, we are aware that the easier portion of the system to be covered by LbL is the exit of the channel of the 2nd flow-focusing junction. The deposition inside the central channel (between the first and the second junction) is more challenging since it requires very precise control of both air and polyelectrolytes flow rates.

Double emulsions are created in the two steps flow-focusing microchip which surface has been locally treated by the previously described layer-by-layer procedure to be hydrophilic at the exit channel of the 2nd junction (Fig. 13). Water containing 2.5%_w of sodium dodecyl sulfate (SDS) is utilized as the aqueous phase while tri(propylene glycol) diacrylate (TPGDA) is employed as the oil phase. The different phases are introduced as represented in Fig. 13. Double W/O/W emulsions are successively prepared by varying the oil and water flow rates (Fig. 14a,b). This confirms the successful deposition of the five bilayers of PAH/PAA on the exit channel. This highlights also the localized treatment of the PDMS surface since the first junction and the central channel are not covered by the polyelectrolytes. Depending on the flow rates of the different phases, the number of water droplets in the double emulsion varies, and in some conditions, the flow rates of the phases to be dispersed are too high to produce the double emulsions (Fig 14c). In details, considering the water drops formed at the first junction (W/O emulsion), the drops size diminishes with the flow rate of the oil ($Q_{O(B)}$). When the oil flow rate $Q_{O(B)}$ is increased from 170 $\mu\text{L/h}$ to 250 $\mu\text{L/h}$, the size of the drop of water reduces from 46 to 35 μm . As far as the double emulsion is considered, two or three water drops are encapsulated inside the oil shell. The size of the oil drops which encapsulated the water drops range between 140 and 160 μm depending on the flow rates. The sizes of the internal water drops (45-35 μm) as well as of the oil shells (140-160 μm) are compatible with those targeted in some pharmaceutical applications such as arterial-injection chemotherapy [44,45], vaccines [46,47], and hemoglobin multiple emulsions as an oxygen delivery system [48].

4. Conclusions

The LbL deposition process of 5 bilayers of poly(acrylic acid) and poly(allylamine hydrochloride) on PDMS surfaces have been successfully performed onto flat surfaces and confined microchannel of the microfluidic systems. We could control the deposition to

conduct the functionalization on localized sections of the microdevice constituted of 2 successive flow-focusing junctions. For flat surfaces, the well-known dipping procedure was performed for the LbL assembly of the polyelectrolytes, whereas for the complete microsystem, the polyanions solutions are introduced manually with a syringe and keep in contact in a stationary state with the channels for 10 min. For the localized treatment, only the exit of the second flow-focusing has been modified with the polyelectrolytes. The use of a constant air flow at counter-flow during the continuous introduction of the polyelectrolyte solutions (PAH or PAA) allows to protect some channels from a contact with the polymer solutions while maintaining the contact of the polyelectrolyte solution with the exit channel.

These protocols give excellent results to create long-lasting (several months) and stable hydrophilic surface on PDMS, PMMA and polycarbonate. The activation steps can be done either by oxygen plasma exposure or by a flow of a piranha solution without significant differences on the hydrophilicity of the coated PDMS. Direct oil-in-water emulsions, and more interestingly, stable double W/O/W emulsions have been produced in PDMS two steps flow-focusing microchips, which opens the way to the production of microcapsules and double emulsions for the pharmaceutical applications.

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FIGURE CAPTIONS

Fig. 1. The structural formulas of (a) poly(allylamine hydrochloride) PAH, and (b) poly(acrylic acid) PAA. PAH is positively charged ($-\text{NH}_3^+$) and PAA is negatively charged ($-\text{COO}^-$) at pH 6.5 where deposition is performed.

Fig. 2. Procedure building the PAH/PAA LbL assembly onto PDMS surface. The last two steps can be repeated several times to obtain a multilayer LbL coating.

Fig. 3. (a) Schematic representation of the microsystem consisting of two successive flow-focusing junctions. After the localized LbL surface treatment, the hydrophilic walls are in red while the hydrophobic walls are in blue. Under this configuration, W/O/W emulsions can be produced. (b) Picture of the microsystem consisting of two successive flow-focusing junctions. The configuration of the pipes corresponds to that used for the formation of simple O/W emulsions. The symbols (i) and (ii) indicate the positions where the pictures of the Figs. 7, 8, 10 and 12 are taken.

Fig. 4. Influence of the PDMS activation prior to the LbL deposition on the temporal evolution of the contact angle of a 5 μL water droplet on the PAH/PAA LbL treated PDMS surface. The water contact angle on untreated PDMS is around 100° .

Fig. 5. Results of the ageing of the PAH/PAA LbL coating on PDMS, polycarbonate (PC), and polymethyl methacrylate (PMMA) surfaces. The temporal evolution of the contact angle of a 5 μL water droplet on the PAH/PAA LbL treated surfaces.

Fig. 6. Solid/drop/liquid contact angles measured on PAH/PAA treated (a,b) PDMS, (c,d) polycarbonate (PC), and (e,f) poly(methyl metacrylate) (PMMA) solid surfaces. (a,c,e) Water drop deposited onto the surface in the presence of oil; (b,d,f) Oil drop deposited onto the surface in the presence of water. (a,b) Oil phase: castor oil + 5%_w Span 20; Aqueous phase: distilled water + 5%_w Tween 80. (c-f) Oil phase: silicone oil; Aqueous phase: distilled water. In the legend, O corresponds to oil while W is related to water phase.

