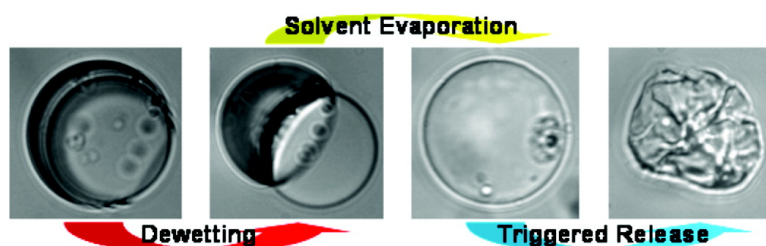


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Microfluidic Fabrication of Monodisperse Biocompatible and Biodegradable Polymersomes with Controlled Permeability

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Abstract: We describe a versatile technique for fabricating monodisperse polymersomes with biocompatible and biodegradable diblock copolymers for efficient encapsulation of actives. We use double emulsion as a template for the assembly of amphiphilic diblock copolymers into vesicle structures. These polymersomes can be used to encapsulate small hydrophilic solutes. When triggered by an osmotic shock, the polymersomes break and release the solutes, providing a simple and effective release mechanism. The technique can also be applied to diblock copolymers with different hydrophilic-to-hydrophobic block ratios, or mixtures of diblock copolymers and hydrophobic homopolymers. The ability to make polymer vesicles with copolymers of different block ratios and to incorporate different homopolymers into the polymersomes will allow the tuning of polymersome properties for specific technological applications.

Introduction

The encapsulation of drugs, flavors, colorings, fragrance, and other active agents is of increasing importance to the pharmaceutical, food, and cosmetic industries.^{1,2} Ideal encapsulating structures should capture the actives as efficiently as possible and should be easily triggered to release the actives. One class of suitable structures includes vesicles, which are microscopic compartments enclosed by a thin membrane often self-assembled from amphiphilic molecules. Due to the hydrophobicity of the membrane, active materials with large sizes cannot readily pass through the vesicle wall; however, small molecules such as water can penetrate the vesicles. Therefore, depending on the osmotic pressure difference between the aqueous core and the surrounding environment, vesicles can be inflated or deflated by varying the water content. The thin membrane that makes up the vesicle wall is often mechanically weak and breaks beyond a certain pressure difference, releasing the actives. This provides a simple mechanism for triggered release. The amphiphiles of choice in Nature are phospholipids, which are a major constituent of cell membranes. Typical phospholipids have molecular weights less than 1 kilodalton, thus phospholipid bilayers have a thickness of only a few nanometers.^{3,4} Because mechanical stability

increases while water permeability decreases with increasing membrane thickness, phospholipid vesicles have poor mechanical rigidity and a high water permeation rate, limiting their use for encapsulation and controlled release.⁵ Synthetic amphiphiles of diblock copolymers with larger molecular weights have also been used to synthesize polymer vesicles, which are known as polymersomes. Their larger molecular weights have led to thicker membranes, resulting in significant improvements in both mechanical and thermodynamic stability.^{3,5} As a result, applications of polymersomes are rapidly emerging in drug delivery⁶ and cosmetics.⁷

Block copolymers of poly (ethylene-glycol)-b-poly(lactic acid) (PEG-b-PLA) are completely biocompatible making them promising for pharmaceutical uses. The hydrophilic PEG block exhibits very low toxicity, and is thus widely used in a variety of cosmetic products.^{8,9} Moreover, PEG is often incorporated in drug carriers for delivery to human bodies, due to its resistance against opsonization, the process through which antibody binding is enhanced to induce phagocytosis.^{10,11} The hydrophobic PLA block is also known to be nontoxic and biodegradable, offering great potential for controlled release.⁹ Therefore, PEG-PLA block polymers have been of great interest as a material for drug delivery. However, due to the difficulty

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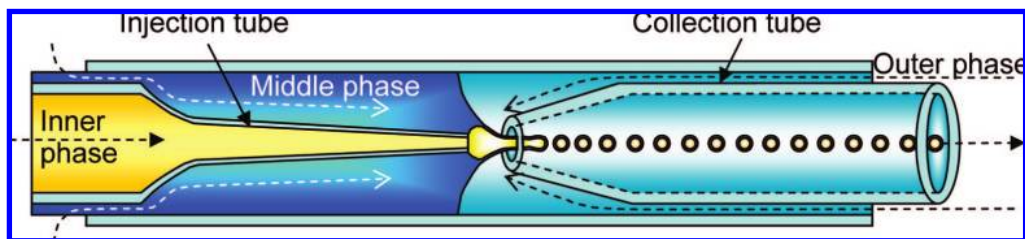


Figure 1. Schematic of the microcapillary geometry for generating double emulsions. The geometry requires the outer phase to be immiscible with the middle phase, which is in turn immiscible with the inner phase. However, the inner phase can be miscible with the outer phase, as it is in our case. Both the injection tube and the collection tube are tapered from glass capillary tubes with an outer diameter of $1000\ \mu\text{m}$ and an inner diameter of $580\ \mu\text{m}$. Typical inner diameters after tapering range from 10 to $50\ \mu\text{m}$ for the injection tube and from 40 to $100\ \mu\text{m}$ for the collection tube.

