Cytomimetic Chemistry

Real-Time Membrane Fission of Giant Polymer Vesicles**

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Vesicle transformations that mimic cellular processes have become a specific topic of interest in recent years.[1] Lipid vesicles (liposomes), surfactant vesicles, and block-copolymer vesicles are conventional and useful model membranes for living cells.[2–5] Most model membranes reported are submicroscopic (20–500 nm) in nature and conclusions concerning these membranes are often based on inference rather than on direct observation. Menger and co-workers have successfully used giant vesicles (5–200 μm), which have the advantage of being microscopic and therefore visible, to directly observe real-time shape transformations of vesicles that mimic cellular morphology. They coined the term "cytomimetic chemistry" to describe the visible cell-like morphologies of giant vesicles.[6] Up to now, many interesting cell-like activities such as birth, budding, endocytosis, exocytosis, fusion, and fission have been reported in studies of cytomimetic behavior.[6–8] However, all of the vesicles that have been used so far in cytomimetic chemistry are giant liposomes, and no real-time transformations of polymer vesicles have been observed. Herein, we present work to develop the cytomimetic chemistry into the field of polymers by using giant polymer vesicles as model membranes ("cytomimetic macromolecular chemistry"). We also describe a new and sequential membrane fission process by displaying a series of high-resolution and real-time transformation images of individual vesicles.

Membrane fission is very important and common in various biological processes such as endocytosis. However, there has been little experimental work on vesicle fission that mimics cell fission at the phenomenological level. Luisi and co-workers found evidence of fission of larger vesicles by adding fresh surfactant into preformed vesicles; they called this phenomenon "the matrix effect" to stress that the pre-existing vesicles or liposomes act as a matrix for the formation of new vesicles.[2] Nolte and co-workers also detected vesicle fission by adding calcium ions into synthetic phospholipid vesicles and presented a possible mechanism.[3b] Eisenberg and co-workers also proposed a fission sequence by carefully arranging several images selected from a large number of TEM micrographs of submicroscopic block copolymer vesicles.[5c,d] The mechanisms for vesicle fission mentioned above are speculative and based on characterization data obtained from TEM and light scattering. In contrast, Döbereiner, Sackmann, and co-workers—the pioneers of cytomimetic chemistry—used optical microscopy to monitor the real-time fission of giant lipid vesicles induced by heating and osmotic deflation.[8] Kitamura also presented images of real-time fission of giant lipid vesicles induced by a laser.[9] Unfortunately, these images in are low-resolution or segmental. To understand similar behavior in living cells, it is necessary to obtain high-resolution, real-time, sequential images of individual vesicles undergoing fission. In addition, the use of giant polymer vesicles as model membranes in cytomimetic chemistry is a new and exciting area of research.

Compared with liposomes, polymer vesicles are very stabile. Eisenberg and co-workers reported a type of polymer vesicle called "crew-cut micelles", which are obtained by the self-assembly of block copolymers that have small hydrophilic fractions ($<20\,\%$) in a mixture of solvents.[5] Discher and co-workers reported another type of polymer vesicle termed "polymersomes" from block copolymers that have a hydrophilic fraction ($35\pm10\,\%$) similar to those of liposomes.[10] Very recently, we reported a new sort of polymer vesicle called "branched polymersomes". These branched polymersomes were synthesized through molecular self-assembly of an ill-defined hyperbranched copolymer, HBPO–star–PEO (HPBO is hyperbranched poly(3-ethyl-3-oxetanemethanol); PEO is polyethylene oxide), which has a higher hydrophilic fraction ($>60\,\%$) in water.[11] HBPO–star–PEO is an amphiphilic multiarm copolymer with a hydrophobic hyperbranched HBPO core and a large population of hydrophilic PEO arms.[11] Three giant polymer vesicles, HB3, HB2, and HB1, with average diameters of 4.0 μm, 22.6 μm, and 112.8 μm, respectively, were obtained[11b] The molecular self-assembly process of vesicles HB1–3 involved directly putting the polymer into stirred deionized water (polymer concen-

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tration 10 mg mL^{-1}) at room temperature. HB1–3 vesicles not only have the advantages of polymer vesicles but are also visible under the microscope as are giant lipid vesicles. Thus, HB1–3 vesicles are ideal model membranes for cytomimetic chemistry.

