

Fabrication of Polymersomes with Controllable Morphologies through Dewetting w/o/w Double Emulsion Droplets^{*}

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Abstract In this work, monodisperse giant polymersomes are fabricated by dewetting of water-in-oil-in-water double emulsion droplets which are assembled by amphiphilic block copolymer molecules in a microfluidic device. The dewetting process can be tuned by solvation between solvent and amphiphilic block copolymer to get polymersomes with controllable morphology. Good solvent (chloroform and toluene) hinders dewetting process of double emulsion droplets and gets acorn-like polymersomes or patched polymersomes. On the other hand, poor solvent (hexane) accelerates the dewetting process and achieves complete separation of inner water phase from oil phase to form complete bilayer polymersomes. In addition, twin polymersomes with bilayer membrane structure are formed by this facile method. The formation mechanism for different polymersomes is discussed in detail.

Keywords: Polymersomes; Double emulsion droplets; Dewetting; Microfluidic device.

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INTRODUCTION

Polymersomes, which are vesicles whose membranes are comprised of amphiphilic block copolymers, have the ability to encapsulate and transport molecules either in the central water pool or sequestered in their hydrophobic bilayer membrane. They provide higher stability and lower permeability than liposomes due to the larger molecular weight of the polymeric amphiphiles. So they are useful for wide range of drug-delivery systems^[1,2] and for mimic biological systems such as cells and artificial organelles^[3].

The most common method used for polymersomes preparation is film hydration^[4,5]. Amphiphilic polymers are dissolved in an organic solvent and then a thin film is prepared on the container wall by evaporation of the organic solvent. Subsequently, the film is hydrated by adding water to form polymersomes. Typically, this method produces polymersomes with broad size distribution and often with large quantities of other metastable phases. An alternative method was proposed by adapting the electroformation of liposomes which is based on the hydration of the amphiphilic film under an external electrical field that enhances water diffusion across the bulk copolymer^[4]. This method can prepare almost exclusively unilamellar polymersomes^[6]. The “phase inversion” technique involves dissolving an amphiphilic block copolymer in an organic phase which is a suitable

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solvent for all the blocks, followed by the injection of an aqueous phase, which causes the non-polar block to become insoluble, triggering the copolymer self-assembly into polymersomes^[7-9]. All these methods generally do not allow efficient encapsulation of water-soluble molecules which is of crucial importance for their application.

In order to improve the encapsulation efficiency, water-in-oil-in-water (w/o/w) double emulsion droplets have been extensively used as template to prepare giant liposomes^[10], microcapsules^[11, 12] or colloidosomes^[13] using lipid, polymer or colloid particles dissolved in solvent as middle phase. With evaporation or diffusing of solvent in middle oil phase, these solutes aggregate to form a shell. Usually, core-shell particles prepared by these processes have thick-shell structure which decreases their permeability^[14]. How to fabricate giant unilamellar polymersomes is a great challenge. Recently, Weitz and co-worker have developed an easy and fast method to prepare monodisperse PEO-*b*-PLA polymersomes with bilayer membrane structure by dewetting of double emulsion droplets. But how to control the morphology of polymersomes is less reported in literature.

Herein, giant polymersomes with controllable morphologies are fabricated by dewetting highly monodisperse w/o/w double emulsion droplets which are prepared in a microfluidic device using amphiphilic diblock copolymer PEG-*b*-PS as stabilizer. By careful selecting solvent and collecting solution to adjust the dewetting process, acorn-like, patched and bilayer polymersomes are fabricated, respectively.

