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Preparation of Monodisperse Block Copolymer Vesicles via Flow Focusing in Microfluidics

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We demonstrate that microfluidic flow devices enable a rapid, continuous, well-reproducible and size-controlled preparation of unilamellar block copolymer vesicles. The PDMS-based microfluidic device consists of perpendicularly crossed channels allowing hydrodynamic flow focusing of an ethanolic block copolymer solution in a stream of water. By altering the flow rate ratio in the water and ethanolic inlet channels, the vesicle size can be tuned over a large size range from 40 nm to 2 μm without subsequent processing steps manipulating size and shell characteristics. The ability of tuning the vesicle mean size over a range of several orders of magnitude with the possibility of *in situ* encapsulation of active ingredients creates new opportunities for the preparation of tailored drug delivery systems in science, medicine and industry.

1. Introduction

Amphiphilic molecules such as lipids and surfactants are able to self-assemble and form vesicles.[1] Applications of lipid vesicles or "liposomes" as model systems for biomembranes as well as in the area of cosmetics and pharmaceutics have been limited due to their insufficient stability and occasionally unregulated release of encapsulated active agents.[2] On this account, block copolymer vesicles or "polymersomes" have attracted increasing interest based on their excellent stability and the potential to control biological, chemical and physical properties by tailoring block lengths, block chemistry and functionalization.[3−6]

Experiments have shown that for drug delivery applications the diameter of polymersomes should range from 50−150 nm to ensure an optimal intake in cells and preserve the cell viability.[7] However, none of the classical vesicle-formation techniques such as film rehydration, electroformation, homogenization, phase transfer, or ultrasonication[8] enables vesicle formation and encapsulation with predefined vesicle diameters in this size range with the possibility of simultaneous *in situ* encapsulation.

Recently, it has been demonstrated that modified inkjet printers allow the preparation and in situ loading of lipid vesicles in the size-range of 50−200 nm.[9,10] However, the capability for modification of inkjet devices is limited, and their usage is restricted to certain solvent systems.

A promising alternative approach for the preparation of polymersomes providing a high degree of flexibility are microfluidic devices fabricated by polydimethylsiloxane (PDMS) based soft lithography. PDMS based soft lithography has developed to the most significant fabrication method for microfluidic flow devices in recent years.[11−16] It allows the fabrication of high quality devices in short time entailing only small manufacturing costs. Moreover, PDMS based channel dimensions in microfluidic devices are adjustable in a wide range from less than 10 nm to several hundred micrometers, providing an environment where reproducible self-assembly processes and nanometer-scale synthesis are well controllable.[17] The combination of diffusion-based mixing and the capability to load vesicles during the formation process *in situ* with active agents has led to very innovative applications of microfluidic devices. This includes the preparation, surface modification and efficient filling of lipid vesicles with active agents[18−22] or the usage of double emulsions as templates to direct vesicular assembly and allowing in situ encapsulation in giant polymersomes.[23−25]

Herein, we report the capability of hydrodynamic flow focusing in microfluidics to exert size control over the spontaneous

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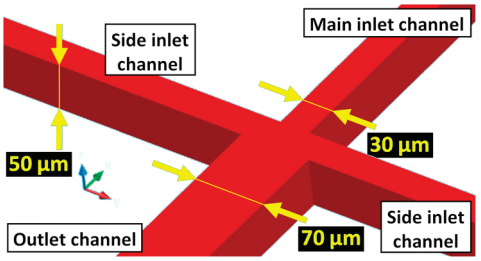

Figure 1. Cross section of the AutoCAD-based microchannel design used for the preparation of block copolymer vesicles via flow-focusing.

self-assembly of unilamellar poly-2-vinylpyridine-*b*-poly(ethylene oxide), P2VP−PEO, vesicles. P2VP−PEO is an extensively studied vesicle-forming amphiphile. While the polybase poly-2-vinylpyridine exhibits a pH-dependent solubility, the PEO-blocks solubility is temperature dependent.[9,26] The amphiphile has been chosen because of its good solubility in ethanol, in which PDMS exhibits a low swelling ratio *S*.[27]

2. Experimental Section

2.1. Materials. Poly-2-vinylpyridine-*b*-poly(ethylene oxide) ($P2VP_{47}-PEO_{29}$, mean M_w 6400, 21 wt % PEO) was synthesized by sequential living anionic polymerization, yielding a block copolymer with narrow polydispersity in molecular weight of $M_w/M_n = 1.06$, where M_w and M_n are the weight- and number-averaged molecular masses. The synthesis and characterization of P2VP−PEO is described in detail elsewhere.[26,28] The dry polymer is stored in the freezer at −32 °C before use.