HB2 vesicles were chosen to investigate the real-time cytomimtetic behavior under an optical microscope. Detailed structures of the HB2 molecules and vesicles are described in the Supporting Information. Membrane fission of a daughter vesicle inside a mother vesicle was observed by adding glucose to the HB2 vesicle solution (Figure 1). The daughter

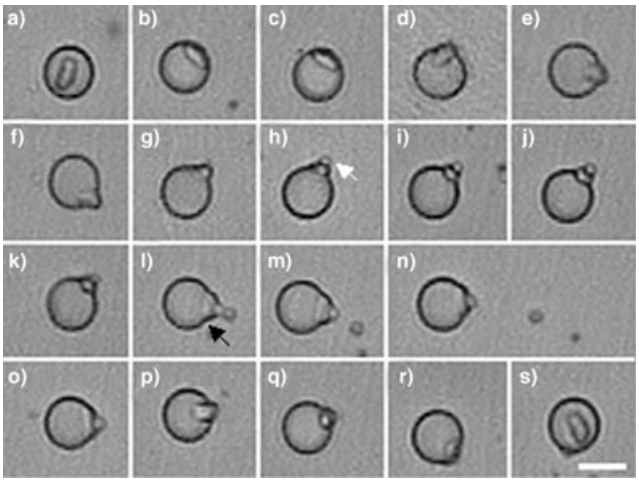

Figure 1. A series of images showing the interaction of a daughter vesicle inside its mother vesicle during fission. The scale bar represents 25 μm. The time of image (a) is set as 0, and the elapsed times for the images are 33 (b), 64 (c), 105 (d), 130 (e), 134 (f), 165 (g), 170 (h), 179 (i), 182 (j), 190 (k), 243 (l), 246 (m), 263 (n), 273 (o), 421 (p), 620 (q), 719 (r), and 920 s (s), respectively. Images (a–g), (h–o), and (p–s) are defined as the prophase, metaphase, and anaphase of fission, respectively. For the fission experiment, glucose (15 mM) was added to an aqueous solution of HB2 vesicles. After the glucose had dissolved, the sample was subjected to ultrasound for 10 min before being transferred to a culture dish for optical microscopy measurements (Leica Dmlp, TMS94) at 20°C.

vesicle underwent five fission steps: suspension inside the mother vesicle (Figure 1 a); coalescence with the mother vesicle (Figure 1 b,c); protrusion from the membrane of the mother vesicle to form a pear shape (Figure 1 d–i); fission (Figure 1 m,n); retraction into the mother vesicle (Figure 1 o–s); suspension of the daughter vesicle for several minutes until the fission cycle ends or repeats itself (Figure 1 s). We have observed one daughter vesicle inside a mother vesicle undergoing three fission cycles, with each cycle taking longer to complete than the previous. We observed at least eight vesicles displaying similar fission behavior to that shown in Figure 1.

Clearly, it is the daughter vesicle that undergoes membrane fission. Experimental evidence to support this claim comes from three sources. First, we have not observed spontaneous budding from a single vesicle without the presence of an inner daughter vesicle. Second, the sequential images (Figure 1 a–l) suggest that the daughter vesicle passes through the mother vesicle to form the pear-shaped structure (Figure 1 h–l), and it is the deformation of this structure that results in membrane fission (Figure 1 l–o). We also find that formation of a pear-shaped structure is not certain to lead to vesicle fission; sometimes the vesicles maintain the pear shape for a relatively long time without further fission. Instead, there is a gradual retraction of pear shape into the mother vesicle and the intact daughter vesicle is formed again (see the Supporting Information). The images in Figure 1 (Figure 1 a–l) combined with those in the Supporting Information confirm that the pear-shaped structure, from which the fission takes place, is formed from the daughter vesicle. The third piece of evidence comes from the change in size of the daughter vesicle before and after membrane fission. The daughter vesicle is irregular but can be regarded as quasi-ellipse. Thus, we can define the daughter vesicle in terms of the length of the major and minor axes. The major axis of the daughter vesicle before fission (≈ 7 μm) is longer than that of the daughter vesicle after fission (< 6 μm), as is the minor axis. Nevertheless, the daughter vesicle is not a regular ellipse, so it would be better to find a more precise way of comparing the volume of the vesicles. We overlapped the daughter vesicle before and after fission by using a computer program (see the Supporting Information) and the result clearly show that the size of the daughter vesicle decreased after fission.