EXPERIMENTAL

Materials

Deionized water (Millipore Milli-Q grade) with resistivity of 18.2 M Ω ·cm was used in all the experiments. Poly(ethylene glycol) methyl ether (PEG2000, M_n 2000) was purchased from Sigma-Aldrich and dried by azeotropic distillation with toluene before use. The RAFT (reversible addition-fragmentation chain transfer polymerization) chain transfer agents (CTA) 4-cyanopentanoic acid dithiobenzoate was synthesized according to literature^[15]. Poly(vinyl alcohol) (PVA, viscosity 4.6–5.4 mPa·s for 4% aqueous solution, hydrolysis 86.5 mol%–89 mol%) was purchased from Kuraray Co. Ltd. Styrene was distilled under vacuum prior to use. Azobis(isobutyronitrile) (AIBN) was recrystallized from ethanol. Poly(ethylene glycol) (PEG6000, M_n 6000) and Coumarin 6 were purchased from Sigma-Aldrich. *N,N'*-Dicyclohexylcarbodiimide (DCC), 4-(dimethylamino) pyridine (DMAP) and *n*-octadecyltrimethoxysilane were purchased from Aladdin Reagent Co. Ltd (Shanghai, China). All the other chemicals were purchased from Beijing Chemical Works (Beijing, China) and used as received.

Synthesis of Amphiphilic Block Copolymer PEG-*b*-PS

Synthesis of macromolecules RAFT chain transfer agent PEG-CTA: Monofunctional PEG2000 (4 g, 2 mmol), CTA (837 mg, 3 mmol), DCC (618 mg, 3 mmol) and DMAP (61 mg, 0.5 mmol) were charged into a 50 mL flask with 20 mL of anhydrous CH₂Cl₂. The mixture was stirred under nitrogen atmosphere at 0 °C for 48 h. Then, the mixture was filtered and precipitated out in cooled ethyl ether for three times and dried under vacuum for 24 h. From the ¹H-NMR spectrum (in CDCl₃, see Fig. S1), the content of PEG-CTA was about 56% by comparing the integrals of the resonance peaks of aromatic rings ($\delta = 7.4$ – 8.0) and the methylene groups of PEG-CTA ($\delta = 3.64$).

Synthesis of PEG-*b*-PS by RAFT polymerization: PEG-CTA (400 mg, 0.112 mmol), styrene (3 mL, 24 mmol) and AIBN (6.4 mg, 0.04 mmol) were dissolved in 5 mL of anhydride dioxane in a 20 mL flask. The reaction mixture was degassed under vacuum for 10 min and refilled with nitrogen gas for three times. The flask was then placed in a pre-heated oil bath at 85 °C for 20 h. After polymerization, the sample was obtained by precipitating in cooled methanol three times and dried under vacuum for 24 h. From the ¹H-NMR spectrum (in CDCl₃, see Fig. S2), the segment ratio of PEG and PS is 45:164 by comparing the integrals of the methylene groups of PEG-CTA ($\delta = 3.64$) and the resonance peaks of the aromatic ring of PS blocks ($\delta = 6.3$ – 7.2).

Assembly of Microfluidic Device

A microfluidic device with coaxial geometry was assembled by aligning three cylindrical glass capillaries on a glass slide as first outlined by Weitz *et al.*^[16] with slightly modification. An injecting capillary (0.76 mm in outer diameter and 0.58 mm in inner diameter) was pulled and cut to a 30–80 μm tip which was treated with *n*-octadecyltrimethoxysilane to render it hydrophobic. A collecting cylindrical glass capillary of the same dimensions was pulled and cut to a 250–400 μm tip which was treated with piranha solution to render it hydrophilic. Both capillaries were inserted into an outer cylindrical glass capillary (1.0 mm in inner diameter). The distance between the two inner capillaries was about 100–200 μm . The three capillaries were carefully arranged in a concentric manner and subsequently fixed with commercial fast-curing epoxy glue. All solutions were injected using gastight syringes connected to the microfluidic device *via* polytetrafluoroethylene tubing. Flow rates were controlled by syringe pumps (Longer, LSP01-1A) during droplets generation, and the process inside the device was observed using an inverted microscope equipped with camera (Fig. S3).