Fig. 7. Emulsions formed in the untreated PDMS microsystem. (a,b,c) Inverse W/O emulsions produced at the second flow-focusing junction. The pictures are taken at the outlet of the second flow-focusing junction. For (a,b), the water phase is introduced through entry C while the oil phase is introduced by the entry A. (a) Water flow rate at the entry C: $Q_{w(C)} = 1 \mu\text{L}/\text{min}$; Oil flow rate at the entry A: $Q_{o(A)} = 50 \mu\text{L}/\text{min}$; (b) Water flow rate at the entry C: $Q_{w(C)} = 10 \mu\text{L}/\text{min}$; Oil flow rate at the entry A: $Q_{o(A)} = 95 \mu\text{L}/\text{min}$. For (c), the water phase is introduced through entry A while the oil phase is introduced by the entry C. Oil phase: castor oil + 5%_w Span 20; Aqueous phase: distilled water + 5%_w Tween 80.

Fig. 8. Direct emulsions of oil-in-water produced in an PAH/PAA LbL treated PDMS microchip. The microsystem is fully covered by 5 bilayers of PAH/PAA. Oil phase: castor oil + 5%_w Span 20; Aqueous phase: distilled water + 5%_w Tween 80 + methylene blue dye. Water flow rate at the entry A (continuous phase): $Q_{w(A)} = 20 \mu\text{L}/\text{min}$; Oil flow rate at the entry C (dispersed phase): $Q_{o(C)} = 1 \mu\text{L}/\text{min}$. The picture depicted in (a) corresponds to the position (i) in the microsystem (Fig. 3b) just after the 2nd flow-focusing junction. The picture reported in (b) is taken at the entry of the outlet reservoir and coincides with the position (ii) highlighted in Fig. 3b.

Fig. 9. Direct O/W emulsions produced in an PAH/PAA LbL treated PDMS microchip. The microsystem is fully covered by 5 bilayers of PAH/PAA. Effect of the continuous water flow

rate (Q_w) on the drop size for different oil flow rates (dispersed phase, Q_o). Oil dispersed phase: castor oil + 5%_w Span 20; Aqueous continuous phase: distilled water + 5%_w Tween 80.

Fig. 10. Long-term stability of the PAH/PAA LbL coating inside PDMS microchip. The microsystem is fully covered by 5 bilayers of PAH/PAA. Pictures of the direct O/W emulsions produced at different ageing times: (a) 1 day, (b) 3 days, (c) 7 days, (d) 10 days, (e) 15 days, (f) 30 days. Oil dispersed phase: castor oil + 5%_w Span 20 ($Q_{O(C)} = 1 \mu\text{L}/\text{min}$); Aqueous continuous phase: distilled water + 5%_w Tween 80 ($Q_{W(A)} = 20 \mu\text{L}/\text{min}$). The pictures are taken in the position (i) of the microsystem (Fig. 3b).

Fig. 11. Long-term stability of the PAH/PAA LbL coating inside PDMS microchip. The microsystem is fully covered by 5 bilayers of PAH/PAA. Size of the oil drops produced at different duration of experiment under continuous flow of oil and water inside the microchip. The continuous experiments are conducted at different ageing times. Oil dispersed phase: castor oil + 5%_w Span 20 ($Q_{O(C)} = 1 \mu\text{L}/\text{min}$); Aqueous continuous phase: distilled water + 5%_w Tween 80 ($Q_{W(A)} = 20 \mu\text{L}/\text{min}$).

Fig. 12. Emulsions formed in the microsystem for which localized PAH/PAA LbL treatment is conducted. The exit of the channel of the second flow-focusing junction is covered by 5 bilayers of PAH/PAA. (A) Inverse W/O emulsion produced at the first flow-focusing junction. The pictures are taken at the entry of the outlet reservoir and coincide with the position (ii) highlighted in Fig. 3b. (B) Direct O/W emulsion produced at the second flow-focusing junction. The picture corresponds to the position (i) in the microsystem (Fig. 3b) just after the 2nd flow-focusing junction. Oil phase: castor oil + 5%_w Span 20; Aqueous phase: distilled water + 5%_w Tween 80 + methylene blue dye.

Fig. 13. Schematic representation of the emulsification process inside the microsystem for which localized PAH/PAA LbL treatment is conducted. The exit of the channel of the second flow-focusing junction is covered by 5 bilayers of PAH/PAA. $Q_{W(C)}$, $Q_{O(B)}$, and $Q_{W(A)}$

correspond to the flow rates of the aqueous phase at the entry C, of TPGDA oil at the entry B, and the aqueous phase at the entry C, respectively.

Fig. 14. Formation of W/O/W double emulsions inside the microsystem consisting of two successive flow-focusing junctions with five PAH/PAA bilayers deposited onto the outlet part of the system. Effect of the flow rates: (a) Water: $Q_{W(C)} = 60 \mu\text{L/h}$, TPGDA: $Q_{O(B)} = 170 \mu\text{L/h}$, Water continuous phase: $Q_{W(A)} = 2000 \mu\text{L/h}$, (b) Water: $Q_{W(C)} = 100 \mu\text{L/h}$, TPGDA: $Q_{O(B)} = 200 \mu\text{L/h}$, Water continuous phase: $Q_{W(A)} = 2000 \mu\text{L/h}$, (c) Water: $Q_{W(C)} = 150 \mu\text{L/h}$, TPGDA: $Q_{O(B)} = 250 \mu\text{L/h}$, Water continuous phase : $Q_{W(A)} = 2000 \mu\text{L/h}$.