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Chitosan as material for the elaboration of lab-on-a-chip

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Abstract — Currently, the fabrication of lab-on-a-chip is dependent on polymers derived from the petrochemical industry, such as PDMS or thermoplastics. To meet the ongoing environmental challenges, we aim to introduce a bio-sourced and biodegradable polymer in the lab-on-a-chip manufacturing process. In this work, we demonstrate the feasibility of producing a microfluidic system from chitosan. Chitosan has been film deposited and neutralized to prevent its dissolution in water and limit its swelling before being micro-structured by micro-drilling. Channels from 100 μm to 1000 μm wide have been obtained. The microfluidic system was finalized by bonding the micro-structured chitosan surface to a glass slide to allow the injection of aqueous solutions in the channels.

Keywords — Chitosan, Microfluidics, Biopolymer, Micro-structuration, Lab-on-a-chip

I. INTRODUCTION

Lab-on-a-chip (LoC) are fluidic microsystems that enable the precise manipulation of fluids or micro-objects such as cells in a controlled environment. [1] The extremely small volumes and minimal sample handling have immediately drawn attention for drug development [2], food and environmental analysis [3,4], bioanalysis techniques [5] or single-cell studies [6]. The introduction of polydimethylsiloxane (PDMS), an optically transparent elastomer with low toxicity, in the LoC fabrication expanded the potential applications by simplifying the manufacturing process. [7,8] Thermoplastics such as poly(methyl methacrylate) (PMMA) or cyclic olefin copolymer (COC), which are also transparent and biocompatible, but also suitable with mass production, have also shown potential for LoC fabrication. [7] However, in medical settings or on on-field testing, LoC must be single-use to avoid cross-contamination. Thus, these different materials, through their petrochemical origin and non-degradability, contribute to the increasing amount of plastic waste. [9]

To face this societal and environmental challenge, bio-sourced materials have emerged as promising candidates for the production of LoC. A few articles reported LoC made notably from polylactic acid [10], silk [11], zein [12] and alginate [13]. Their biocompatibility is an alluring property

particularly for cell experiments. [9,14-16] However, as for now, none of these biopolymers produce microfluidic systems with the same characteristics as conventional materials in term of transparency, water tight channels or easy manufacturing. [11,17-21]

Chitosan is industrially derived from chitin, the most abundant polysaccharide in marine environment and only second to cellulose on Earth. Its production valorizes wastes from the seafood industry which amount to several million tons per year. [22] This biopolymer is biocompatible, non-toxic, biodegradable, film-forming and antimicrobial. [23-28] Thanks to its intrinsic properties, chitosan is already incorporated in applications ranging from selective coating for biosensors, scaffold for cell regeneration to food packaging and controlled drug release. [23,29-32]

Several methods have already been proven effective to microstructure the surface of chitosan films, although not for microfluidic systems. Mattotti *et al.* [23] exploited soft lithography to produce grooves of 2 and 10 μm in width and 1 μm in depth in order to compare the growth of cells in patterned or non-patterned substrate. Chitosan was patterned with 50 nm lines with e-beam lithography by Caillau *et al.* [33] to obtain a water developable bio-resist. With oxygen plasma etching, Cheng *et al.* [34] got 2 μm patterns on chitosan films. By electrodeposition, the positively charged chitosan polymer chains were attracted to the patterned surface of cathode resulting in patterns from 100 nm to 20 μm . [35] All these methods aimed to achieve the smallest resolution of patterns, whereas, for microfluidics, even if for some applications a high resolution is necessary, it is initially a question of micro-structuring channels of a few tens of μm deep and a few hundred μm wide. A major drawback of these patterning methods is the considerable swelling of chitosan films in water that could even extend to their dissolution. The swelling can be decreased with a neutralization step, although with the downside of changing the shape and mechanical properties of the film. [36]

In this paper, we introduced, to our knowledge, the first fabrication process of LoC from chitosan. The neutralization protocol has been optimized to reduce the swelling over time. We implemented micro-structuration on neutralized chitosan films. Through micro-drilling, micro-channels were produced on the surface. The microfluidic system was sealed

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by bonding, using an adhesive tape, the patterned chitosan film to a glass slide and tested for aqueous solution injection.

II. MATERIALS AND METHODS

A. Preparation of chitosan films

Chitosan (244LG, batch no. 20140503) from shrimp shells was obtained from Mahtani Chitosan Pvt. Ltd. with a weight average molecular weight of 192 kg.mol⁻¹, a dispersity of 1.85 and a degree of *N*-acetylation of 0.8%.

Chitosan was dissolved at 4% (w/v) in a 2.4% (v/v) acetic acid solution. The chitosan solution was centrifugated at 8000 rpm for 6 min to remove bubbles.

The solution was then casted into a 47 mm petri dish and let to dry overnight at 50°C. Then a second layer of chitosan solution was added into the petri dish and let for 24h at 50°C to obtain a dried chitosan film.