Ⓜ Movie S1 in AVI format shows the generation of double emulsions in a glass microfluidic device. Details are provided in the Supporting Information.

in forming stable PEG-PLA vesicles, PEG-PLA is often only used in the form of micelles and nanoparticles,^{11,12} which have low encapsulation efficiency and loading levels. The elusiveness of stable PEG-PLA vesicles is often attributed to the limited copolymer designs available and to the narrow hydrophilic-to-hydrophobic block ratio required for the vesicle phase.^{5,13}

Traditionally, micron-sized polymersomes are prepared by electroformation.³ This method uses an alternating electric field to generate the vesicles from the polymer film-coated electrodes. Alternatively, polymersomes can also be formed by rehydration.⁴ In this technique, an aqueous phase containing the actives is added to a dried polymer film, which, upon rehydration, buckles to form the vesicles. However, vesicles prepared by these simple techniques, which rely on the spontaneous assembly of the diblock copolymers, typically have a large size distribution and capture only small proportions of the desired contents.¹ Alternatively, polymersomes can be formed by evaporating the solvent of a copolymer-stabilized double emulsion.^{14,15} During the solvent evaporation, the assembly of the dissolved diblock copolymers is directed into vesicle structures using the water-in-oil-in-water (W/O/W) double emulsions as templates. By employing monodisperse double emulsions prepared by microfluidics as templates, monodisperse polymersomes can be prepared. However, the ability to encapsulate hydrophilic actives has not yet been demonstrated. To date studies of this technique have focused on diblock copolymers that are not compatible with biological systems; however, the true attractiveness of polymersomes for encapsulation relies on the use of biocompatible diblock copolymers, such as PEG-b-PLA. By confining the amphiphilic diblock copolymer molecules to the two water–oil interfaces during the assembly process, it might be possible to form polymersomes from diblock copolymer with copolymer designs that are difficult to achieve with the spontaneous self-assembly approach.

In this article, we describe a microfluidic approach for fabricating monodisperse biocompatible PEG-PLA polymersomes that selectively encapsulate hydrophilic solutes with high encapsulation efficiency. We use monodisperse double emulsion as templates to direct the assembly of PEG-b-PLA during solvent evaporation. The polymersomes prepared encapsulate a fluorescent hydrophilic solute, which can be released by application of a large osmotic pressure difference. We also show that this technique can be used with diblock copolymers with different molecular weight ratio of the hydrophilic and the hydrophobic blocks. Depending on the ratio, the wetting angle of the polymer-containing solvent phase on the polymersomes changes in the emulsion-to-polymersomes transition. The property of the polymersome wall can also be tuned by changing

the block ratio. Thus, our technique enables the fabrication of PEG-b-PLA polymersomes with excellent encapsulation efficiency, high levels of actives loading and tunable wall properties. These polymersomes provide an ideal biocompatible carrier for encapsulating actives in biomedical, food, and cosmetic industries.

Results and Discussion

Formation of Block Copolymer-Stabilized Double Emulsions. Monodisperse W/O/W double emulsions stabilized by a diblock polymer of PEG(5000)-b-PLA(5000) are prepared in glass microcapillary devices,¹⁶ as shown schematically in Figure 1. The fluorescence dye-containing inner drops are formed in the dripping regime¹⁷ from the small injection tube in a coflow geometry while the middle oil stream containing the inner drops is flow-focused by the outer continuous phase and breaks up into double emulsion drops (see Movie S1). Because the inner phase is only in contact with an immiscible middle oil phase, fluorescence dyes are retained in the inner phase without leakage to the outer continuous phase during the emulsion fabrication. The middle phase consists of PEG(5000)-b-PLA(5000) dissolved in a mixture of toluene and chloroform in a volume ratio of 2:1. The appropriate solvent should be highly volatile and dissolve the diblock copolymer well. While the PEG(5000)-b-PLA(5000) has a high solubility in chloroform, double emulsions with chloroform alone as the middle oil layer have a higher density than the aqueous continuous phase. The double emulsion drops therefore sink to the bottom of the container, which is often wetted by the chloroform layer and destabilizes the emulsions. Toluene has a lower density than the continuous phase, but it does not dissolve the copolymer as well. The mixture of toluene and chloroform in a 2:1 volume ratio provides a suitable combination of the properties.

