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Bursting and Reassembly of Giant Double Emulsion Drops Form Polymer Vesicles

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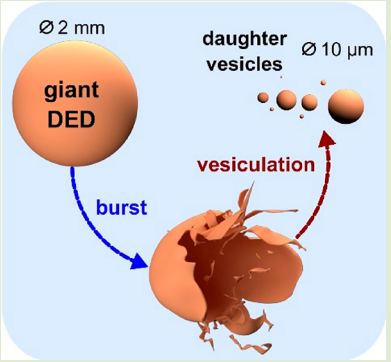
ABSTRACT: Polymeric vesicles are excellent building blocks of synthetic compartmentalized systems such as protocells and artificial organelles. In such applications, the efficient encapsulation of materials into the vesicles is an essential requirement. However, common encapsulation techniques can be time-consuming, demand special equipment or have limited efficiency for large components, such as proteins and nanoparticles. Here, we describe a simple method to create cargo-filled polymer vesicles based on bursting and reassembly of giant double emulsion droplets (DED). Due to their large average diameter of 2 mm, DEDs eventually burst in the aqueous medium, producing polymeric film fragments. These fragments rapidly reassemble into smaller vesicles in a process involving folding, fusion and vesiculation. The daughter vesicles have an average diameter of 10 μm, representing a two-order of magnitude size reduction compared to the original DED, and can efficiently encapsulate components present in solution by entrapment of the aqueous medium during vesicle reassembly.



Polymeric vesicles are tough and chemically versatile compartments that can be used as basic frameworks for supramolecular systems such as protocells, artificial organelles, and microreactors.[1−5] Similar to biological cells, the functions of these vesicular systems are defined by the components encapsulated in the vesicles.[6−11] For that reason, it is desirable that the methods used for vesicle formation also feature a mechanism for efficient encapsulation of materials. Techniques for vesicle formation include microfluidics and phase-inversion, which offer excellent encapsulation efficiency. However, they can be time-consuming, often requiring special equipment and additives.[12−14] Other approaches based on film hydration, although simpler, can show limited encapsulation efficiency for large components such as proteins and nanoparticles.[15,16]

Here, we introduce the bursting and reassembly (BnR) of giant double emulsion drops (DED) as a new method that combines vesicle formation, efficient encapsulation of large components, and minimum equipment requirement. In contrast to other techniques, BnR explores the inherent instability of large DEDs and the unique reassembly properties of amphiphilic films to create new vesicles.

BnR designates the chain of events that leads to the formation of small vesicles through the folding and fusion of membrane fragments created when a vesicle is ruptured. For instance, certain cells can burst, creating membrane fragments that reassemble into smaller vesicles.[17,18] Liquid films stabilized by amphiphilic molecules can also undergo BnR.[19,20] Perhaps the most illustrative example of this phenomenon is the formation of small soap bubbles as result of bursting of larger ones.[21]

At a first glance, the BnR of giant DEDs resembles the BnR observed in large soap bubbles (Scheme 1). But, in contrast to soap bubbles, which vanish once their aqueous film evaporates, DEDs leave behind a robust polymeric membrane that grants daughter vesicles their valuable high stability and chemical properties.

Our first challenge was to find a convenient way to create giant DEDs. To prevent premature bursting of the large drops, it was critical to keep shear forces to a minimum during the formation of the DEDs. This was achieved by using the phase-transfer method detailed in Figure 1a.

First, a liquid interface between water and toluene was prepared in a microcentrifuge tube. The low molecular weight copolymer poly(butadiene)$_{22}$-*b*-poly(ethylene oxide)$_{14}$ (BD_{22}-EO_{14}) was used as a macromolecular surfactant to reduce the interfacial tension (σ) between the immiscible liquids, which facilitated the formation of the giant DEDs (Figure 1c).[22,23] Additionally, the copolymer formed the membrane of the vesicles after toluene removal. The formation of each giant DED started with the addition of single drops of heavy water (D_2O) containing sucrose on top of the toluene layer. Due to their higher density, the drops rapidly sank and crossed the low σ liquid interfaces. This process created giant DEDs (Figure

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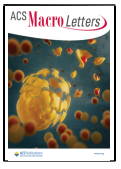
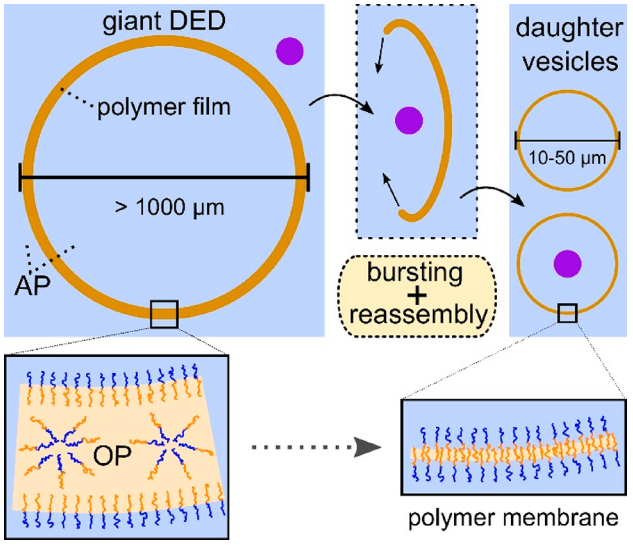