B. Neutralization of chitosan films

The dried chitosan films were neutralized by immersion in a sodium hydroxide solution of 0.1 M or 1 M for 10 min or 45 min. The films were then rinsed with deionized water several times until pH reaches 7. The neutralized chitosan films were finally dried overnight at 50°C. Both the immersion time and the concentration of sodium hydroxide have been studied to improve the neutralization process of chitosan films.

C. Microstructuration of chitosan films

The neutralized chitosan films were micro-patterned by drilling with a 100 μm or 200 μm diameter microdrill onto a Desktop 3-Axis micro-milling machine (Minitech Machinery Corp.) controlled by a Naknishi E3000 controller. The drilling resolution was characterized by measuring the width of channels from Olympus BX51M optical microscope with an Olympus SC50 camera. The depth of the channels was measured with a Veeco Dektak 150 Stylus profiler with a tip radius of 12.5 μm.

D. Lab-on-a-chip assembly

Once channels were engraved on the surface of the chitosan films, the microfluidic systems were sealed by adhesion onto a glass slide. A solid resin (SY310, ORDYL) was laminated on the micro-structured film at 50°C. Then, the films covered in resin were pressed to the other substrate by a hydraulic press (JAS 105 BM 6181, RONDOL Hi-Force Hydraulic tools) at 65°C and at 1 kN for 10min. Once the system was cooled below 40°C, the resin was crosslinked by UV light.

E. Swelling measurement

Dried films (non-neutralized and neutralized) were cut into 2×3 cm² pieces. Their initial weight (W_0) were determined before immersing the samples in deionized water. Swelling (S_t) was calculated from the weight measurements (W_t) after various soaking time and removing the excess of water on the film surface.

$$S_t (\%) = (W_t - W_0)/W_0 \quad (1)$$

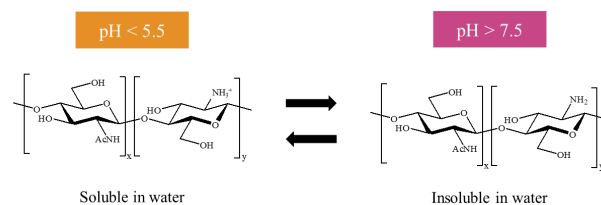


Fig. 1. Protonated and deprotonated chitosan macromolecules.

F. Thickness measurement

Thickness of chitosan films was measured after neutralization with a digital caliper (MITUTOYO).

G. Contact angle measurement

The water contact angle was measured by a PixeLINK camera with a AF Micro Nikkor 60 mm objective and the Windrop++ software. 0.8 μL deionized water droplets were examined at five different spots.

III. RESULTS AND DISCUSSION

Chitosan, linear copolymer of D-glucosamine and *N*-acetyl D-glucosamine units, is water-soluble as cationic polyelectrolyte. (Fig. 1) Thus, it is dissolved in acidic conditions in order to protonate the amino groups of the D-glucosamine units to $-NH_3^+$. [35] Chitosan films were obtained after evaporation of the solvent at 50°C. They appeared optically transparent (75% on average between 400 and 800 nm) with light yellow coloration, with a flat and homogenous surface.

To prevent dissolution of chitosan films in aqueous solutions, ammonium groups of glucosamine units have to be deprotonated to $-NH_2$. This occurs by immersing chitosan films in sodium hydroxide solution. The neutralized films maintain a similar aspect as before their neutralization, with the same transparency and coloration. We have tested two molar concentrations of sodium hydroxide solutions (0.1 M or 1 M) and two immersion times (10 min or 45 min) for neutralizing chitosan films. The swelling of neutralized films and non-neutralized ones were evaluated as a function

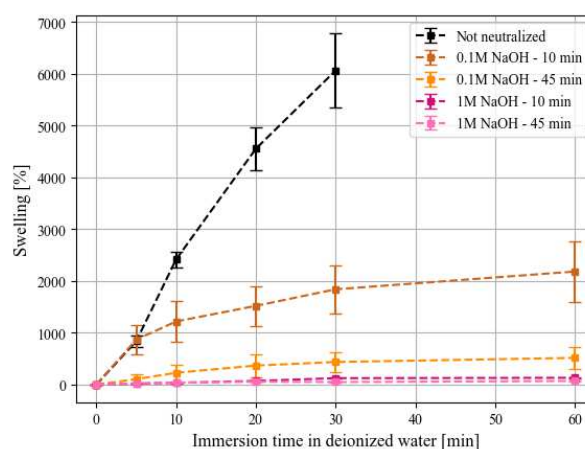


Fig. 2. Swelling of chitosan films in function of the immersion time in deionized water. For the neutralized films, the concentration of sodium hydroxide is denoted by x NaOH, followed by the immersion time in alkaline solution (y min). For each neutralization method, 3 samples were studied.

