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Self-assembled polysulfone nanoparticles using microfluidic chip

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ABSTRACT

This article reports a microfluidics-based method for the preparation of polysulfone nanoparticles. Upon mixing polysulfone in a good solvent with pure water, polysulfone undergoes a coil-globule transition and forms nanometer-sized soft particles. A key to achieving small nanoparticle sizes is to change the solvent quality within a time frame shorter than the typical time of aggregation between polysulfone. Accordingly, we devised microfluidic devices relying on hydrodynamic flow focusing and we managed to obtain nanoparticle sizes below 100 nm, which is a significant improvement compared to latest nanoprecipitation methods We demonstrated the continuous encapsulation of FITC dyes into polysulfone nanoparticles as well and we envision the use of these biocompatible fluorescent polysulfone nanoparticles for biological imaging.

1. Introduction

Technology has evolved to the stage where it is now possible to manipulate the basic subunits of matter, i.e., molecules, in order to form elaborate structures at the nanoscale [1]. Self-assembly is an efficient route in terms of accuracy and throughput to produce supramolecular and nanometer-scaled systems. The nature of the noncovalent driving forces is twofold: either weak (H bond, hydrophobicity, entropic effects) or strong (electrostatics, van der Waals forces) [2]. Strong interactions generally lead to far-from-equilibrium assembly with kinetic traps and metastable states where the system exhibits many structural defects and uncontrolled properties [3]. Even though the equilibrium state is the most stable by definition, it is not necessarily the one that confers the most attractive properties to the system and it can require a lot of time or energy to be reached. For a number of applications, the relaxation time of the system in a nonequilibrium state is long enough compared to the typical timescale for practical use and the system is then kinetically frozen. In that case, it can be advantageous to guide the system towards a far-from-equilibrium state with desired features such as narrow size distribution.

Polymeric nanoparticles have been exploited for many purposes such as drug delivery [4,5], biomedical diagnosis [6], and heterogeneous catalysis [7], to name a few. They are synthesized either by polymerization or by dispersion of the polymers. Polymerization is a tedious process, which requires to control accurately the conditions of synthesis and to remove residual monomers as well as catalysis agents. Dispersion of the polymers is an attractive alternative, in particular, via nanoprecipitation. This route exploits the precipitation of polymer chains resulting from the change of solvent quality. The particles are thus formed through a nucleation-growth process and their final size distribution depends upon the kinetics of solvent exchange. In a seminal study, Johnson and Prud'homme [8] demonstrated that the size of diblock copolymer nanoparticles was minimal when the time for mixing copolymers with a poor solvent was shorter than their typical aggregation time. The fine tuning of the mixing time can be achieved by confined impinging jets mixer [8] or by using microfluidics-based strategies [9–16]. In the latter case, hydrodynamic flow focusing revealed itself to be a good approach for rapid, controllable and mild mixing. It was successfully used for the nanoprecipitation of block copolymers and lipids [17,18] as well as by us for the directed self-assembly of polyelectrolyte complexes [19–23].

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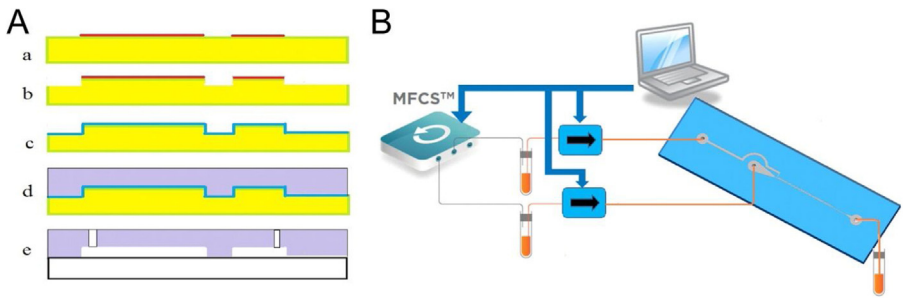

Fig. 1. Fabrication process of the device and experimental setup. (A) Main steps of the fabrication process: (a) photolithographic process, (b) 50-μm-deep Si etch using DRIE, (c) coating of the Si mold, (d) PDMS casting, (e) PDMS/glass bonding. (B) Schematics depicting the microfluidic device with one inlet for polysulfone in NMP and another one for pure water. The outlet contains the nanoparticles. The device is connected to a MFCS pumping system controlled by computer.