Scheme 1. Polymer Vesicles by Bursting and Reassembly (BnR)[a]



[a]A giant DED bursts, producing fragments that rapidly fold to form daughter vesicles. In the process, materials (purple dot) present in the aqueous phase (AP) can be encapsulated. Continuous removal of the organic phase (OP) results in thin vesicle membranes.

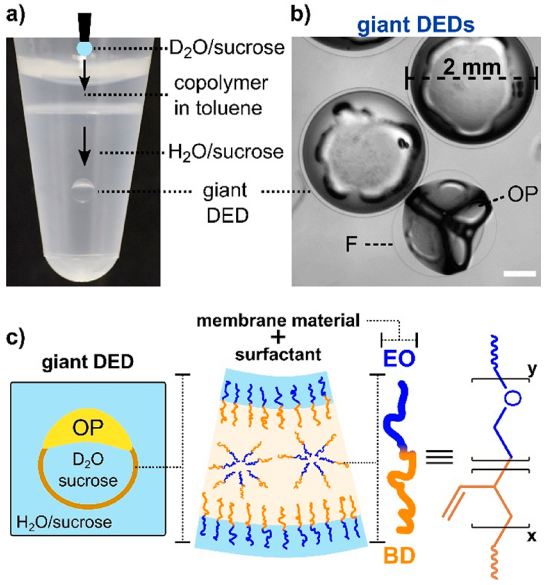


Figure 1. Formation of giant DEDs by the phase-transfer method. (a) Photograph of the experimental setup showing a single giant DED. (b) Top view of a bright field micrograph with three giant DEDs. F: polymer film; OP: copolymer/toluene solution. Scale bar: 500 μm. (c) Scheme of the cross-section of a giant DED and the composition of the corresponding copolymer film. BD: 1,2-polybutadiene; EO: poly(ethylene oxide).

1b) with an average diameter of 1.9 ± 0.6 mm. Micrographs taken immediately after DED formation revealed drops at an advanced state of dewetting, as indicated by the clear separation between the polymer-rich film (F) and the toluene-rich phase (OP) on top of the drops.

Next, a series of $(BD)_x$-$(EO)_y$ copolymers with different block lengths (x, y) were tested to determine the effect of copolymer molecular weight and concentration on giant DED formation. Figure 2a shows the results for each copolymer.

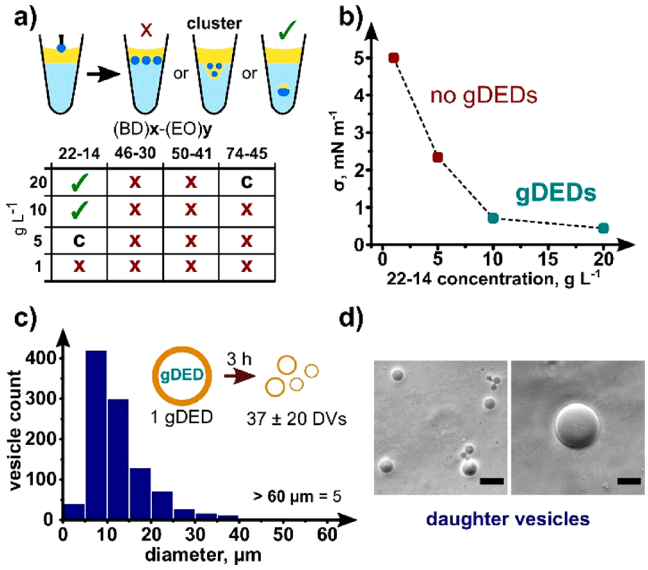


Figure 2. Effect of BD-EO type on giant DED formation and vesicle yield. (a) Copolymer type and concentrations required for giant DED formation. (b) Change on interfacial tension (toluene/water) as a function of $(BD)_{22}$-$(EO)_{14}$ concentration. Method: spinning drop tensiometry. (c) Size distribution of daughter vesicles and average vesicle yield per giant DED. Average represents the combined results of 15 giant DEDs in triplicate. (d) Phase contrast micrographs of daughter vesicles. Scale bar: 50 μm.