Fabrication of Double Emulsion Droplets in Microfluidic Device

The PVA aqueous solution or PEG aqueous solution was used as the inner phase; PEG-*b*-PS dissolved in solvent was used as the middle oil phase; PVA aqueous solution was used as the outer phase. The three fluids were injected into the microfluidic device to prepare w/o/w double emulsion droplets at flow rates 0.5–1.0 mL/h, 0.5–3.0 mL/h and 1.0–3.0 mL/h respectively. The resultant double-emulsion droplets were collected into different aqueous solutions for further observation of the evolution process.

RESULTS AND DISCUSSION

Fabrication of w/o/w Double Emulsion Droplets

The microfluidic technique is an effective method to fabricate monodisperse single^[17, 18], double^[19], or multiple emulsion droplets^[20] which can be used as templates to synthesize different kinds of microspheres or microcapsules. In this work, three cylinder glass capillaries are used to assemble a coaxial microfluidic device. Single or double emulsion droplets can be fabricated in our device by turning the different phase off temporarily. When the middle phase is stopped, no droplets are formed because the inner phases coalesce with outer phase (Fig. 1a). By turning the outer phase off, we can fabricate w/o droplets of the inner phase in the middle phase (Fig. 1b). By stopping the inner phases, stable o/w droplets of the middle phase in the outer phase are formed (Fig. 1c). When the inner phases are switched on again, stable w/o/w double emulsion droplets are formed as shown in Fig. 1(d) and Movie S1. The ability of changing emulsion structures by switching the flow on and off sequentially in the microfluidic device allows flexible on-demand generation of droplet templates with desired structures.

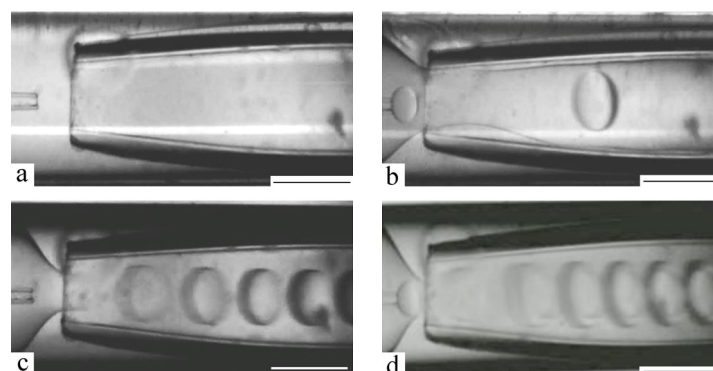


Fig. 1 Optical images of droplets formation: (a) inner phase on, middle phase off, outer phase on, no droplets formed; (b) inner phase on, middle phase on, outer phase off, w/o droplets formed; (c) inner phase off, middle phase on, outer phase on, o/w droplets formed; (d) inner phase on, middle phase on, outer phase on, w/o/w double emulsion droplets formed (Movie S1) (The scale bars are 500 μm .)

The droplets can be formed by dripping or jetting mechanism at the orifice of the tapered injecting capillary according to the relative flow rate of different phases. Generally, the sizes of droplets fabricated by dripping mechanism are more uniform than those of jetting mechanism. So we adjust the flow rate to insure both inner droplets and middle droplets are simultaneously in dripping regime to form uniform double emulsion droplets. Droplets are formed in dripping regime by two competing forces, namely, the surface tension holding the droplets to the tip and the drag force pulling the droplets downstream^[21]. The drag force grows with the droplets growing, the droplets detach from the tip when the drag force becomes comparable with the surface tension.

During fabricating double-emulsion droplets, both inner water droplet size and shell size can be precisely controlled by adjusting the relative flow rate. The drag force to the inner phase increases with increasing the middle phase flow rate which results in smaller inner droplets when the viscous drag force and surface tension reach a balance. When the flow rate of middle phase increases from 0.5 mL/h to 2 mL/h, the size of inner droplets decreases from 328 μm to 266 μm (Table S1 and Figs. 2a–2d). At the same time, the ratio of outer phase flow rate to middle phase flow rate decreases with increasing the flow rate of middle phase, this results in an increased outer droplet size from 353 μm to 402 μm . The number of droplets encapsulated in each larger drop also can be precisely controlled by adjusting the flow rates. When the dripping frequency of inner phase is equal to that of the middle phase, the annulus and core break simultaneously, generating double emulsion droplets with a single internal core. By increasing the flow rate of middle phase and decreasing the flow rate of outer phase, the middle phase dripping frequency decreases. When the dripping frequency of inner phase is twice of that of middle phase, droplets containing two small cores are fabricated (Table S1 and Fig. 2e).

