Effect of gelation method and microchannels structure for alginate microparticles production in microfluidics

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Abstract: The aim of this work was to investigate the effect of gelation kinetic and microchannels structure for the production of alginate microparticles in microfluidics. The generation of alginate microparticles in microfluidics leads to the generation of small particles and narrow distribution size, which can be employed for cell encapsulation with application in biological areas. At this way, we explored the alginate formation using methods with fast and slow gelation kinetics. From experimental data, we noticed that faster gelation can affect the microparticles formation in microchannels. Moreover, microchannels configuration has also influenced in the uniform microparticles production. Then, we constructed a T-junction device that generated homogeneous droplets. From this, it was possible to produce alginate microparticles with diameter of 63.53 ± 1.77 μm and a coefficient variation below 3 %, which indicate the microparticles monodispersity. Therefore, the use of the slow gelation in this microfluidic system was efficient for alginate microparticles production.

Key-Words*: droplet microfluidics, alginate, microparticles, gelation kinetic*

Introduction: The generation of homogeneous alginate microparticles is influenced by gelation process. In microfluidics, the gelation kinetic has great importance since the gelation can affect the formation of alginate microparticles inside of microfluidic system [1], [2]. The alginate hydrogel is due to crosslinking of a divalent cation to make the bond to the carboxylic group. This crosslinking process can occur via by external or internal gelation methods. By external method, an active ion makes an instantaneous alginate gelation, while by internal; the crosslinking is slow because of an inactive ion that is released gradually into the polymeric matrix [3]. Based on this, we evaluated the alginate microparticles production using a T-junction microfluidic system and compared the gelation alginate by the two methods: external and internal methods, taking into account that the gelation kinetic.

Experimental: The droplet microfluidic system was constructed with glass-PDMS by soft lithography technique [4]. The gelation of alginate in microfluidics was evaluated regarding the microparticles struture. The gelation process via external method, it was used solutions of sodium alginate at 2 % (w/v) (Sigma-Aldrich, USA), distillated water and calcium chloride at 30 mM (Sigma-Aldrich, USA) at flow currents of dispersed phase using a flow rate of 0.4 – 1 μL min^{-1} (Figure 1a). The production of alginate via internal gelation, we used the complex ligand exchange crosslinking ion (CLEX) method, described by BASSETT et al. (2016)[1], using flow rate for dispersed phase between 0.4 and 1.5 μL min^{-1} (Figure 1c). For the continuous phase, we used in both methods, HFE 7500 oil (3M, USA) with 1% (v/v) fluorosurfactant (Ran Biotechnologies, USA). The flow rate of continuous phase were between 4 and 10 μL min^{-1} for these experiments. The production of alginate microparticles by cited methods were evaluated at microchannles nozzles and after the emulsion breaking, using perfluorooctanol (PFO) 20% (v/v) (Alfa Aesar, USA), and after that, the alginate structure was observed on confocal microscope (Leica TCS SP5, USA).

Results and discussion: We evaluated the alginate microparticles production using a T-junction microfluidic system and compared the gelation alginate by the external and internal methods. From the experimental data, we noticed that the faster the alginate gelation, less uniform is the alginate microparticles.

As observed in Figure 1a-b, the alginate microparticles produced by external gelation showed an irregular shape. Also it was possible to verify some alginate particles presented an elongate shape, which are similar to design channel and non-uniform size (Figure 1b). The non-uniform microparticles can be caused by the fast gelation kinetic, since there is a direct crosslinking by active Ca^{2+} ion that diffusing to the interior of

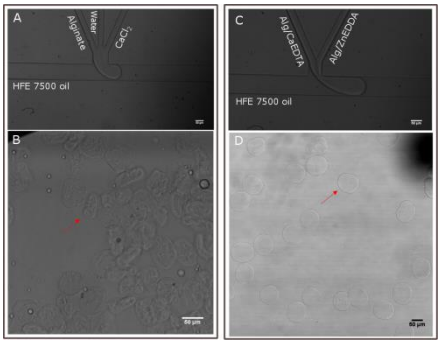

Figure 1: Alginate microparticles produced using T-junction device by: a-b) external gelation method; c-d) internal gelation method.

polymeric matrix until to get the complete gelation [3]. Furthermore, the contact between alginate and $CaCl_2$ solutions at the nozzle of microchannels, even with a central flow of water, it favored the gel formation before the droplets breaking and caused a clog of channels in microfluidics (as observed in Figure 1b).