How does the vesicle fission shown in Figure 1 take place? We believe that the osmotic pressure and the mother vesicle induce the fission. Similar to biological membranes, liposomes are semipermeable and are much more permeable to water than to glucose. It is well known that polymer vesicles are less permeable than liposomes.[5] Therefore, it is conceivable that water can easily permeate the HB2 vesicle membrane, however, permeation of glucose, a larger molecule, through the vesicle membrane is more difficult. In fact, the semipermeable membrane property of the HB1–3 vesicle has been verified by a vesicle-shrinkage experiment in another hyperosmotic solution (results not shown). So the addition of glucose greatly increases the difference in osmotic pressure between the aqueous interior of the HB2 vesicle and the bulk solution, which drives water and the daughter vesicle out of the mother vesicle.

During the fission, the daughter vesicle moves towards the mother vesicle and often part of the daughter breaks through (Figure 1 a–d). However, it does not directly penetrate the mother vesicle because of the intrinsically high stability of the polymer vesicle.[5,10] The membrane of the mother vesicle works as a tough elastic barrier, thus preventing the daughter vesicle from completely breaking out. The daughter and mother vesicles make contact and at that point the mother vesicle, driven by the movement of the daughter vesicle, deforms into a bud structure (Figure 1 e–g). A comparison of the mother vesicle before and after deformation (Figure 1 a and f; see also the Supporting Information) shows that the surface of the mother vesicle increases after forming the bud. This expansion of the membrane should lead to an increase in surface tension.[12] Figure 2 a–f shows the models to illustrate the deformation of the daughter vesicle and mother vesicle to form the bud. Details of the interaction between the mother and daughter vesicles are still under investigation in our lab and will be reported in due course.

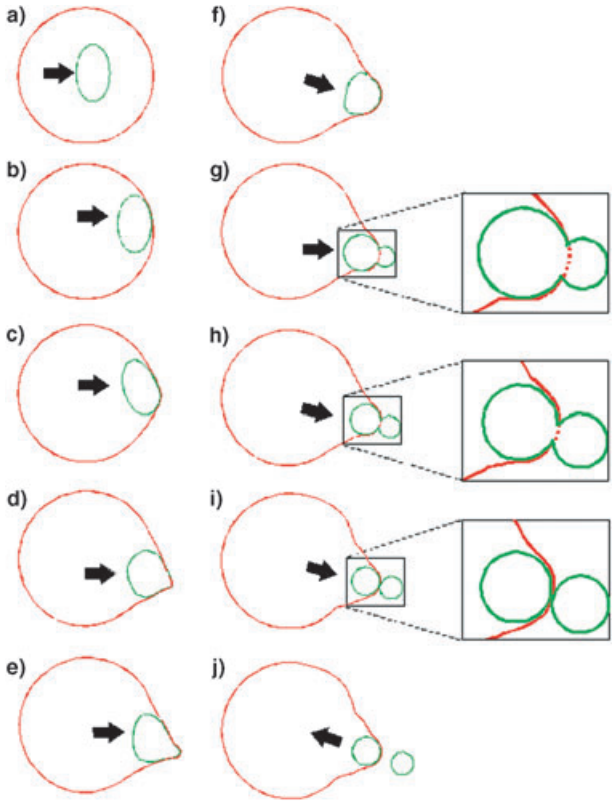

Figure 2. Models of vesicle transformations during membrane fission shown in Figure 1. The mother vesicle is in red and the daughter vesicle is in green. Black arrows show the movement of the daughter vesicle. The red dashed curves in (g) and (h) show the intersecting line resulting from the penetration of the mother vesicle by the daughter vesicle.