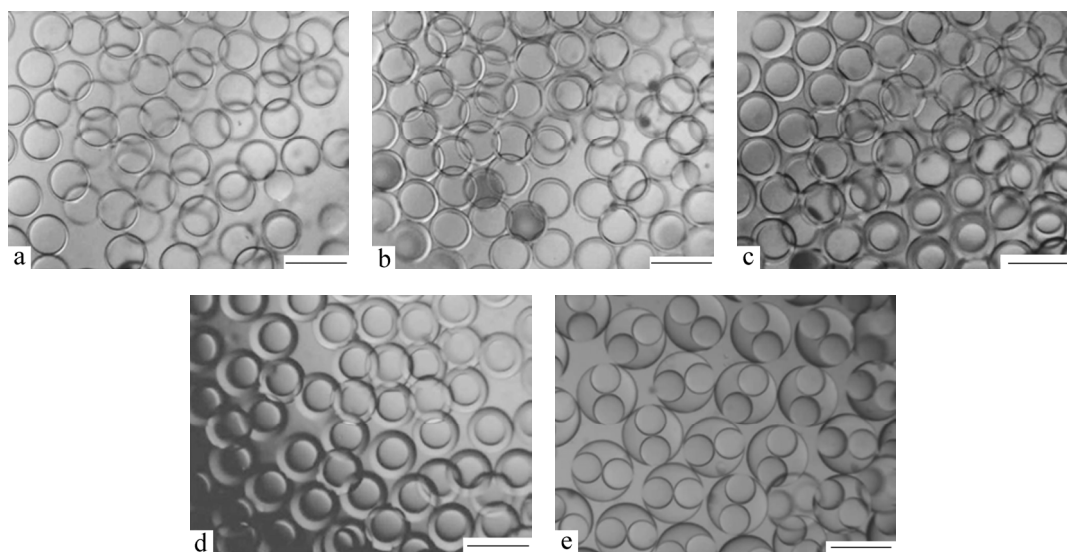


Fig. 2 Optical images of double emulsion droplets fabricated with different flow rates of inner phase, middle phase and outer phase: (a) 0.8 mL/h, 0.5 mL/h and 2.0 mL/h, (b) 0.8 mL/h, 1.0 mL/h and 2.0 mL/h, (c) 0.8 mL/h, 1.5 mL/h and 2.0 mL/h, (d) 0.8 mL/h, 2.0 mL/h and 2.0 mL/h, (e) 0.8 mL/h, 3.0 mL/h and 1.0 mL/h. Inner phase 2 wt% PVA, middle phase 10 mg/mL PEG-*b*-PS in toluene and chloroform (volume ratio is 62:38), outer phase 10 wt% PVA. Scale bar is 500 μm .

Good Solvent Evaporation/Diffusion Induced Acorn-like or Patched Polymersomes

The PEG-*b*-PS amphiphilic diblock copolymer was dissolved in a mixture of toluene and chloroform with a volume ratio of 67:33 at a concentration of 10 mg/mL and used as middle phase to fabricate double emulsion droplets. The double emulsion droplets have off-center core-shell structure because the density of mixed solvent is larger than that of the inner phase (Fig. 3a). When these off-center droplets are collected into 10% PVA aqueous solution, they float on the interface and expose to the air due to their lower density. Through quick evaporation of toluene and chloroform, the droplets are transformed into acorn-like (half thin shell and half thick shell) polymersomes (Fig. 3b and Fig. 3d left).