2.2. Fabrication of Microfluidic Devices. The device consists of two perpendicular crossed channels which have a depth of 50 μm. The side channels as well as the main channel section leading to the intersection have a width of 30 μm (cf. Figure 1). Not shown is the meander-shaped channel leading away from the intersection which has a width of 70 μm. As pumps, three Nemesys units from CETONI GmbH, Korbussen, Germany, were used.

2.3. Vesicle Preparation. Depending on the experimental requirements, $P2VP_{47}-PEO_{29}$ is dissolved in ethanol (0.05−0.1 wt %), filtered through a 0.2 μm PTFE filter, and injected into the main channel. Millipore-quality water is injected into the side channels and hydrodynamically focuses the polymer stream. The vesicle solution is directly collected in microcuvettes with a minimum volume of 40 μL.

2.4. Vesicle Characterization. Dynamic light scattering (DLS) is performed on a Zetasizer Nano ZS from Malvern Instruments Ltd., U.K., at $\lambda = 632$ nm with a scattering angle of 173° (noninvasive back scatter technology). Cryo-transmission electron microscopy (cryo-TEM) is carried out on a TEM LEO912 electron microscope from Zeiss, Oberkochen. Confocal laser scanning microscopy (CLSM) was performed on an Olympus FluoView 1000.

2.5. FEM Simulations. In order to adapt the AutoCAD based channel structure to experimental parameters, simulations based on the finite element method (FEM) were performed, which are well-suited for the understanding of the hydrodynamics present during the polymersome formation process as well as to quantify the influence of viscosity effects (*simulation-based rapid prototyping*).[29] We utilize COMSOL 3.5 applying 20346 finite elements for 3D simulations and 117146 for 2D simulations.

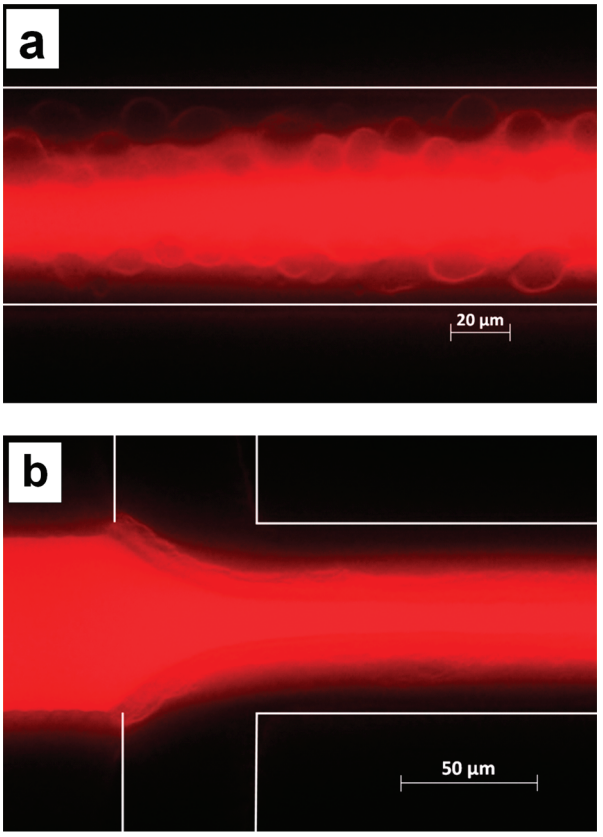

Figure 2. Fluorescence images of a Rhodamine B labeled P2VP−PEO stream, hydrodynamically focused with Millipore-quality water: (a) accumulated giant polymersomes on the periphery of the focused stream at high volumetric flow ratio; (b) dense layer of small accumulated polymersomes at the periphery of the focused stream at small volumetric flow ratio.