Transition from Double Emulsions to Polymersomes. Double emulsion drops stabilized by the PEG(5000)-b-PLA(5000)

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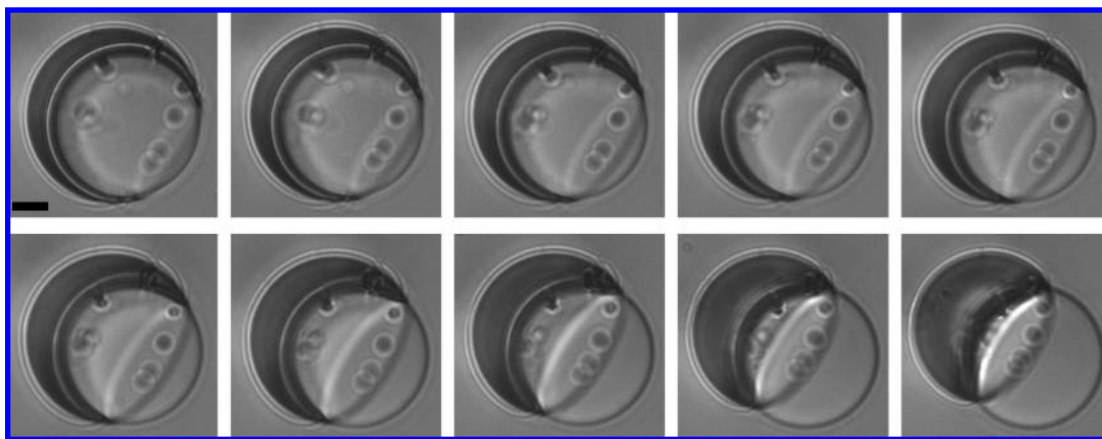


Figure 2. Bright-field microscope images of a double emulsion drop undergoing dewetting transition. The double emulsion drop consists of an aqueous drop surrounded by a shell of $10 \text{ mg} \cdot \text{mL}^{-1}$ PEG(5000)-b-PLA(5000) diblock copolymer dissolved in a toluene/chloroform mixture (2:1 by volume). At the end of the transition, the drop adopts an acorn-like structure with the organic solvent drop on the left and the aqueous drop on the right. Successive images are taken at intervals of 910 ms. Scale bar is $10 \mu\text{m}$.

Ⓜ Movie S2 in AVI format shows the shrinkage of a dewetted inner drop. Details are provided in the Supporting Information.

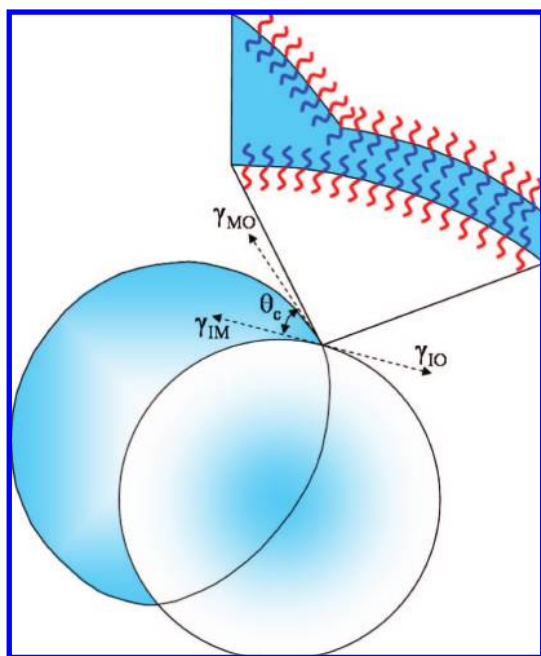


Figure 3. Schematic of the proposed structure of a double emulsion drop with partial wetting of the organic phase on a thin layer of block copolymer.

copolymers typically go through various stages of dewetting transition, as shown in Figure 2. The organic solvent layer, which initially wets the entire inner drop, dewets from the inner drop, resulting in an acorn-like structure. The contact angle, θ_c , at the three-phase contact point is 56° , as schematically illustrated in Figure 3. The acorn-like equilibrium structure has been predicted from an analysis of the three interfacial tensions between various different pairs of three immiscible liquids.¹⁸ The final morphology of a core–shell system is determined by the relative surface energies. If the interface between the core and the external phase has a larger surface energy compared with that between the core and the shell, the shell completely

wets the core, forming a stable core–shell structure.¹⁹ If the relative surface energy between the core and the shell phase is very high, the core and the shell separate from each other to avoid wetting. In the case of comparable surface energies, partial wetting between the core and the shell occurs, leading to formation of acorn-like structures. Each of the morphologies has been observed experimentally in a three-phase system of oil, water and polymer.²⁰ In our case, the PEG(5000)-b-PLA(5000) copolymer acts as a surfactant and migrates to the two interfaces. The formation of acorn-like structures suggests that the surface energy of the copolymer–oil interface is comparable to that of the copolymer bilayer. From a simple force balance at the three-phase contact point shown in Figure 3, for this partial wetting to occur, there must be a negative spread coefficient, S , such that

$$S = \gamma_{IO} - \gamma_{IM} - \gamma_{MO} \quad (1)$$

where γ_{IO} , γ_{IM} , and γ_{MO} are the surface tensions of the inner–outer, the inner–middle, and the middle–outer interfaces, respectively. In our experiments, the measured value of the spreading coefficient is -2.1 mN/m . Associated with S is an attractive adhesion energy between the inner and outer phases, and the driving force for the attraction has been shown to arise from depletion effects.⁹