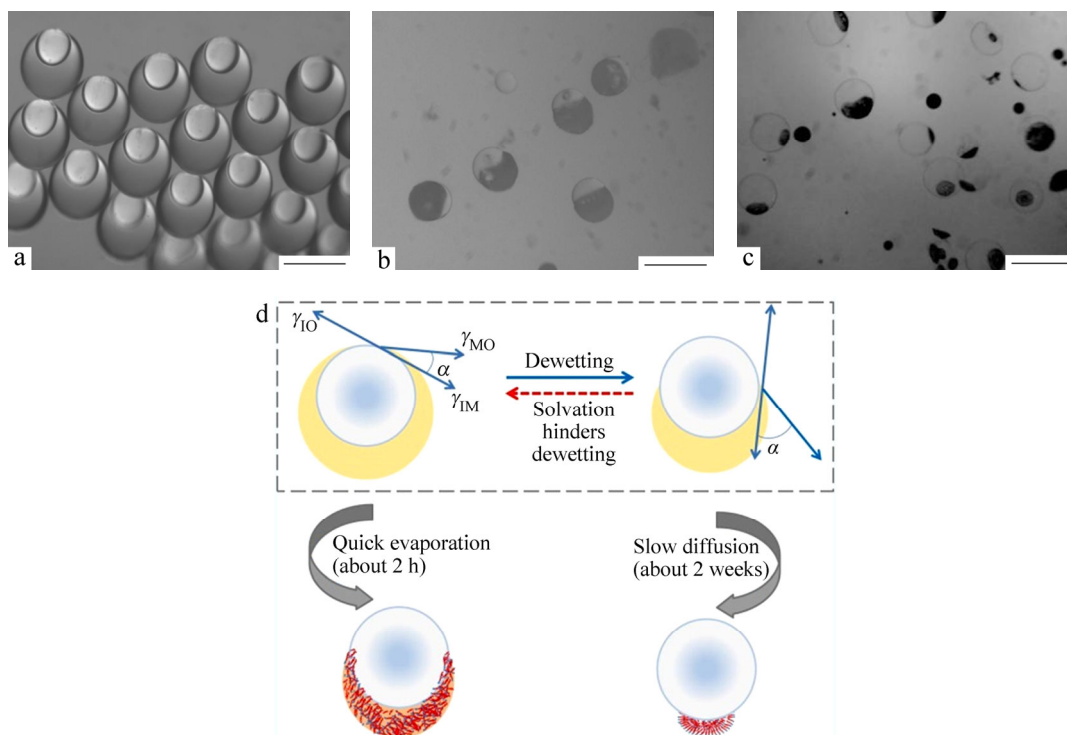


Fig. 3 (a) Off-center droplets (side view), (b) acorn-like vesicles collecting in 10 wt% PVA, (c) patched vesicles collecting in water, (d) illustration of droplets to polymersomes transformation with solvent evaporation and diffusion (Scale bars are 500 μm .)

This process is explained by using a dewetting and solvation mechanism. Amphiphilic PEG-*b*-PS molecules which adsorbed on the interfaces of both inner-middle and middle-outer phases are of crucial importance to stabilize the double emulsion droplets. But these double emulsion droplets are metastable systems and they have a dewetting tendency due to the negative spreading coefficient (S)^[22, 23], $S = \gamma_{IO} - \gamma_{IM} - \gamma_{MO} = \gamma_{MO} (\cos\theta - 1)$, where γ is the interfacial tension between the aqueous phase and the solvent phase, θ is the contact angle as illustrated in Fig. 3(d). This dewetting tendency prompts the separation of inner phase from oil droplets. Meanwhile, the solvation between amphiphilic macromolecules and solvent influences the stability of double emulsion droplets. Both toluene ($\delta_{\text{toluene}} = 18.2 \text{ J}^{1/2}/\text{m}^{3/2}$) and chloroform ($\delta_{\text{chloroform}} = 19.0 \text{ J}^{1/2}/\text{m}^{3/2}$) have the similar solubility parameter with PS ($\delta_{\text{PS}} \approx 18.2 \text{ J}^{1/2}/\text{m}^{3/2}$) or PEG ($\delta_{\text{PEG}} \approx 17.3 \text{ J}^{1/2}/\text{m}^{3/2}$) blocks. So toluene and chloroform are good solvents for the PEG-*b*-PS copolymer. This solvation inhibits the dewetting process. Thus, during quick evaporation of toluene and chloroform, these droplets have no enough time to further dewet, *i.e.* only partial dewet, and transform into the polymersomes with half thin shell and half thick shell (acorn-like).

