Biodegradable Polymersomes

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Polymersomes, i.e., polymer vesicles, have received great interest in recent years. It has been shown that polymersomes can be formed from block copolymers using organic solvents,[1] organic solvent/water systems,[2−5] or aqueous media.[6−10] Polymersomes are promising in biomedical applications for instance as artificial cells or vehicles for drug delivery and also for mimicking cell membranes. For in vivo applications of polymersomes the membrane-forming materials should be preferably biodegradable. Biodegradable block copolymers based on poly(ethylene glycol) (PEG) and polylactide (PLA) have been used to prepare carriers such as micelles, nano- or microparticles, and gels for controlled drug delivery. However, to our knowledge, no biodegradable polymersomes have been reported yet. This communication is the first report of the preparation of biodegradable polymersomes from amphiphilic block copolymers based on PEG and polyesters or polycarbonates.

All block copolymers were synthesized by ring-opening polymerization of cyclic esters, e.g., DL-lactide (DLLA), ϵ-caprolactone (CL), or carbonates, e.g., trimethylene carbonate (TMC), in the presence of zinc bis[bis(trimethylsilyl)amide] (97%) and monomethoxy poly(ethylene glycol) (methoxy PEG, 5800 and 1200) at room temperature. Similar to the calcium bis[bis(trimethylsilyl)amide] catalyst system,[11] zinc bis[bis(trimethylsilyl)amide] combined with methoxy PEG initiated the ring-opening polymerization of lactides and lactones with high conversion, affording block copolymers with a controlled molecular weight and low polydispersity index (Table 1). Besides its high activity, its low toxicity renders the zinc-based catalyst attractive for the synthesis of copolymers for in vivo applications.

Polymersomes were made by injecting a solution of a polymer in an organic solvent into an aqueous phase. Both water immiscible and water miscible organic solvents were applied. For visualization of polymersomes by fluorescence microscopy and confocal laser scanning microscope (CLSM), hydrophobic Nile red was dissolved in the polymer solution or hydrophilic fluoresceinamine was dissolved in the aqueous phase prior to polymersome preparation.

As a typical example, a polymer solution in a water immiscible solvent, e.g., chloroform (10 mg/mL, 0.1 mL), was added to 5 mL of an aqueous phase, e.g., deionized water (DI water) or phosphate buffered saline (PBS), under vigorous stirring. Typically within 3 min this resulted in an opaque dispersion, which was further stirred for 1−2 h. Using the chloroform/water system all copolymers listed in Table 1 formed polymersomes,

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Table 1. Characteristics of Diblock Copolymers[a]

diblock copolymer	M_n(NMR)[b]	M_n(GPC)[c]	M_n(theor)[d]	PDI[c]
PEG-PDLLA1.2-4.8	6 600	9 000	6 100	1.1
PEG-PDLLA1.2-12	14 800	16 300	13 200	1.1
PEG-PDLLA5.8-17	20 400	16 900	22 400	1.1
PEG-PDLLA5.8-24	28 200	26 200	30 200	1.1
PEG-PDLLA5.8-48	50 500	37 800	53 000	1.5
PEG-PCL5.8-24	29 800		29 700	
PEG-PTMC5.8-24	30 900		29 700	

[a] Copolymers are denoted by PEG-polyester or PEG-polycarbonate followed by molecular weights of the blocks ($\times 10^{-3}$). [b] Determined by ^{1}H NMR (300 MHz) using the integrals of the methylene peak of PEG and the main peaks of polyester or polycarbonate block. [c] Determined by GPC. [d] Calculated from the initial ratio of monomer to PEG hydroxyl groups and the conversion.