3. Results and Discussion

In a typical experiment (see scheme in Figure 1), ethanol containing the dissolved block copolymer (0.05−0.1 wt %) flows through the main inlet channel, and demineralized water (Milli-Q, Millipore) flows through the two side inlet channels. The volumetric flow rate for each channel ranges between 5 and 40 nL/s, which corresponds to an outlet flow velocity of 4.28−34.3 mm/s. The flow rates in the main channel (MC) and the two side channels (SC) are adjusted to control the degree of hydrodynamic focusing. The width of the ethanol stream in the outlet channel depends on the ratio of the volumetric flow rates of the main channel (MC) to the two side channels (SC_1, SC_2), where the flow rates of the side channels are kept equal. With volumetric flow ratios ($MC:SC_{1,2}$) ranging from 4 to 0.13, the width of the central ethanol stream in the outlet channel can be adjusted in the range 8−42 μm.

For high volumetric flow ratios the formation of vesicles can directly be observed by fluorescence microscopy. To facilitate the visualization of the polymersomes, Rhodamine-B was added to the ethanol stream. The fluorescent dye readily solubilizes into the polymersome bilayer thereby labeling the bilayer. Figure 2a shows that the polymersomes are all located along the phase boundary between the focused polymer solution and the aqueous phase. For smaller volumetric flow ratios the obtained polymersomes are smaller (Figure 2b). Their size cannot be determined by fluorescence microscopy any more.

For determination of their size distribution and structure, the collected polymersome solutions are directly characterized

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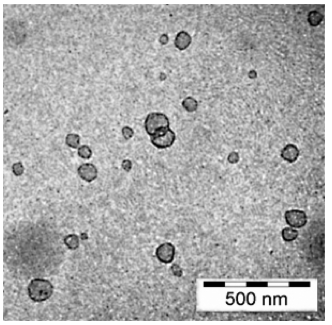

Figure 3. Cryo-TEM images of P2VP−PEO vesicles, prepared from a 0.1 wt % ethanolic solution, which was hydrodynamic focused with Millipore-quality water at a flow velocity of 30 nL/s in each channel. All vesicles are unilamellar.

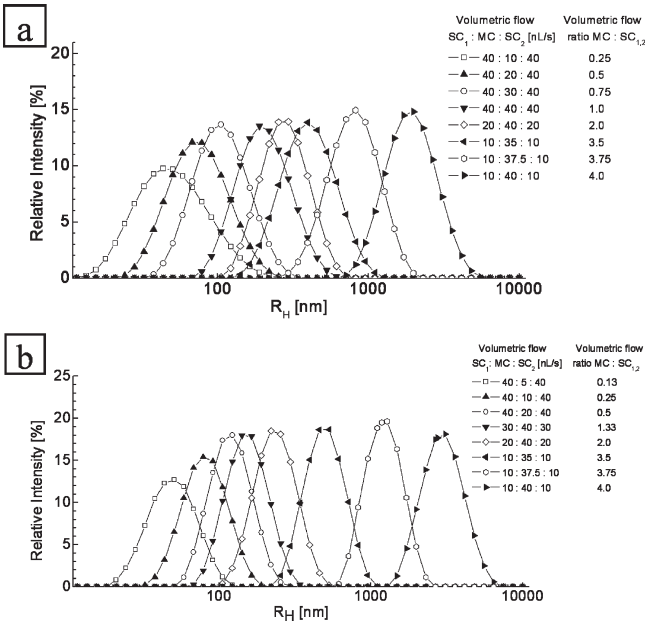

Figure 4. Size distributions of P2VP−PEO polymersomes determined by dynamic light scattering. The polymersome size is adjusted by altering the flow rate ratio between main and side inlet channel with a high degree of control.

by dynamic light scattering (DLS) and cryo-transmission electron microscopy (cryo-TEM), without subsequent processing steps such as purification and manipulation of the polymersome size distribution. Figure 3 shows a typical cryo-TEM image of polymersomes prepared from a 0.1 wt % ethanolic polymer solution at low flow ratios. We observe unilamellar polymersomes with a unimodal, well-defined size distribution.

Figure 4 shows that the size of the polymersomes can be easily adjusted over a wide range of 40 nm to 2 μm by altering the flow rate ratio between the main inlet channel (MC) and the side channels ($SC_{1,2}$). This is possible for both concentrations investigated in this study, (a) 0.1 and (b) 0.05 wt %. We would like to point out that the size distributions of all P2VP−PEO polymersome solutions prepared in our microfluidic device are more monodisperse compared to P2VP−PEO polymersomes prepared by us by any of the above-mentioned conventional methods. The relative standard deviation of the vesicle size as determined by dynamic light scattering is in the range 0.05−0.2.