Monodisperse Polymersomes for Encapsulation. One bulb of the acorn-like dewetted drop consists of a volatile organic solvent, which continues to evaporate after the dewetting transition. The evaporation rate is adjusted to ensure that the double emulsion remains stable throughout the evaporation process. After evaporation of the organic solvent for a day, the excess diblock copolymer forms an aggregate on the side where the organic solvent drop attaches (Figure 4A). The aggregates remain attached to most polymersomes. The size of the aggregates attached to the polymersomes can, in principle, be controlled by varying the amount of excess diblock copolymer in the organic solvent layer. Occasionally, we have observed that the oil drop, as it is drying, can break off the polymersome, carrying the excess diblock copolymer and leaving behind a

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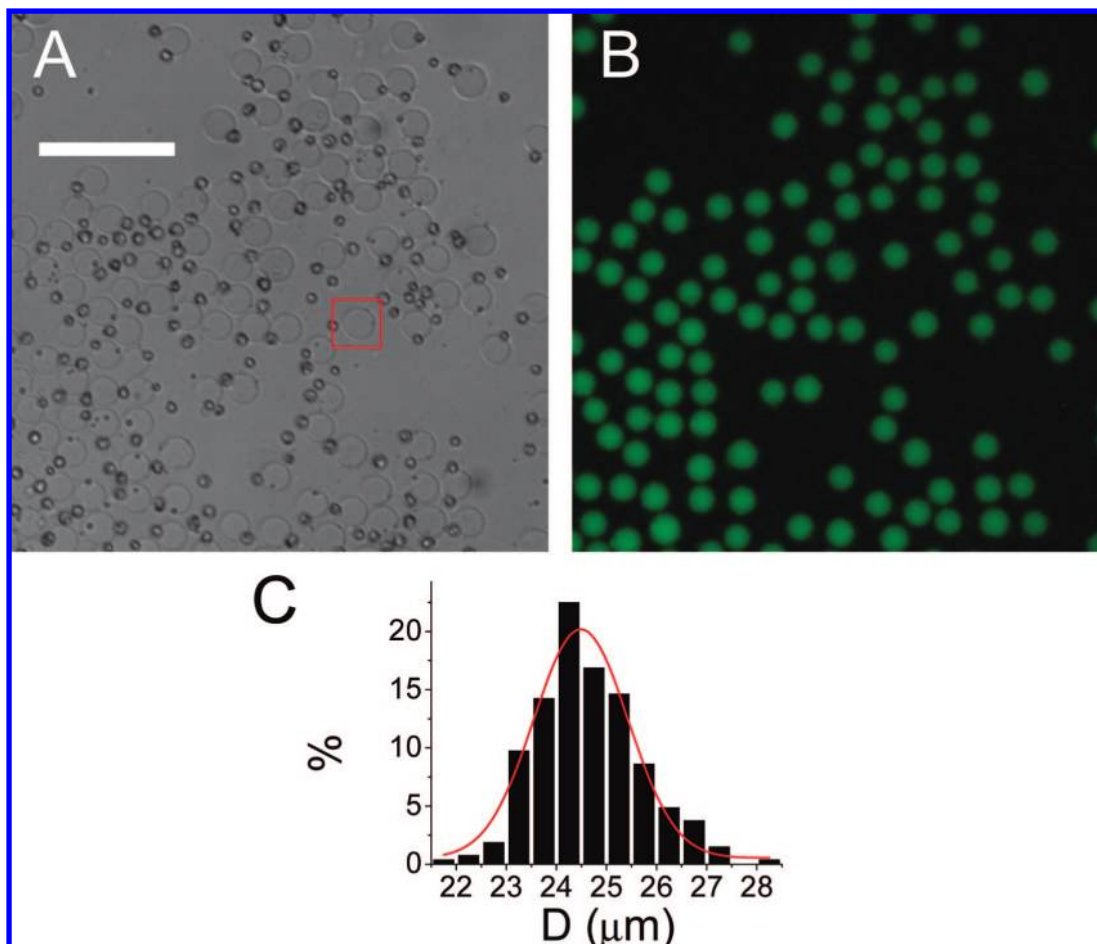


Figure 4. (A) Bright-field microscope image of the PEG(5000)-b-PLA(5000) polymersomes formed after dewetting transition and solvent evaporation. The excess diblock copolymer contained in the dewetted organic solvent drop forms aggregates, which attaches to the polymersomes. Occasionally, the aggregates are detached from the polymersomes, as shown in the red box. Scale bar is 100 μm . (B) Fluorescence microscope image of the same area as in (A). The fluorescent HPTS solutes are well-encapsulated inside the polymersomes without leakage to the continuous phase. (C) Size distribution of the PEG(5000)-b-PLA(5000) polymersomes. Polydispersity of polymersomes is 4.0%. The experimental data is fitted with a Gaussian distribution in red.