Here, we developed a microfluidic device based on hydrodynamic flow focusing for the nanoprecipitation of polysulfone nanoparticles. Polysulfone is a thermoplastic polymer with excellent thermal, mechanical and chemical properties. It has been widely used as a biomaterial, notably for orthopaedic implants, and more recently, in filtration membranes for bioartificial kidney [24] or as optical probe for second harmonic generation microscopy [25]. Due to its high biocompatibility, polysulfone is an ideal material for polymeric nanoparticles in biological imaging or labelling. Nanoprecipitation is a method of choice to produce submicrometer-scaled polysulfone particles. Latest methodologies using metal membrane contactor [26] achieved nanoparticle sizes comprised between 200 nm and 400 nm, and provided a high throughput with a set of equipment easy to scale up for mass production. Here, we advanced a step further by obtaining nanoparticle sizes below 100 nm, which is more suitable for intracellular imaging, and we demonstrated the possibility to encapsulate fluorescent dyes simultaneously with continuous nanoprecipitation.

2. Materials and methods

2.1. Chemicals

Polysulfone (average molecular weight ~22 kDa) was purchased from Sigma-Aldrich without further treatment. It was dissolved in *N*-methylpyrrolidone (NMP) and stored at room temperature. 4′,6-diamidino-2-phenylindole (DAPI; Ex/Em = 358/461 nm) and fluorescein isothiocyanate (FITC; Ex/Em = 494/512 nm) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Device fabrication

The device was fabricated using soft lithography. The main steps of the fabrication process are depicted in Fig. 1A. First, a mask with the microfluidic channels was transferred on a 4" silicon (Si) wafer using a classical photolithographic process, which involved positive resist AZ4620. In the next step, a 50-μm-deep etching process was performed through the photoresist mask using a classical Bosch process in a deep reactive ion etching (DRIE) reactor. After removal of the photoresist mask in NMP, a double layer of SiC (obtained by plasma-enhanced chemical vapor deposition) [27] and Teflon was used for an easy demolding. The method was successfully used for plastic demolding from Si stamp [28]. The classical PDMS casting was performed according to [29]. The inlet/outlet holes were punched, and the casted PDMS was separated in individual devices, which were bonded onto the glass slide after a 30-s activation of PDMS surface in O_2 plasma.

2.3. Nanoparticle assembly

The experimental setup is shown on Fig. 1B. The nanoparticle assembly was driven by a MFCS-FLEX pumping system (Fluigent, France) connected to the microfluidic device. The system comprised mass flow controllers for the two inlets enabling to fine-tune the applied pressure and subsequently the flow rate of each stream individually. The flow rate of the polysulfone stream was between 5 and 10 μL/min while the flow rate of the water stream ranged from 20 to 60 μL/min. The microfluidic device was flushed with NMP then deionized water for a few minutes for complete removal of polysulfone and contaminants. Clogging of the device could appear for large size nanoparticles (over 300 nm). However, for bioimaging application the targeted size of nanoparticle is below 200 nm, large size nanoparticle could be easily obtained using classical methods [26]. We experienced some precipitation problems until we figured out suitable concentrations of polysulfone.

For FITC-loaded polysulfone nanoparticles, FITC was first dissolved in anhydrous DMSO (Sigma-Aldrich) at 1 mg/mL and further diluted in pure water at 50 μg/mL as the side stream reagent for one inlet; polysulfone was dissolved in NMP at 10 between two side streams containing fluorescent dyes. The central polysulfone stream was then hydrodynamically focused nanoparticles were dialyzed against Williams' medium E in order to remove free dyes. The nanoparticles were then incubated with HaCaT cells [30] for 4 h and imaged by fluorescence microscopy.

2.4. Nanoparticle characterizations

The hydrodynamic diameter of the polysulfone nanoparticles was measured by dynamic light scattering (DLS) with a Nano ZS-90 instrument (Malvern Instruments) at a backscattering angle of 173° and the temperature was maintained at 25 °C. Hydrodynamic diameter and polydispersity index were deduced from the cumulants method, and all the measurements were performed in triplicate.

Polysulfone nanoparticles were imaged by scanning electron microscopy (SEM, JSM-7400, JEOL) at 10 kV and 10 mA.

3. Results

Polysulfone was initially dissolved in NMP, which is a good solvent, and accordingly, polysulfone adopted a coil conformation. The quality of the solvent was rapidly changed by hydrodynamic flow focusing [17,19–22]. Briefly, a stream of polysulfone in NMP was sandwiched between two lateral streams of pure water. As a result, the stream of polysulfone was pinched and its width was reduced to a few micrometers depending on the flow rate ratio, while the channel was 100-μm wide (see inset of Fig. 2A). Water then diffused across a thin layer of polysulfone solution triggering a coil-globule transition. A rough estimate of the mixing time τ_{mix} can