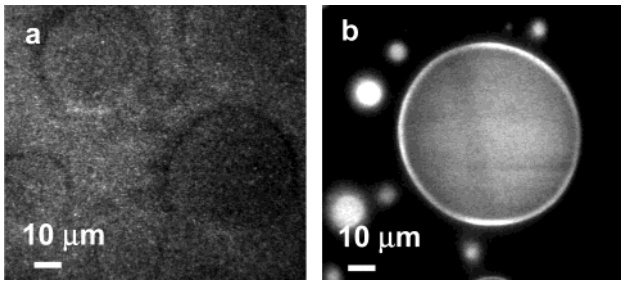

Figure 1. CLSM images of polymersomes prepared by adding a solution of PEG-PDLLA1.2-12 in chloroform to an aqueous phase in the presence of fluoresceinamine or Nile red as a fluorescent probe: (a) polymersomes with incorporated fluoresceinamine in DI water; (b) polymersomes with incorporated Nile red in PBS.

whose size covered a broad range from 70 nm to 50 μm. CLSM (Insight Plus) images (Figure 1) of giant polymersomes confirmed their vesicular nature. In the case of fluoresceinamine incorporation (Figure 1a), the dark rings represent the hydrophobic part of the membrane. In the case of Nile red incorporation (Figure 1b), the bright rings with high fluorescent intensity reflect the hydrophobic membrane, and the less intense center regions point to the interior of the polymersomes. Scanning of a giant polymersome (20−50 μm) by changing the focal plane confirmed the vesicular structure of the polymersomes.

TEM micrographs revealed that polymersomes prepared from chloroform/water coexist with other bilayer structures, e.g., tubules. Upon storage, polymersomes prepared via the chloroform/water system disappeared, lost their structure, or aggregated quickly, which was probably due to the presence of the organic solvent, which fluidizes the membrane. After removal of the organic solvent from the dispersions, polymersomes were stable for at least 1 month. Furthermore, they remained spherical as seen in SEM or TEM measurements.

For the preparation of polymersomes from copolymers in solvents that are fully water miscible, e.g., acetone and tetrahydrofuran (THF), DI water or water saturated with ethyl acetate or benzyl alcohol (named ea.water and ba.water, respectively) was used as the aqueous phase. Typically, injection of 0.05 mL of a polymer solution (10 mg/mL) into 2.5 mL of an aqueous medium resulted in instantaneous formation of a dispersion that is transparent, turbid, or cloudy. For the water miscible

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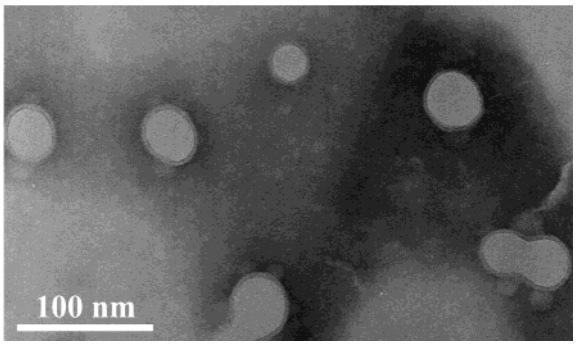

Figure 2. TEM micrograph of polymersomes made by injecting a polymer solution of PEG-PDLLA1.2-12 in acetone into DI water. Polymersomes on a carbon-coated copper grid were stained with phosphotungstic acid (2 wt %) solution and imaged with a Philips CM30 Twin STEM at an acceleration voltage of 200 kV.

solvent/ba.water system, polymersomes were formed by further dilution with DI water or dialysis against DI water. Only copolymers that contain PEG5800 as hydrophilic block can form stable polymersome dispersions. The majority of the polymersomes obtained were smaller (submicron, actual size depends on preparation conditions), and the size distribution of polymersomes was much smaller than that of polymersomes prepared from the chloroform/water system. Figure 2 demonstrates a TEM image of polymersomes, prepared from acetone/DI water system, with well-defined walls and a narrow size distribution (PDI < 0.15 as determined by dynamic light scattering, DLS). After staining, the interior of the particles has a pronounced lower electron density compared to the wall, proving that the particles are indeed polymersomes. The polymersomes do not show any significant deformation or collapsed structure, possibly due to the fact that the T_g of the PDLLA in the membrane is higher than room temperature. The polymersomes prepared using acetone as solvent are generally smaller than those using THF as solvent. In addition, using the same water miscible solvent, polymersomes prepared from ethyl acetate saturated water were smaller than those from benzyl alcohol saturated water.