Figure 1 h shows an important intermediate: the partial penetration of the daughter vesicle through the mother vesicle. Figure 1 shows the mother vesicle in black and the daughter vesicle in gray. The black profiles of the vesicle image in Figure 1 a–g indicate that the daughter vesicle is enveloped by the mother vesicle during these changes in morphology. However, in Figure 1 h the gray vesicle has partly passed through the black vesicle (white arrow). From a comparison of the volume of the daughter vesicle in Figure 1 h with that in Figure 1 a, it is clear that the gray membrane located outside the black vesicle in Figure 1 h comes from the daughter vesicle. Evidently, the daughter vesicle has partly penetrated the mother vesicle by the force of osmotic pressure. It is conceivable for the daughter vesicle to break through the mother vesicle if the tension induced by the movement of the daughter has exceeded the tolerance of the mother. Further evidence of the penetrating ability of the daughter vesicle came from experiments with a higher glucose concentration (25 mM): the daughter vesicle was observed to completely pass through the mother vesicle forced by the higher osmotic pressure (see the Supporting Information). This process is similar to the birthing process reported by Menger and Gabrielson.[6b,c]

Figure 1 i–l shows the sequential deformation of the daughter vesicle inside and outside the mother vesicle. The penetration by the daughter leads to a "wound" in the mother vesicle and the mother heals the wound spontaneously.[6b,c] In addition, the daughter vesicle continues to penetrate the mother vesicle driven by osmotic pressure. Figure 2 g–i shows the possible changes of the daughter vesicle during the penetration. Figure 2 g shows that the daughter vesicle is divided into two by the mother vesicle and a neck is formed in the interface, which shapes the daughter vesicle into a pearlike structure. As the wound in the mother vesicle heals and the penetration by the daughter vesicle continues, the neck becomes narrower and narrower (Figure 2 h). Then the daughter becomes two vesicles that are in contact with each other as shown in Figure 1 l and Figure 2 i.

The strong deformation of the mother vesicle in Figure 1 l is of particular interest. Figure 1 l shows the mother vesicle at the point of greatest deformation (indicated by the black arrow; see also Figure 2 i), which leads to the large tension that retracts the part of the daughter vesicle retained inside the mother. Thus, complete fission takes place (Figures 1 m, 1 n, and 2 j). We have found that vesicle fission will only occur after the deformation of the mother and daughter vesicles has reached the state shown in Figure 1 l, which is the critical morphology for vesicle fission. The deformation of the mother vesicle decreases after releasing a small vesicle (Figure 1 m–o), the mother vesicle gradually recovers its spherical morphology (Figure 1 p–s), and the daughter vesicle is fully retracted.

Fission processes reported previously involve only one vesicle, whereas the fission described herein is new as it involves two vesicles. There is a strong interaction between mother and daughter vesicles during fission and deformation of the mother occurs with concomitant fission of the daughter. We have called this process "cooperative fission".

The "spontaneous curvature" and "area difference elasticity" models developed by Döbereiner, Sackmann, Seifert, and others have been successfully used to forecast, explain, and analyze the budding and fission of lipid vesicles.[8] The theoretical models mostly deal with a fission process that involves only one vesicle. The participation of a mother vesicle greatly increases the complexity of the studied "cooperative fission". A modified physical model that takes into account the geometrical and mechanical constraints of the mother vesicle is needed. Nevertheless, since both the theoretical and experimental results with lipid vesicles have stressed the pear structure and its importance in subsequent membrane fission, the behavior of lipid vesicles and polymer vesicles overlap here in membrane fission.

In conclusion, we have presented a novel real-time membrane fission process, called cooperative fission, by using giant polymer vesicles as model membranes. A mother vesicle with a daughter vesicle within shows a cooperative effect during membrane fission, which is caused by osmotic pressure combined with the stability of the mother polymer vesicle. Our finding extends the field of cytomimetic chemistry into polymers. These results along with previous reports on liposomes and surfactant vesicles,[2–6,8,9] lead us to the conclusion that membranes—regardless of the composition, whether they involve small molecules or macromole-

cules, whether synthetic or natural—can undergo this cytomimetic process in the absence of a protein.

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