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High-Throughput Fabrication of Nanocomplexes Using 3D-Printed Micromixers

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ABSTRACT

3D printing allows a rapid and inexpensive manufacturing of custom made and prototype devices. Micromixers are used for rapid and controlled production of nanoparticles intended for therapeutic delivery. In this study, we demonstrate the fabrication of micromixers using computational design and 3D printing, which enable a continuous and industrial scale production of nanocomplexes formed by electrostatic complexation, using the polymers poly(diallyldimethylammonium chloride) and poly(sodium 4-styrenesulfonate). Several parameters including polymer concentration, flow rate, and flow ratio were systematically varied and their effect on the properties of nanocomplexes was studied and compared with nanocomplexes prepared by bulk mixing. Particles fabricated using this cost effective device were equally small and homogenous but more consistent and controllable in size compared with those prepared manually via bulk mixing. Moreover, each micromixer could process more than 2 liters per hour with unaffected performance and the setup could easily be scaled-up by aligning several micromixers in parallel. This demonstrates that 3D printing can be used to prepare disposable high-throughput micromixers for production of therapeutic nanoparticles.

Introduction

Advances in nanomedicine in the last decades have resulted in an unmatched opportunity to improve the treatment of many diseases. Nanomedicine is the design and development of therapies and diagnostic tools distinguished by the nanoscale of its delivery vehicles, typically drug-loaded colloids of diameter less than 200 nm.[1,2] Such nanomaterials have the ability to encapsulate drugs and potentially release them in a controlled manner at the disease site. Thus, they result in an improved pharmacokinetic profile and facilitate transport across biological barriers such as in the mucosal membrane of the gastrointestinal tract or the lungs.[3,4]

Most therapeutic nanoparticles are composed of lipids or polymers and are prepared via self-assembly processes including nanoprecipitation and emulsification-solvent diffusion. These are generally labor-intensive and time-consuming methods, which are noncontinuous and not compatible with a commercial production setting.[5,6] The need for more scalable and controllable nanoparticle manufacturing processes is evident from the literature as well as statements from the industry.[7-9] The most recent development in the preparation of nanoparticles involves the application of microfluidics, where microscale channels are used to manipulate small volumes of fluids and perform well-defined mixing.[10-12] Microfluidic mixers provide a continuous process with better control, improved functionalization, and higher reproducibility compared with conventional manual preparation methods.[13]

Recent microfluidics chip and micromixer designs also demonstrate that high-throughput production of nanoparticles can be achieved using single micromixers and this can be further optimized or scaled up by combining identical devices in parallel.[14]

Abbreviations used: 3D, three-dimensional; PDDA, poly(diallyldimethylammonium chloride); PDI, polydispersity index; PDMS, poly(dimethylsiloxane); PSS, poly(sodium 4-styrenesulfonate).

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All authors have contributed to the conception and design of the study and acquisition, analysis, and interpretation of data. All authors have drafted the article and revised it critically for important intellectual content. All authors have approved the final article.

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A key driver to increase the commercial application of nanoparticle-based products is the development of inexpensive fabrication processes by taking advantage of microfluidics technology. The scalability of these methods is essential for producing large quantities of nanoparticles necessary for a regular therapeutic treatment.[15,16] Future manufacturing lines should also include *in silico* design of a given pharmaceutical product.[17]

The present study designed micromixers allowing for achieving flow patterns with high Reynolds numbers (i.e., efficient mixing) using 3D printing. Different process parameters with influence on the characteristics of the nanocomplexes were investigated via numerical simulation of the mixing and formation process using a finite element platform combined with experimental studies. The nanocomplexes synthesized by micromixing were compared with those prepared by manual bulk mixing as well as those prepared using another microfluidics device. The micromixer was further used for a high-throughput production of nanocomplexes alone and in a parallelized setup of several micromixer devices.

Materials and Methods

Materials

Poly(diallyldimethylammonium chloride) (PDDA; $M_w = 200$-350 kDa) and poly(sodium 4-styrenesulfonate) (PSS; M_w ~1000 kDa) were acquired from Sigma-Aldrich (Poole, UK). Polylactide feedstock tailored for 3D printing was purchased from Makerbot (Makerbot, New York, NY) and used for printing of the micromixers. The feedstock is supplied as extruded strand (diameter, 1.75 mm) and is based on polylactide resin (semi-crystalline, transparent general-purpose film grade, $T_g = 60$-65°C, $T_m = 145$-160°C, and a density of 1.24 g/cm^3). All other chemicals and solvents were of analytical grade and used without further purification.