However, when double emulsion droplets are collected in water, they sink to the bottom of the reservoir. The solvent in droplets cannot evaporate to the air directly but only diffuse into water slowly. With slow diffusion of chloroform and toluene into water, the droplets have enough time to dewet. After about 2 weeks, the solvent diffuses into water completely, and patched polymersomes (bilayer polymersomes with nearly uniform thickness and a small thicker patch of excess polymer) are available (Fig. 3c and Fig. 3d right).

Poor Solvent Induced Bilayer Polymersomes

In order to accelerate the dewetting process, hexane is added to the middle phase of double emulsion droplets. Hexane has a solubility parameter ($\delta_{\text{hexane}} = 14.9 \text{ J}^{1/2}/\text{m}^{3/2}$), which is much less than the solubility parameter of PS and PEG. So hexane is poor solvent of amphiphilic PEG-*b*-PS block copolymer. However, the solubility parameter (δ_{mix}) of the mixture of chloroform and hexane (volume ratio 38:62) can be calculated by equation:

$$\delta_{\text{mix}} = \varphi_{\text{hexane}} \delta_{\text{hexane}} + \varphi_{\text{chloroform}} \delta_{\text{chloroform}}$$

which is about $16.5 \text{ J}^{1/2}/\text{m}^{3/2}$, so the mixture can well dissolve the PEG-*b*-PS amphiphilic diblock-copolymer. The droplets using this mixed solvent as middle phase have off-center structure with the core at the bottom of the droplets because of density difference. The prepared droplets are collected in a 10 wt% or 2 wt% aqueous solution of PVA. They float on the surface and expose to air (Fig. 4a). With solvent quick evaporation, inner droplets bud from the bottom of the droplets and separate from oil droplets (Figs. 4b–4d) and finally complete bilayer polymersomes are formed (Fig. 4e). The dewetting process is about 1 min. Obviously, the process differs from that of the above-mentioned chloroform-toluene system. Chloroform has lower boiling temperature than hexane, so it is more quickly to evaporate. With evaporation of solvent in middle phase of droplets, the ratio of hexane in mixed solvent gradually increases. The PEG-*b*-PS molecules tend to aggregate at the two interfaces to form bilayer membranes. This aggregation accelerates the dewetting process and finally the wholly bilayer polymersomes are formed due to complete dewetting (Fig. 4f). Double emulsion droplets containing two inner droplets can also dewet to form twin polymersomes. During the dewetting process, the two droplets neither burst nor coalesce (Figs. 4a–4e and Movie S2).