of time and compared. The results are shown in Fig. 2. As expected, non-neutralized chitosan films displayed the highest swelling rate and dissolved completely after 30 min in water. Concerning neutralized chitosan films, the concentration of sodium hydroxide and immersion time in sodium hydroxide solution impacted the behavior of the films. The swelling decreases for higher concentration of sodium hydroxide and longer immersion time. The most efficient neutralization method was obtained with 1 M sodium hydroxide for 45 min, leading to minimal swelling rate of $49\% \pm 1\%$ after 30 min in deionized water. The neutralization reaction in the 1 M sodium hydroxide takes place in the first few minutes as a 10 minutes immersion gave results close to a 45 minutes immersion. Therefore, in the following studies, chitosan films were neutralized using the 45 minutes immersion in 1 M sodium hydroxide. High hydrophobicity of neutralized chitosan films was confirmed by contact angle measurement to be $99.5^\circ \pm 2.4^\circ$. Their thickness was measured to $0.33 \text{ mm} \pm 0.04 \text{ mm}$.

Microchannels were successfully fabricated from micro-drilling. The attractiveness of this microfabrication technique is based on the flexibility of design of the microchannels. [37] As a matter of fact, it is possible to customize and vary the width and depth of the channels. As for now, we have produced channels of 200 μm depth and 250, 500 or 1000 μm width with a micro-drill of 200 μm diameter (Fig. 3) or 105 μm or 200 μm width with a 100 μm diameter micro-drill on neutralized chitosan films. The inputs and outputs to connect the channels to the outside have been drilled with the micro-drilling machine.

The micropatterned chitosan films were then bonded to a glass slide or a chitosan film, using an adhesive tape, to seal the microfluidic devices. The different steps of the adhesion process were monitored by optical microscope to detect any resin deposition in the channels. To verify the tightness of the microfluidic channels, we injected water colored with blue or red food dye into the channels (Fig. 4). The water filled the channels with a flow of $1 \text{ mL}\cdot\text{h}^{-1}$. After removing the water and rinsing the channels with ethanol, we could notice that they were slightly colored. This is consistent with the remaining swelling of chitosan film after neutralization.

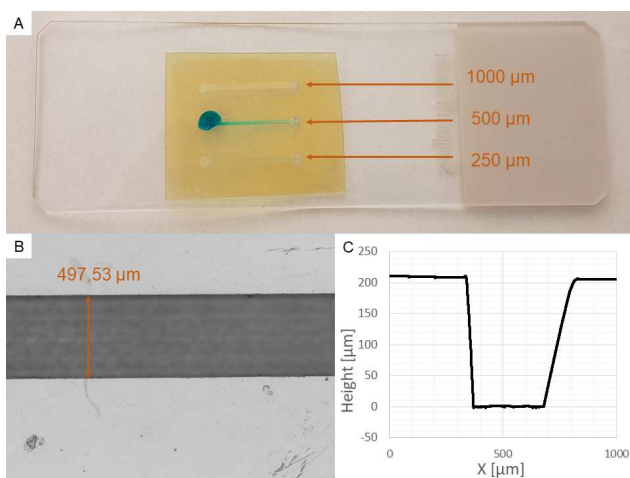


Fig. 3. A) Neutralized chitosan film. Microfluidic channels of 250, 500 and 1000 μm width and 200 μm depth with holes of 1.4 mm. – B) Photo of the top of the 500 μm wide channel taken by optical microscopy. – C) Height profile of the 500 μm wide channel determined by Dektak profiler.

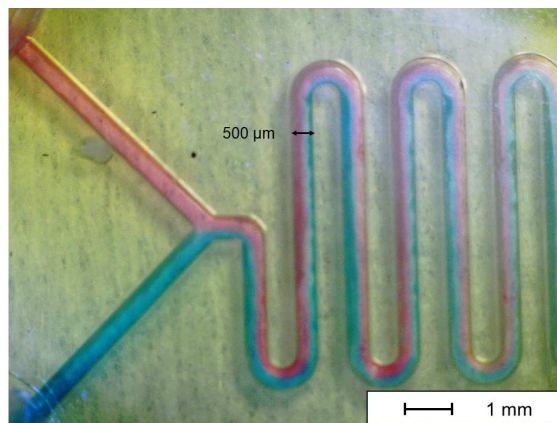


Fig. 4. Microfluidic system in chitosan with 500 μm width and 150 μm depth channels bonded to a glass slide. Red and blue food dyed water flowed at $1 \text{ mL}\cdot\text{h}^{-1}$.

As the observed swelling of chitosan stabilizes after 30 min, a channel preparation step, which could be associated with channel passivation, will probably have to be integrated in the process for using chitosan devices.

IV. CONCLUSION

In this paper, we demonstrated a novel process using chitosan, a bio-sourced and biodegradable polymer, for the fabrication of LoC. After chitosan film formation, a neutralization step was necessary to reduce the swelling of chitosan films. Microfluidic channels were patterned onto a chitosan film by micro-drilling and a first microsystem in chitosan was obtained. This proof of concept paves the way for eco-responsible LoC integrating more complex functions including sorting functions allowing to isolate cells or molecules, micro-reactors for chemical or biological reactions.

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