HYDRODYNAMIC FLOW FOCUSING MICROFLUIDIC CONFIGURATION FOR CHITOSAN NANOPARTICLES SYNTHESIS

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Abstract: *Microfluidic platforms enable the development of low-cost, feasible processes for nanoparticles synthesis with adequate properties for nanomedicine applications, including drug and gene delivery. This work describes a simple and reproducible microfluidic configuration to modulate the synthesis of chitosan (CHI) nanoparticles through exploiting molecular diffusion. We used a polydimethylsiloxane (PDMS)/glass hydrodynamic flow focusing device, applying a central water stream and side streams of CHI and adenosine triphosphate (ATP). This system resulted in CHI/ATP nanoparticles with around 100 nm, rounded shape and low polydispersity. Further, we investigated the effects of the system flow rate ratio (FRR, on nanoparticles synthesis and their resultant physicochemical characteristics. The proposed system represents an interesting strategy to contribute to a better understanding about the microfluidic production of CHI/ATP nanoparticles to be applied in the most varied research fields.*

Key-Words*: microfluidics; chitosan; nanoparticles*

Introduction: Chitosan (CHI) nanoparticles have been widely investigated in various research areas. This cationic polysaccharide is well-known for advantageous features such as low toxicity, biocompatibility, biodegradability and mucoadhesivity, which allow for ample pharmaceutical and biomedical applications, including wound healing, tissue engineering, and drug and gene delivery [1,2]. As a result of its low immunogenicity and adaptable functionality, CHI has been widely explored as non-viral nanoparticulate vectors for drug and gene delivery [3]. Microfluidic devices have remarkable features that contribute to amplify the monitoring and control over the production process of nanoparticles. Benefiting from microscale, microfluidics explores the manipulation of small amounts of fluids along with the development of unique hydrodynamic properties, such as laminar flow regime and minimized heat and mass transfer resistances [4]. As a result, microfluidics emerges as a resourceful tool for nanoparticles production. This work aims to investigate the synthesis of low molecular weight CHI nanoparticles through ionic gelation with adenosine triphosphate (ATP) within hydrodynamic focusing microfluidic plarforms. We assessed different microfluidic process configurations to explore distinct diffusion paths, and the effects of the introduction of a central aqueous stream on the microfluidic synthesis of CHI/ATP nanoparticles.

Experimental: We used a polydimethylsiloxane (PDMS)/glass hydrodynamic flow focusing microfluidic device for the production of CHI/ATP nanoparticles, with dimensions of 140 µm width and 50 µm height. The synthesis was carried out maintaining some standard output parameters: total flow rate (Q_T) of 90 µL/min, CHI/ATP mass ratio ($R_{CHI/ATP}$) of 0.5 and final CHI concentration (C_{fCHI}) of 0.14 mg/mL. We assessed two microfluidic approaches denominated: **(i)** Regular configuration, with a central CHI stream focused by ATP streams (Fig. 1A), and **(ii)** Central Aqueous Stream (CAS) configuration with a central inlet stream of acidified water and two side streams composed by CHI and ATP (Fig. 1B). All solutions were prepared in pH 4 taking into account CHI's extended conformation and solubility in this pH [5].

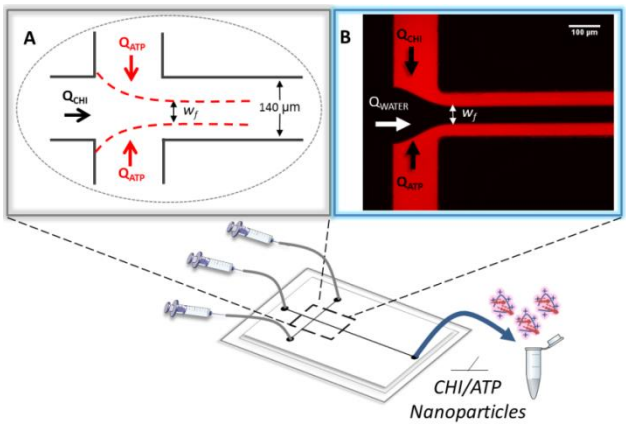

Fig 1. Schematic diagram of (A) Regular and (B) Central Aqueous Stream configurations for CHI/ATP nanoparticles synthesis.

The samples were measured by Dynamic Light Scattering (DLS) and laser Doppler anemometry techniques using a Zetasizer Nano ZS (Malvern Instruments Ltd.).