Giant DEDs only formed with $(BD)_{22}$-$(EO)_{14}$, the shortest copolymer in the series, and only at concentrations of 10 and 20 g·L^{-1}. At 5 g·L^{-1}, the D_2O drops only crossed the water–toluene interface as clusters and at 1 g·L^{-1} the drops could not cross. These results were expected since the presence of the surfactant at the interface depends on the initial copolymer concentration and how fast the copolymer can diffuse in solution. Therefore, concentrations below 5 g·L^{-1} were just too low to provide the required drop in σ. In addition, copolymers with chains longer than $(BD)_{22}$-$(EO)_{14}$ could not rapidly adsorb at the interface due to their relatively limited diffusion in solution, and giant DEDs could not be formed independently of concentration.[24] The minimum σ required for the successful formation of giant DEDs was determined for $(BD)_{22}$-$(EO)_{14}$. As shown in Figure 2b, giant DEDs were only observed for σ < 1 mN m^{-1}.

The giant DEDs were stable only for a short time, inevitably bursting within 5 min from the moment they were formed. Our hypothesis was that bursting of the giant DEDs would result in the production of large membrane fragments, which could fold to form new and smaller vesicles. Indeed, we observed new vesicles by optical microscopy after bursting of giant DEDs prepared with $(BD)_{22}$-$(EO)_{14}$. The daughter vesicles had an average diameter of 10 μm, corresponding to a two-orders of magnitude size reduction compared to the original giant DEDs (Figure 2c,d). On average, each giant DED produced 37 daughter vesicles.

Next, we showed that the daughter vesicles formed by a BnR type mechanism. This was demonstrated through a sequence of images covering the time span from bursting until vesicle formation. The images were obtained at a rate of one per second. Figure 3a shows selected frames from one sequence.

The first frame shows two giant DEDs that were about 5 min old when the image was taken. After 55 s, the DEDs burst in response to the instabilities caused by solvent separation.[25,26]

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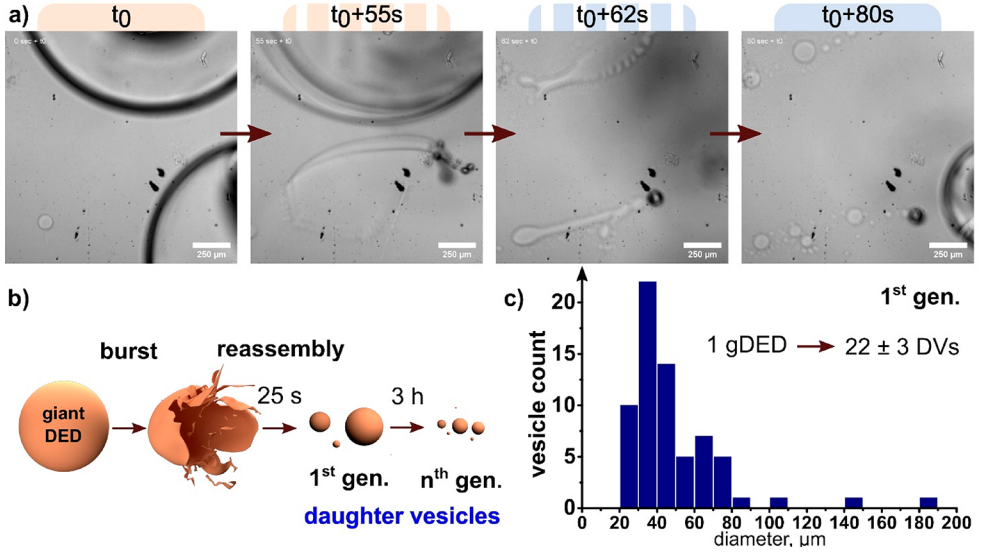

Figure 3. Bursting and reassembly of giant DEDs. (a) Bright field images of selected frames from the time-lapse acquisition during BnR of giant DEDs. Scale bars: 250 μm. (b) Schematic depiction of distinct events that occurred during BnR. (c) Vesicle diameters of the first generation of daughter vesicles. Statistical and experimental details are in the Supporting Information.