homogeneous polymersome (See red box in Figure 4A). This suggests the possibility of obtaining homogeneous polymersomes with gentle stirring. Due to the small difference between the refractive indices of the inner and the outer phases, the polymersomes can barely be seen in bright field microscopy. In fluorescence microscopy, however, the polymersomes can be clearly seen as bright green spots, as shown in Figure 4B. The large contrast in fluorescence intensity between the inner drop and the outer continuous phase demonstrates the excellent encapsulation efficiency of the fabrication process. Not only is the FITC-Dextran, with an average molecular weight of 4000 Da, well encapsulated, but, remarkably, the fluorescent HPTS dye, with a very small molecular weight of less than 600 Da, also stays encapsulated inside the polymersomes. This highlights the low membrane permeability to small hydrophilic solutes. After going through the processes of dewetting and solvent evaporation, the polymersomes still shows a low polydispersity of only 4% or lower (Figure 4C), as determined by image analysis.

Responsive Polymersomes to Osmotic Shock. In the polymersome fabrication process, the osmolalities of the inner phase and the outer phase must be balanced to maintain the polymersome size. In some initial experimental runs where sodium chloride salt is not added to balance the osmolality with the outer solution, the polymersomes shrink considerably after

dewetting (see Movie S2). Although the membrane is impermeable to the small HPTS salts, water molecules can diffuse in and out of the polymersomes. The osmotic pressure, π_{osm} , is related to the concentration of solutes,

$$\pi_{\text{osm}} = cRT \quad (2)$$

where c is the molar concentration of the solutes, R is the gas constant and T is the temperature. Due to osmotic pressure difference, water diffuses from regions with a low salt concentration to regions with a higher concentration. Osmotic pressure can therefore be used to tune the sizes of our polymersomes (see Figure S1, Supporting Information). If the osmotic pressure change is sudden and large, the resulting shock can break the polymersomes. The kinetics of the polymersomes' response following a large osmotic shock is too fast to visualize; we therefore slow down the process for visualization by gradually increasing PVA concentration through water evaporation. Initially, the polymersomes are suspended in a 10wt% PVA solution, which is left to evaporate in air on a glass slide. As water evaporates, the PVA concentration becomes higher and higher and so water is squeezed out from the inside of the polymersome. As a result, the polymersome becomes smaller, and its wall buckles, as shown in Figure 5. When subjected to a sufficiently high osmotic shock, the polymersome wall can break (see Figure S2, Supporting Information). This provides a

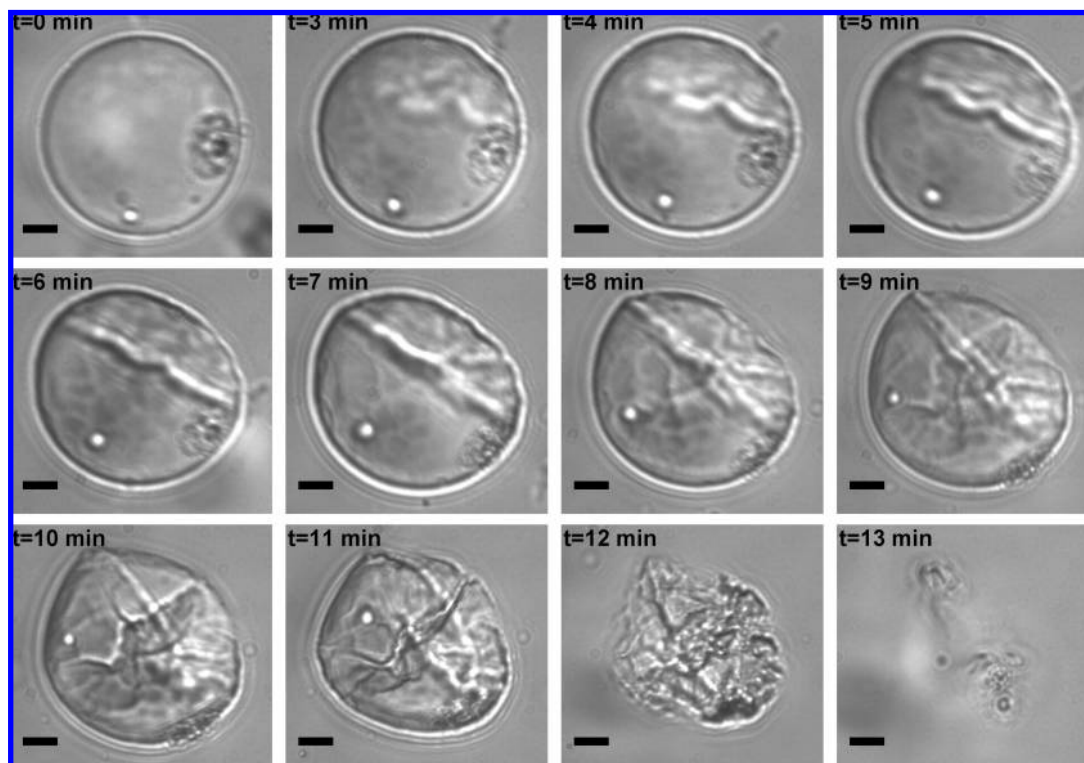


Figure 5. Bright-field microscope images showing the shrinkage and breakage of a PEG(5000)-b-PLA(5000) polymersome after an osmotic shock. As a result of water expulsion from its inside, the polymersome shrinks and buckles. When subjected to a sufficiently high osmotic shock, the polymersome wall can break. A detailed analysis of such breakage is shown in Figure S2 (Supporting Information). Scale bar is 10 μm .