For the alginate microparticles by internal method (Figure 1c), it was produced alginate microparticles more homogeneous and rounded shape (Figure 1d). In this case, the alginate microparticles presented uniformity due to the exchange crosslinking of ions between Ca-EDTA and Zn-EDDA that releasing the Ca ion into the alginate matrix [5]. As observed in Figure 1b-d, because of the slower gelation kinetic, the structure of alginate gels is more homogenous than external gelation method [3].

Despite of homogeneous alginate microparticle production by CLEX method, the structure of microchannels could be disturbing the spherical shape of microparticles, as is possible to see the shape of microparticles in Figure 1b-d. At this way, we constructed a new T-junction geometry with fluid filters and fluid resistor to improve the fluid distribution in order to improve the fluid distribution [6]. The fluid resistor structures in the microchannels avoided any flow fluctuation caused by the syringe pump and the filters was important to prevent the impurity entrance inside of microchannels and produce homogeneous alginate microparticles (Figure 2a-c).

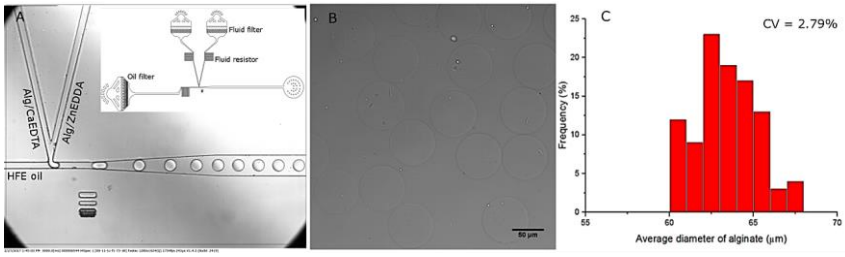

Figure 2: Alginate microparticles produced by CLEX method using new T-junction geometry: a) new T-junction geometry and droplet generation; b) alginate microparticles produced in new T-junction geometry; c) profile of size distribution of microparticles using 10 μL/min for continuous phase and 1 μL/min for dispersed phase.

The new T-junction geometry generated more spherical and uniform alginate microparticles using the CLEX method (Figure 2a). For assay using flow rates of 10 μL min^{-1} for continuous phase and 1 μL min^{-1} for dispersed phase, it was obtained homogeneous alginate microparticles (Figure 2b). The alginate microparticles presented an average diameter of 63.53 ± 1.77 μm with coefficient variation (CV) of 2.79 % that indicated a homogeneous size distribution of microparticles produced by this T-junction microfluidic system (Figure 2c). Thus, this new T-junction microfluidic device showed efficiency for droplet generation and consequently, for production of alginate microparticles by internal gelation method.

Conclusion: The gelation kinetics of alginate is extremely relevant when it is generated inside of microfluidic microchannels [1]. The controlled gelation by internal method generated alginate microparticles more uniform and the microchannels structure allowed the production of spherical and homogeneous microparticles. This methodology to produced alginate in microfluidics may be applied to the development of protocol for cell encapsulation in alginate microparticles.

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References

[1] D. C. Bassett, A. G. Håti, T. B. Melø, B. T. Stokke, and P. Sikorski, J. Mater. Chem. B, **4**, 37 (2016).
[2] H. Hirama, T. Kambe, K. Aketagawa, T. Ota, H. Moriguchi, and T. Torii, Langmuir, **29,** 2 (2013).
[3] I. Donati and S. Paoletti, in Alginates : Biology and Applications, Germany: Springer US (2009)
[4] J. C. Mcdonald and G. M. Whitesides, Acc. Chem. Res., **35**, 7 (2002).
[5] T. Braschler, R. Johann, M. Heule, L. Metref, and P. Renaud, Lab Chip, **5**, 5 (2005).
[6] L. Mazutis, J. Gilbert, W. L. Ung, D. A. Weitz, A. D. Griffiths, and J. A. Heyman, Nat. Protoc., **8**, 5 (2013).