Results and discussion: When applying the Regular configuration (Fig. 2A-B), we detected, after a few minutes of processing, the generation of microfiber-like structures that extended throughout the entire microchannel length over time. This polymeric microstructures generation may hinder not only the control over the resultant output process conditions, such as the final $R_{CHI/ATP}$ for the synthesized CHI/ATP nanoparticles, but also the process reproducibility [6]. On the other hand, the CAS microfluidic process configuration with a central water stream was developed to explore the gradual transverse diffusion of CHI and ATP molecules across the aqueous stream along spatial time. As the flow continues, the molecular diffusion process provides balanced local concentrations of the molecules within the microchannels and, as a result, the contact and the ionic gelation process between CHI and ATP is gradual, favoring predominantly the synthesis of CHI/ATP nanoparticles (Fig. 2C-D). This process resulted in CHI/ATP nanoparticles with 85 ± 15 nm of particle size, 0.10 ± 0.02 of PDI and +13.2 ± 1.4 mV of zeta potential [6].

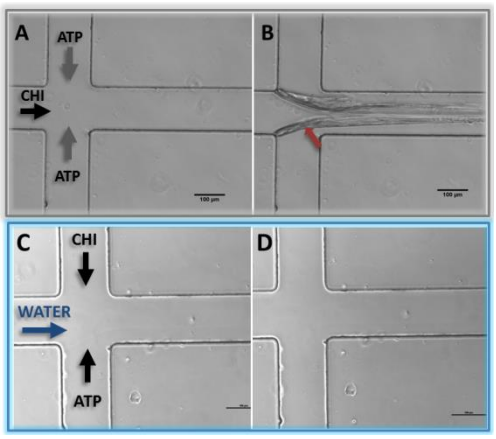

Fig. 2. CHI/ATP nanoparticles synthesis applying (A-B) Regular and (C-D) CAS configurations. (B) Microfiber-like structures formed after 15 min. (D) Absence of microstructures accumulation after 10 min.

Depending on the ratio of the volumetric flow rates of the side streams to the central stream, the width of the focused central water stream (w_f) in the outlet microchannel would be altered, leading to the immediate encounter between CHI and ATP and the formation of microstructures. To confirm this hypothesis, we assessed the effects of reducing the central water stream flow rate (Q_{WATER}), increasing the Flow Rate Ratio (FRR), while adjusting the concentrations of CHI and ATP inlet solutions to maintain the same output standard parameters previously stated. In this case, FRR is defined as the ratio of the sum of the volumetric flow rates of the side streams (Q_{CHI} + Q_{ATP}) to the volumetric flow rate of the central stream (Q_{WATER}) [6].

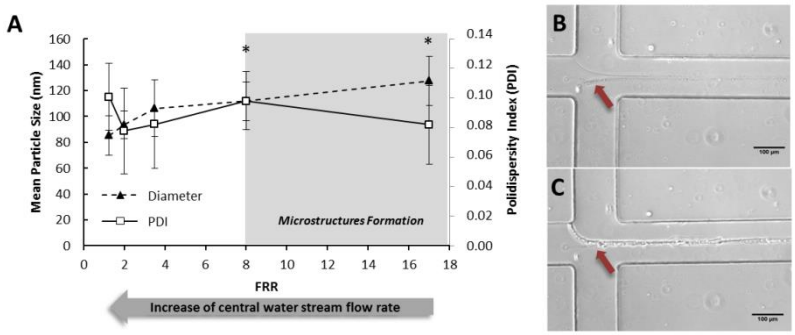

Fig. 3. (A) Effects of Flow Rate Ratio (FRR) on CHI/ATP nanoparticles hydrodynamic diameter and polydispersity index (PDI). Microstructures formation for FRR of (B) 8 and (C) 17.

Differences on size were observed for the conditions of FRR above 8 (Fig. 3A, shaded area), in which $Q_{WATER} \leq 10$ µL/min. These circumstances led to the formation of microstructures within the channels (Fig. 3B-C) that may be justified by the immediate encounter of CHI and ATP molecules at the streams frontier, thus generating regions that favor an unbalanced molar charge condition for electrostatic interaction. This study highlighted the important role of the central water stream in avoiding the formation and retention of microstructures by slowing down the diffusion process, reflecting on the encounter between CHI and ATP molecules for ionic gelation [6].

Conclusion: These findings elucidated that in microfluidic platforms appropriate diffusive barriers (represented by the central water stream) can modulate the electrostatic association between CHI and the crosslinking agent to form nanoparticles, avoiding the formation of microfiber-like structures. This strategy enables the synthesis of CHI nanoparticles in a simple and reproducible microfluidic approach to be applied in various research areas.

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