simple trigger for the release of the encapsulated fluorescent HPTS. Thus, by tuning the properties of the polymersome wall, it might be possible to adjust the level of osmotic shock required to break the polymersomes. Alternatively, release can also be triggered by diluting the continuous phase and thus reducing its osmotic pressure. The simple triggered release mechanism makes our polymersomes a promising candidate for encapsulation and release of actives.

Copolymers with Different Block Ratios. We also apply the same technique to diblock copolymers of different block ratios. With a PLA-rich diblock copolymer of PEG(1000)-b-PLA(5000), double emulsions collected do not form the acorn-like structures observed in the case of PEG(5000)-b-PLA(5000) (Figure 6A). As the organic solvent evaporates, the middle solvent phase gets thinner and thinner. Eventually, after most of the organic solvent is evaporated, dewetting of the middle phase occurs and aggregates are seen attached to the final capsules, similar to those attached to the PEG(5000)-b-PLA(5000) polymersomes (Figure 6B). However, the contact angle of the middle phase at the three phase contact point is much smaller ($\sim 17^\circ$). The spreading coefficient associated with it is -0.4 mN/m. This suggests that the organic solvent with the PLA-rich diblock copolymer wets the inner drop more than that with PEG(5000)-b-PLA(5000). Like the PEG(5000)-b-PLA(5000) polymersomes, these capsules show excellent encapsulation of both the FITC-Dextran (Figure 6D) and the low molecular weight HPTS (Figure 6C), which can be released by application of an osmotic pressure shock. We demonstrate the release of FITC-Dextran from the PEG(1000)-b-PLA(5000) polymersomes by diluting the continuous phase with water. Before dilution, FITC-Dextran is encapsulated inside the polymersomes, as shown by the green fluorescent compartment in Figure 6D. After dilution with water, the green fluorescence of the polymersome disappears even

though the polymersome is still observed in bright field, as shown in Figure 6E. To ensure that this is not an artifact due to photobleaching of the FITC-Dextran, the fluorescent shutter remains closed at all times except when the polymersomes are imaged about ten minutes after dilution with water. The contrast in fluorescence intensity is too low for the polymersomes to be observed with fluorescence microscopy after the osmotic shock. Instead, we must observe the polymersome with bright-field microscopy. The images suggest that the polymersome remains intact after the osmotic shock; nevertheless, the FITC-Dextran is released when the osmotic pressure outside the polymersomes is suddenly decreased. We believe that the FITC-Dextran is released from the polymersomes through cracks or pores that are too small to observe; however, the exact release mechanism has yet to be established. The versatility of our technique to diblock copolymers of different PEG/PLA ratios allows customization of polymersomes for different technological applications. By changing the PLA/PEG ratio, blends of PLA and PEG exhibit different properties such as morphology, crystallinity, mechanical properties and degradation properties.²¹

Despite its applicability to diblock copolymers with different block ratios, our technique is limited to systems where stable double emulsions can be formed. In the case of a highly hydrophilic diblock copolymer of PEG(5000)-b-PLA(1000), polymersomes cannot be directly formed with our technique. The diblock copolymer, PEG(5000)-b-PLA(1000), is clearly surface active and lowers the interfacial tension significantly, as suggested by the highly nonspherical shape of the droplets in Figure 7A–C; an interface with a higher interfacial tension would otherwise relax to the surface-minimizing spherical shape