In Figure 5, the mean hydrodynamic radii as determined by dynamic light scattering are plotted as a function of the flow rate

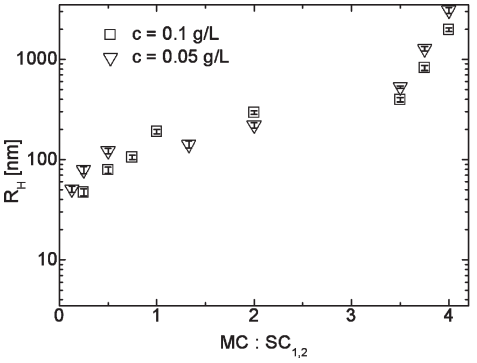

Figure 5. Flow ratio dependence of the mean hydrodynamic radii (R_H) of P2VP−PEO vesicles prepared using different polymer concentrations for three repeated experiments (blue, 0.1 wt %; red, 0.05 wt %).

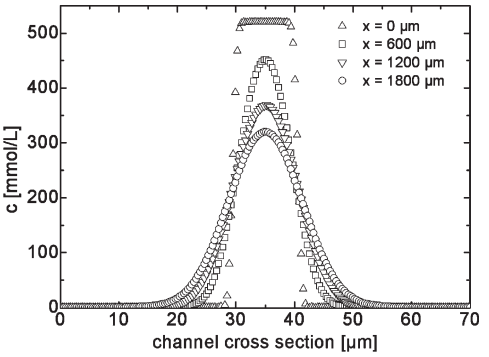

Figure 6. FEM-simulated concentration profiles for Rhodamine B dissolved in the central stream are shown for a flow rate ratio of 0.5 at different positions down the outlet channel (x = 600, 1200, 1800 μm). With increasing distance from the channel cross the concentration profile broadens, developing into a Gaussian distribution.

ratio. Similar to a previous study on lipid vesicles,[30] we observe that the polymersome size increases with increasing flow rate ratio.

Our results suggest that a hydrodynamically well-controlled nucleation and growth process leads to the observed dependence of vesicle size on the flow rate ratio. The flow rate ratio directly determines the width of the focused stream. This is shown in Figure 6 where the simulated concentration profiles for Rhodamine B dissolved in the focused stream are shown for a flow rate ratio of 0.5 at different positions down the outlet channel. Directly after the cross-junction at the entrance to the outlet channel (x = 0 μm) there is a sharp drop of the Rhodamine concentration at the periphery of the focused stream. Further down the outlet channel (x = 600, 1200, 1800 μm), the concentration profile broadens, developing into a Gaussian distribution. With decreasing flow rate ratio, the width of the focused stream becomes smaller.

In our experiments the focused stream contains the ethanolic block copolymer polymer solution. With decreasing ethanol concentration, the solvent quality for the polymer decreases. At the periphery of the focused ethanol/polymer stream, the ethanol concentration decreases to a level, below which the P2VP-block becomes insoluble. Polymersomes are then nucleated at the periphery of the focused stream in agreement with our experimental observations (Figure 2a).

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The polymersome nuclei formed at the periphery of the focused stream will then grow by uptake of polymers from the central part of the focused stream. Since the number of such polymers is proportional to the width of the focused stream, larger polymesomes are grown from focused streams with larger widths. This is in agreement with the results in Figure 5 where the width is controlled via the flow rate ratio. Whereas at lower flow rate ratios the size of the polymersomes is roughly proportional to the flow rate ratio, allowing good control of the size distribution, at the highest ratio the polymersome size is more strongly increasing. This indicates that a different polymersome growth mechanisms exists, which is the topic of ongoing studies. Yet, for both mechanisms, by tuning the flow rates at the confluence of the main inlet channel and the side channels, the self-assembly process as well as the size of the vesicles can be well controlled and is well reproducible.

4. Conclusions

Summarizing, we have shown that hydrodynamic flow-focusing microfluidic devices can be used to control the size of polymersomes over a wide range of sizes from 40 nm to 2 μm with narrow size distributions with excellent reproducibility. The polymersomes are formed at the ethanol/water boundary interface. A simple nucleation and growth model is proposed to explain the observed relation between polymersome size and focused stream width. This example shows the versatile use of current PDMS-based microfluidics for the formation of polymersomes, whose diameter can be well controlled for an optimal intake in cells for applications in drug delivery systems.

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