Bursting occurs because the membrane could not efficiently relax the surface tension gradients that arose during dewetting.[20,27] Toluene being less dense than water was pushed toward the top of the DED and created an upward force that strained the membrane, which eventually ruptured. As shown in Figure 3a, the DED burst produced fragments that rapidly folded in order to eliminate their hydrophobic and highly curved edges.[28,29]

Before vesicles could form, the folded fragments first produced short-lived tubular structures. These tubes rapidly collapsed due to a "pearling" instability, which resulted in the formation of the daughter vesicles.[30] Overall, the formation of daughter vesicles occurred through a BnR mechanism that took less than 30 s. The main events are illustrated in Figure 3b. Full movies and images of selected frames are also available (Movies 1−3, Figures S5−S27). BnR of giant DEDs produced, on average, 22 daughter vesicles per DED immediately after burst (first generation). The mean diameter of the first generation was about 50 μm. However, measurements made after 3 h showed a reduction in average diameter, which stabilized at about 10 μm. This size reduction is consistent with vesicle budding, which is induced by the excess of copolymer in the membrane of the first generation of daughter vesicles.[31] Micrographs taken several hours after BnR clearly showed that vesicle replication continued through vesicle budding (Figure S1). All vesicles featured the expected hollow structure surrounded by a thin polymer membrane (Figure S2). GC-MS measurements showed that less than 10% of the toluene remained in the system after 2 h of solvent separation, falling below 4% after 4 h (Figure S3).

One essential characteristic of BnR, as illustrated in Figure 3b, is that its mechanism allows substances to be captured by the daughter vesicles during the reassembly step. BnR is not limited by the typical low water permeability of polymer membranes (ca. 2.5 μm s^{-1}).[4] That contrasts with hydration-based methods, where encapsulation of materials into vesicles requires an efficient transport of the aqueous solution through the polymer membrane.[32] To demonstrate the encapsulation properties offered by BnR, we performed the dye-entrapment experiment illustrated in Figure 4.

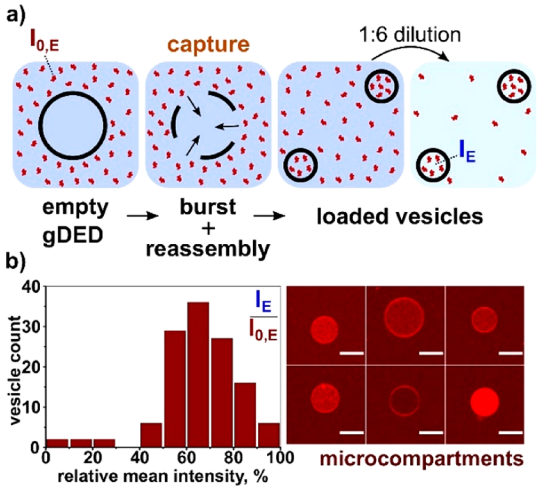

Figure 4. Encapsulation of a macromolecule by BnR. (a) Scheme of the dye-entrapment experiment. Giant DEDs were formed in a medium containing a fluorescent macromolecular tracer (in red). $I_{0,E}$: tracer emission intensity of the external medium. I_E: tracer emission intensity inside the daughter vesicles. (b) Relative mean fluorescence intensity (RMF, $I_E/I_{E,0}$) inside the daughter vesicles. Scale bars: 20 μm. Tracer: bovine serum albumin Alexa Fluor 647 conjugate (BSA-AF 647). Vesicle membrane stained with Nile Red. Data acquired by confocal laser scanning microscopy.

The experiment consisted of determining by confocal laser scanning microscopy the relative amount of a bulky macromolecular fluorescent tracer (BSA-AF 647) that was entrapped in the vesicles formed by BnR. To quantify the encapsulation efficiency, the relative mean fluorescence intensity of the tracer (RMF) was calculated, as shown in Figure 4. Our results showed that only a small fraction (2%) of the vesicles were essentially empty (RMF < 5%), while most of them (over 75%) were able to encapsulate the tracer in high amounts as indicated by RMFs values greater than 50%.

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The fact that a few vesicles were empty or contained only small amounts of the tracer is consistent with the low encapsulation efficiency of vesicles formed through budding. Vesicles formed by BnR, on the other hand, were much more efficient at encapsulating the tracer, because the encapsulation mechanism involved the entrapment of the surrounding aqueous medium inside the new vesicles. If the tracer was initially present in the giant DED, most of it was lost after burst (Figure S4). Therefore, the encapsulation efficiency highly depended on the location of the materials (inside or outside the DEDs) before BnR took place.

In summary, we have shown that polymer vesicles can be formed by bursting and reassembly of giant DEDs. We explored its remarkable mechanism as new way to encapsulate materials present in an aqueous medium. Moments before the vesicular structure was restored, the daughter vesicles were able to capture and entrap the macromolecular tracer present in solution. Our results demonstrate that BnR methods, such as the one described here, could be developed into a completely new way to create vesicular compartments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmacrolett.0c00849.

Sections 1 and 2 contain details of materials and characterization methods. Section 3 describes the BnR method and include additional statistical information. Figure S1 illustrates the budding effect. Figures S2−S4 contains additional data. Figures S5−S27 show bright field images of the BnR process over time (PDF)

Movie SI of vesicle formation by BnR (AVI)

Movie S2 of vesicle formation by BnR (AVI)

Movie S3 of vesicle formation by BnR (AVI)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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