3D Printing of Micromixers

A 3-inlet micromixer was designed using Comsol Multiphysics software version 4.4 (Stockholm, Sweden) and exported as a .stl file. These were subsequently loaded into the MakerWare software version 2.4.1 (Makerbot) and 3D printed using Makerbot Industries, MakerBot Replicator 2 (Makerbot) at the highest resolution, using a 0.1-mm layer height.

Bulk Mixing

PDDA-PSS nanocomplexes were prepared via bulk mixing at different concentration and volume ratios (Table 1). Briefly, PDDA and PSS solutions were prepared in water at different concentrations and the PDDA solution was pipetted into the PSS solution under vortexing, at different volume ratios.

Table 1
Nanocomplex Production Yield (grams per hour) as a Function of the Polymer Concentration and Total Flow Rate

Polymer Concentration (mg/mL)	Total Flow Rate (mL/min)			
	6	12	24	48
0.5	0.18	0.36	0.72	1.44
1	0.36	0.72	1.44	2.88
2	0.72	1.44	2.88	5.66
4	1.44	2.88	5.66	11.32

The calculated values were verified experimentally by determining the recovery of nanocomplexes by measuring the mass of lyophilized nanosuspensions.

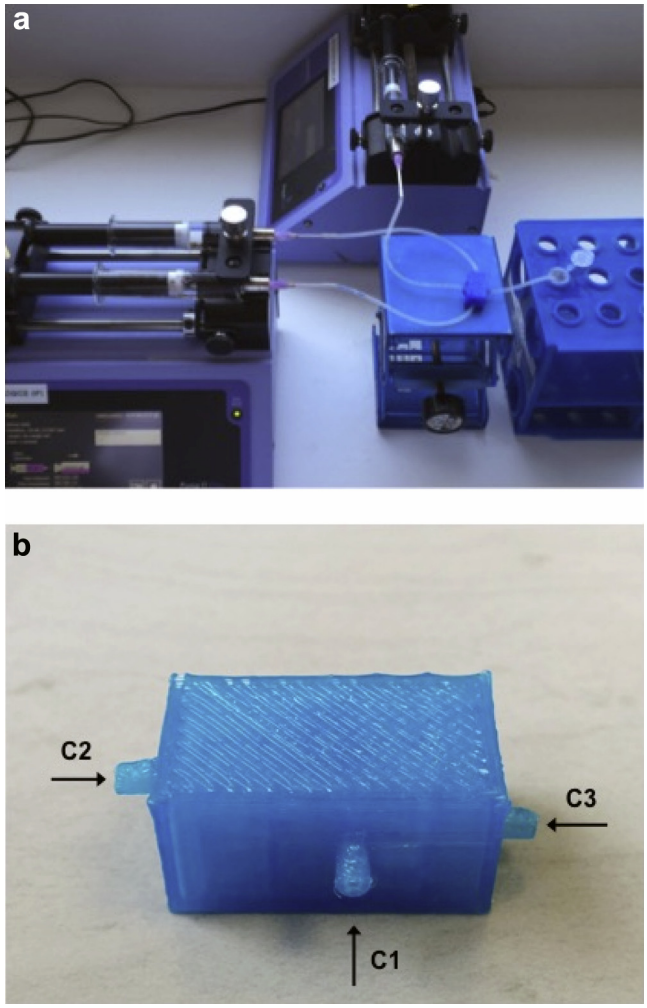

Figure 1. Photographs of the micromixing setup (a and b).

Simulation of Microfluidic Mixing

Simulation of flow patterns in the micromixer was modeled using the finite element method. COMSOL multiphysics was used to construct the inner geometry of the micromixer, and boundary conditions in form of the fluid velocities of the inlets were applied alongside the fluid outlet boundary condition of zero pressure.

$$\text{Fluid inlet} \quad u = u_0 \tag{1}$$

$$\text{Fluid outlet} \quad P_0 = 0 \tag{2}$$

Here, u_0 is the velocity of the inlet flow (m/s). u is the velocity of the flow at a given position in the mixer cell (m/s), and P_0 is the fluid outlet pressure (Pa).

The partial differential equations were solved in a mesh consisting of 87,448 elements using the direct PARDISO (parallel direct solver) solver. The flow is essentially coupled to the mass balance equation

$$\text{Mass balance equation} \quad p\nabla \cdot u = 0 \tag{3}$$

where p is the density of the solvent (kg/m^3) and governed by the Navier-Stokes equation