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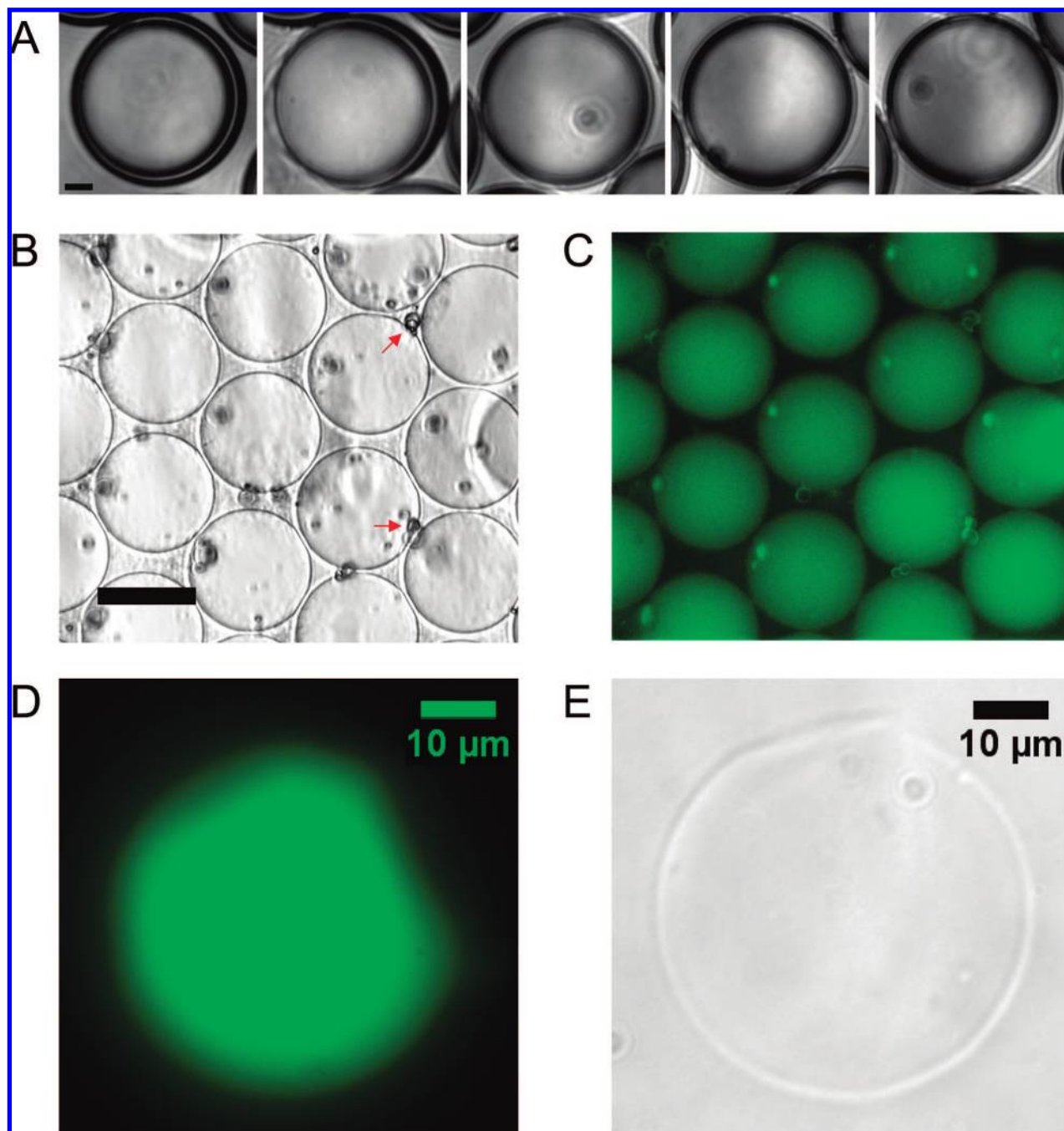


Figure 6. Synthesis of polymersomes with a PLA-rich diblock copolymer of PEG(1000)-b-PLA(5000). (A) Bright-field microscope image following the evaporation of the organic solvent shell of a double emulsion drop. The double emulsion drop consists of an aqueous drop surrounded by a shell of $10 \text{ mg} \cdot \text{mL}^{-1}$ PEG(1000)-b-PLA(5000) diblock copolymer dissolved in a toluene/chloroform mixture (2:1 by volume). The shell gets thinner and thinner, as the toluene/chloroform mixture evaporates. Scale bar is $10 \mu\text{m}$. The images are taken at intervals of 1 h. (B) Bright-field microscope image of a dried capsule formed from the PEG(1000)-b-PLA(5000) diblock copolymer. The red arrows indicate aggregates of excess diblock copolymer. Scale bar is $50 \mu\text{m}$. (C) Fluorescence microscope image of the same area as in (B). As in the case of the PEG(5000)-b-PLA(5000), the fluorescent HPTS solutes are well-encapsulated inside, without leakage to the continuous phase. (D) Fluorescence microscope image of a PEG(1000)-b-PLA(5000) polymersome encapsulating the green FITC-Dextran in a 1 M Trizma hydrochloride buffer solution (pH 7.2). The polymersome is slightly deflated initially when the salt concentration in the continuous phase is higher due to water evaporation. (E) Bright-field microscope image of a PEG(1000)-b-PLA(5000) polymersome after dilution of the continuous phase by about five times with deionized water. Even though the polymersome is clearly visible in bright field, no fluorescence can be observed in fluorescence microscopy, indicating that the FITC-Dextran has been released after dilution of the continuous phase with water. To ensure that this is not an artifact due to photobleaching of the FITC-Dextran, the fluorescent shutter was closed at all times except when the polymersomes were imaged about 10 min after dilution with water.