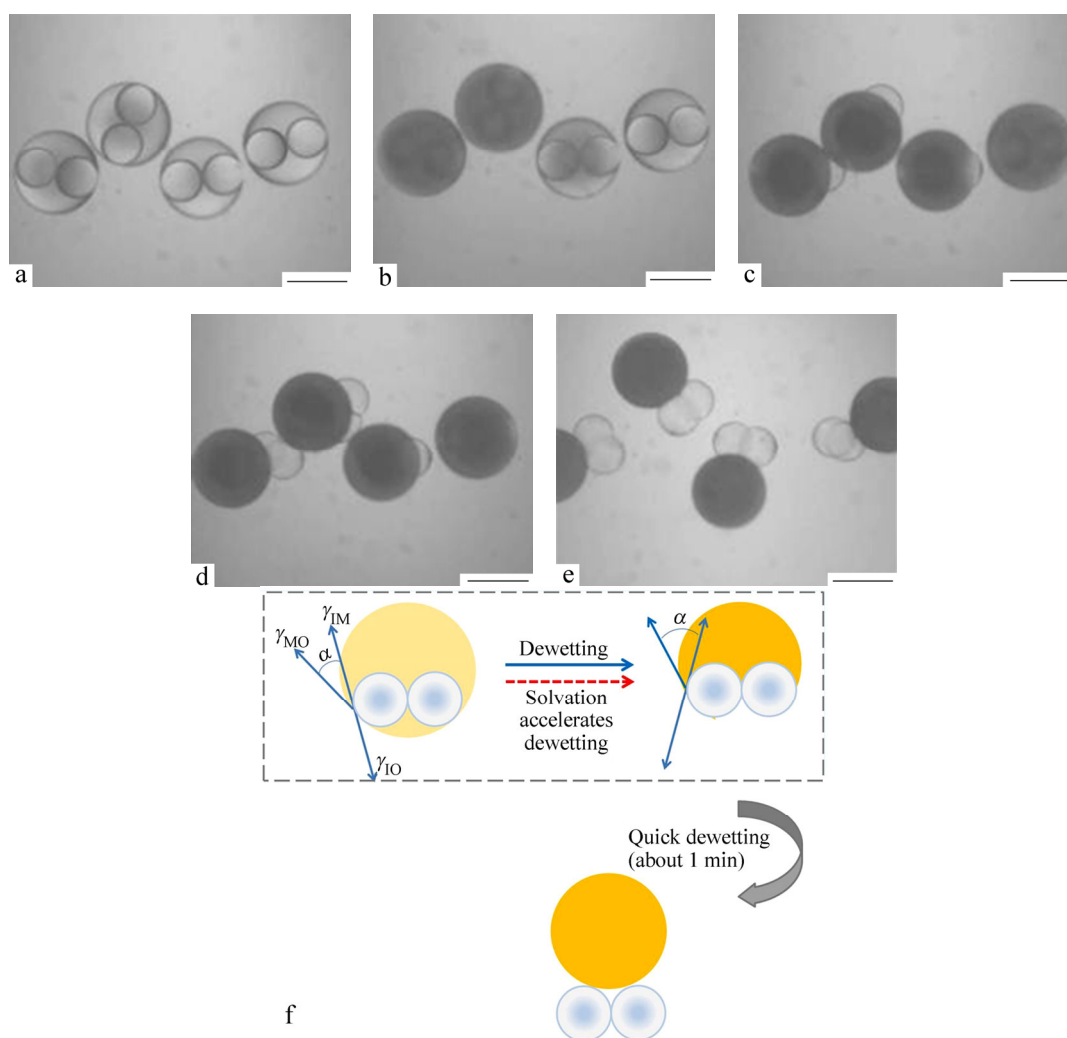


Fig. 4 (a–e) Sequence images of w/o/w droplets containing two cores undergoing dewetting transition with intervals of 10 s (Movie S2); (f) Illustration of the droplets to polymersomes transformation with dewetting (Scale bars are 500 μm .)

In order to detach the oil droplets and polymersomes after dewetting, A 10% PEG solution serves as inner phase and a 3% PEG solution serves as collecting solution. Polymersomes after dewetting immediately sink to the bottom, however the oil drops keep floating on the surface due to their low density as shown in Figs. 5(a)–5(f) and Movie S3. The uniform polymersomes are collected at the bottom of container (Figs. 5g–5h).