The organic solvent was removed from the polymersomes by dialysis of the dispersions against DI water. CLSM (Zeiss LSM 510) measurements confirmed the vesicular structure of the particles prepared from the water miscible solvent/ba.water/dialysis system. In Figure 3, the upper figure shows typical polymersome images of PEG-PDLLA5.8-48 prepared in the presence of fluoresceinamine. The lower two figures display intensity scans of white light (A) and fluorescence (B) following the line as indicated in the upper image. The high fluorescence intensity corresponds to the border of the particles, confirming the encapsulation of fluoresceinamine inside the polymersomes.

The majority of the dispersions prepared from the water miscible solvent/aqueous medium after dialysis consists of vesicles and hardly any precipitate was present. After dialysis the polymersomes were stable (in terms of vesicle integrity) for 3 months as indicated by visual observation and by DLS measurements. The CLSM image of Figure 3 was taken after ca. 2 months storage of fluoresceinamine encapsulated polymersomes. The low fluorescent intensity of the outer aqueous phase indicates the low permeability of the polymersome wall for fluoresceinamine. The PDLLA component in the

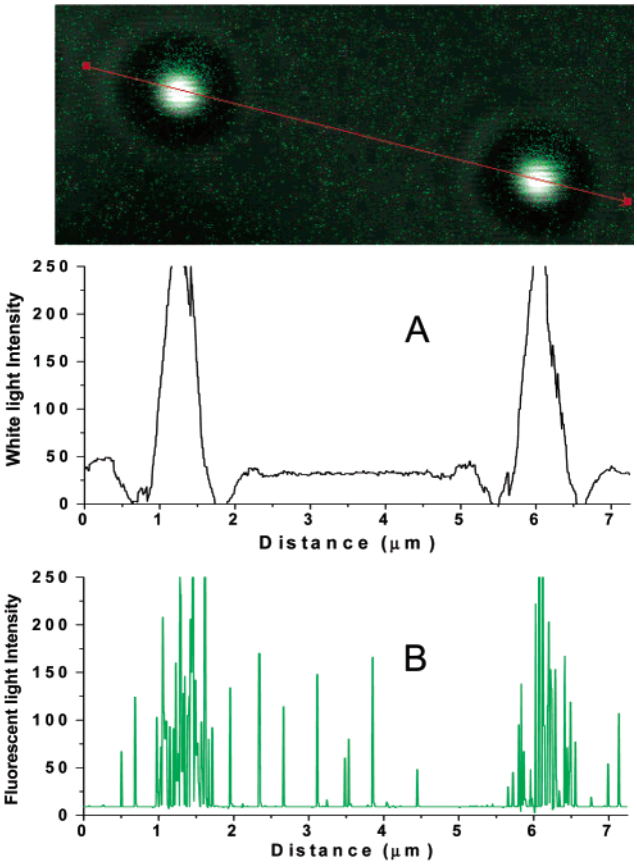

Figure 3. CLSM image and intensity profiles of polymersomes from THF/ba.water/dialysis system. Intensity profiles of white light (A) and fluorescence (B) following the line as indicated in the upper image were shown. PEG-PDLLA5.8-48 in THF (10 mg/mL, 0.04 mL) was injected into 5 mL of saturated ba.water that contains fluoresceinamine (~0.1 mg/mL). After 10 min emulsification, the sample was dialyzed against water.

membrane is biodegradable. However, the rate of degradation is sufficiently low to prevent the loss of integrity of the polymersomes during the 2 month study. It has been reported that complete mass loss of pure PDLLA takes place between 12 and 16 months depending on the molecular weight and the device dimension.[12]

In summary, biodegradable polymersomes based on block copolymers of PEG and polyester or polycarbonates were prepared from a variety of organic solvents and water combinations. Biodegradable polymersomes with sizes ranging from 70 nm to 50 μm could be readily prepared. The subsequent removal of the organic solvents from the polymersomes increased their stability, which may make it possible to tailor the size of the polymersomes, for instance, by extrusion through membranes with well-defined pore sizes. These biodegradable polymersomes have a high potential for biomedical applications. The degradation of the polymersomes is currently under investigation.

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