quickly (see Movie S1). However, using this diblock copolymer, double emulsions are not stable unless additional PLA homopolymer is added to the middle phase; then double emulsion drops are generated (Figure 7A) and the inner drops remain

stable inside the middle drops (Figure 7B). Without the PLA homopolymer, the inner drops break through the middle phase almost immediately after generation of double emulsion drops, as shown in Figure 7C; as a result, only a simple emulsion of

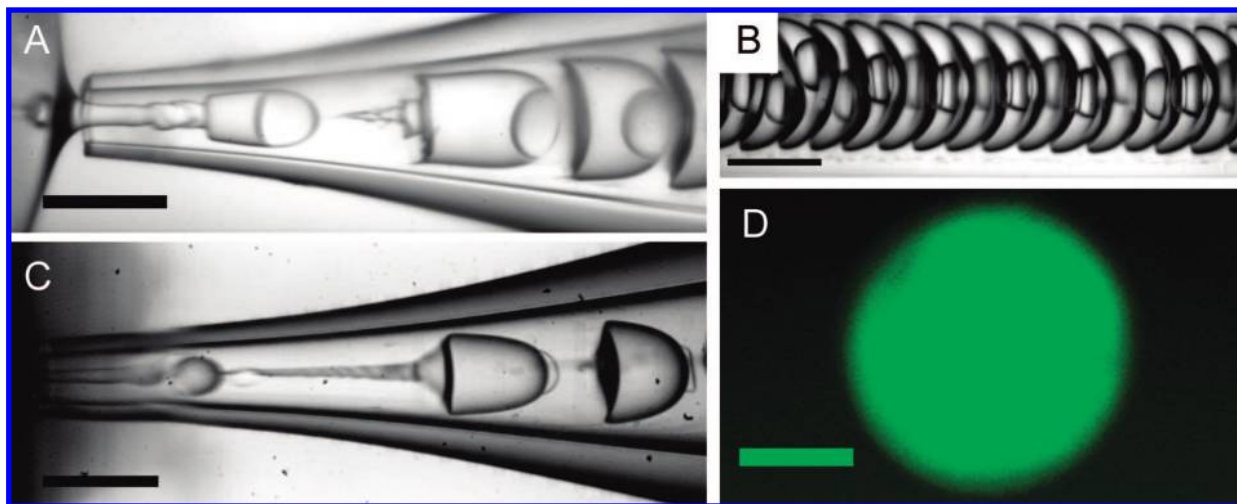


Figure 7. Influence of the homopolymer on double emulsion stability. (A), Middle phase that forms the shell consists of $5 \text{ mg} \cdot \text{mL}^{-1}$ PEG(5000)-b-PLA(1000) and $2 \text{ mg} \cdot \text{mL}^{-1}$ PLA homopolymer dissolved in pure toluene. (B) Inner drops remain stable inside the middle drops. However, the double emulsion drops are not stable without the PLA homopolymer. (C) Inner phase breaks through the middle phase, and only a simple emulsion of the middle phase is collected. In all cases, the diblock copolymer, PEG(5000)-b-PLA(1000), clearly lowers the interfacial tension significantly, as suggested by the highly nonspherical shape of the droplets in panels A, B, and C, which would otherwise relax to the surface-minimizing spherical shape quickly (see Movie S1). (D) Polymersome encapsulating fluorescent HPTS solutes obtained from the double emulsions shown in panel A and B after solvent evaporation. Scale bar is $300 \mu\text{m}$ for A, B and C, and $30 \mu\text{m}$ for D.

the middle phase is collected. This suggests that addition of the PLA homopolymer increases the double emulsion stability significantly. The resulting polymersomes demonstrate excellent encapsulation behavior (Figure 7D). The concept of tuning polymersome properties by incorporating homopolymer has been demonstrated using common polymersome formation techniques such as rehydration.²² However, our technique enables the fabrication of more uniform polymersomes with a simple and efficient way of actives encapsulation. By incorporating different homopolymers to modify the properties and morphology, we can apply our techniques to engineer uniform macromolecular structures with controllable properties.

Conclusions

In this paper, we demonstrate a versatile approach for fabricating monodisperse biocompatible and biodegradable polymersomes with semipermeability using microfluidics. We show that double emulsions stabilized by a diblock copolymer with a hydrophilic-to-hydrophobic block ratio of one undergo a dewetting transition to form polymersomes that encapsulate small hydrophilic solutes and remain permeable to water. Breakage of the polymersomes can be triggered by an osmotic

shock, suggesting a simple mechanism for triggered release of encapsulated actives. Our approach also enables us to fabricate capsules using the same diblock copolymer with a different block ratio as well as polymersomes with a homopolymer incorporated within the membrane. By tuning the block ratios of the block copolymer and/or the homopolymer, the technology will allow precise control of polymersome properties such as membrane thickness, mechanical response, permeability, thermal stability, and other properties as may be required for particular technological applications.

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Supporting Information Available: Experimental section, images of polymersomes showing their structures at different salt concentrations and the effect of an osmotic shock on their structures, as well as details of Movies S1 and S2. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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