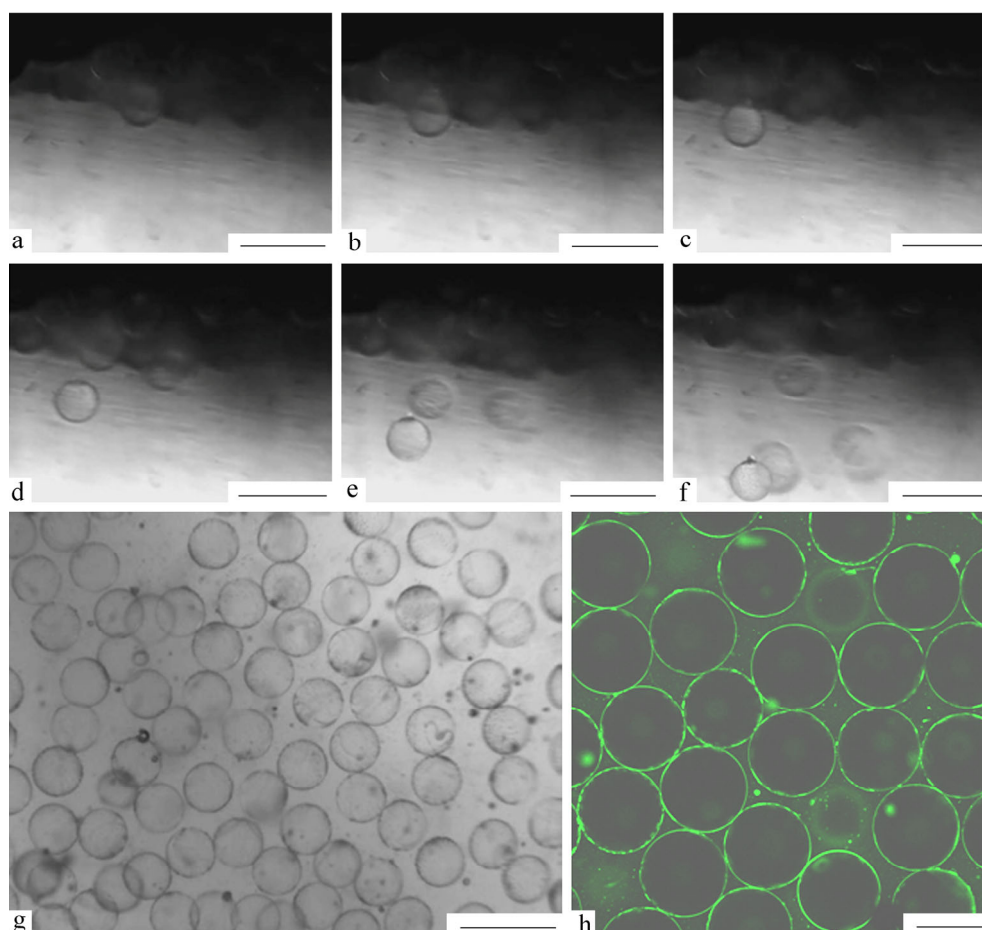


Fig. 5 (a–f) Sequence images of polymersomes detaching from oil droplets taken at intervals of 1 s (side view, Movie S3); (g) Sinking of polymersomes to the bottom of container; (h) Confocal laser scanning micrograph of polymersomes containing dye coumarin 6 (The scale bars are 500 μm for (a–g) and 200 μm for (h).)

CONCLUSIONS

Monodisperse w/o/w double emulsion droplets with one inner core or two inner cores are fabricated in a microfluidic device. The sizes of inner core and outer shell are tuned by changing the relative flow rate. Morphology-controllable giant polymersomes are available through a dewetting process of the double emulsion droplets. It is found that good solvent in oil phase inhibits the dewetting, and acorn-like polymersomes or patched polymersomes are obtained; but poor solvent in oil phase accelerates the dewetting tendency and results in a quick formation of nearly uniform bilayer polymersomes. Twin polymersomes are also prepared by this quick dewetting process. It is considered that the formation of polymersomes is related to both dewetting of double emulsion droplets and solvation between amphiphilic macromolecules and solvent. In addition, only adjusting the densities of inner phase and collecting solution, effective separation between oil droplets and polymersomes is realized readily. Definitely, this paper provides a facile approach to prepare giant and uniform polymersomes with controllable size and morphology.

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