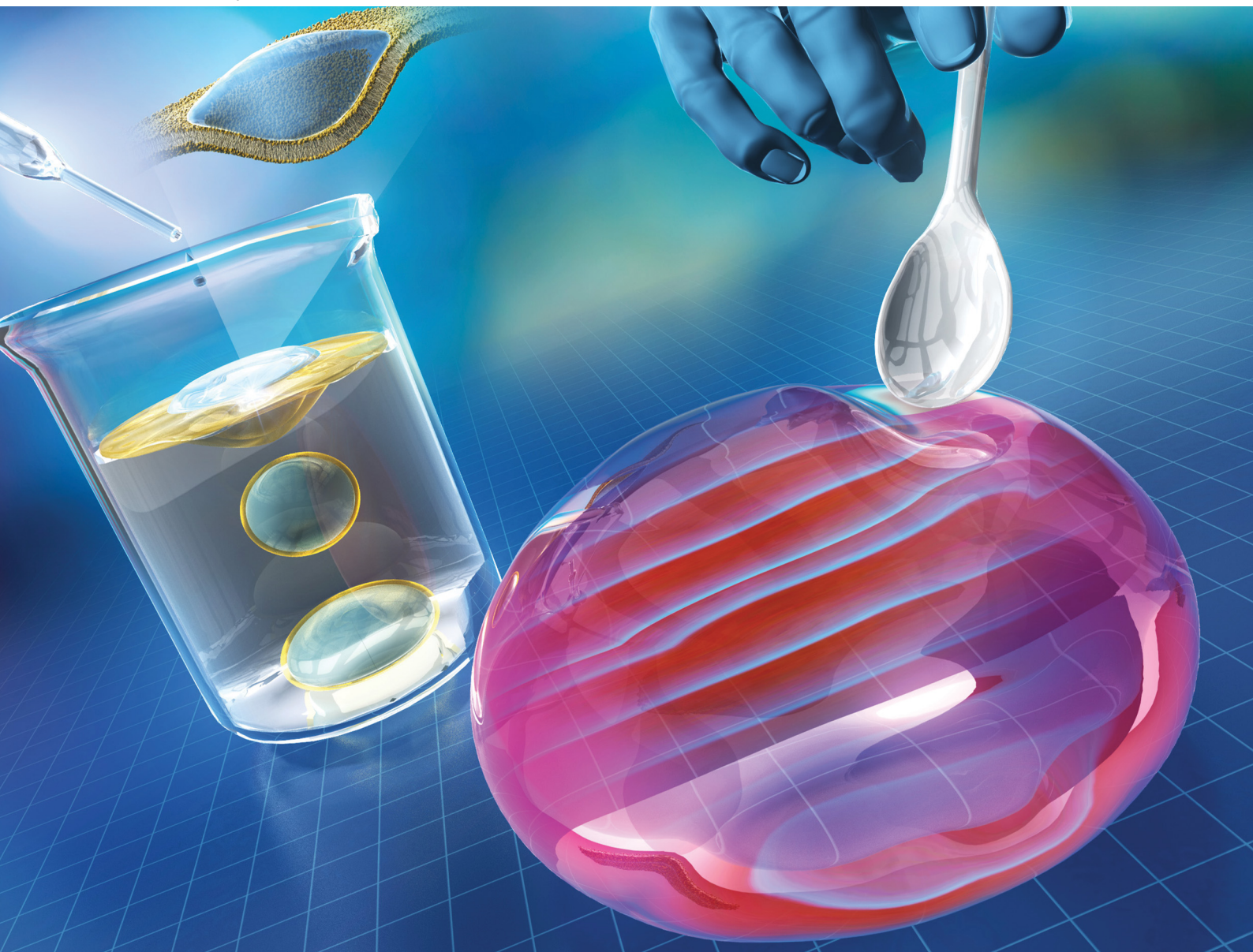


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PAPER

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Ultra-giant lipid vesicles functioning as a centimeter-sized
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Ultra-giant lipid vesicles functioning as a centimeter-sized smart chemical reactor

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We report a simple and robust method for generating centimeter-sized lipid vesicles (ultra-giant lipid vesicles, UGLVs) by dripping an aqueous solution onto a thin lipid layer floating on the surface of an aqueous phase. A mixture of oleic acid and phospholipids was used to form an interfacial lipid layer. During dripping, the aqueous droplet initially remained above the lipid layer but gradually sank owing to gravity as its volume increased. Once the droplet volume reached several cubic centimeters, it detached from the air–liquid interface and spontaneously formed a stable vesicle with a diameter of several centimeters. Notably, the UGLV remained stable even under mechanical perturbation using a spatula. Furthermore, UGLVs encapsulating the Belousov–Zhabotinsky oscillatory reaction medium were successfully prepared. Neighboring UGLVs exhibit spontaneous synchronization of chemical waves upon contact, demonstrating that UGLVs can function as a unique type of smart chemical reactor.

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Introduction

Living cells use lipid membranes to separate their interior from the surrounding environment. Phospholipids are primary constituents of cellular membranes in both eukaryotic and prokaryotic cells. The amphiphilic nature of phospholipids, with appropriate molecular shapes, facilitates the formation of a bilayer structure that serves as a stable barrier for living cells in cooperation with various proteins.^{1–6} Numerous studies have focused on the reconstitution of vesicles enclosed by lipid membranes to create water-in-water confinements with a stable barrier.^{7–21} Such reconstituted lipid vesicles with unilamellar and multilamellar structures are usually classified into three different types based on their size: small vesicles (20–100 nm), large vesicles (100 nm to 1 μm), and giant vesicles (GV, 1–100 μm). Recently, the preparation of super-giant vesicles (100–1000 μm) with lipid membranes of a single unilamellar structure, as well as multilamellar structures, has gained increasing interest.^{22–28} Kubatta and Rehage²² reported

the successful production of vesicles with diameters of 3–5 mm using sulfosuccinate sodium salt and olive oil. Takahashi and Ogawa^{26,27} reported the formation of a super-giant vesicle with a diameter of 1.6–4.4 mm by adopting the phase-transfer methodology of emulsion droplets from the oil phase into the aqueous phase. To the best of our knowledge, the formation of vesicles—either unilamellar or multilamellar—with diameters larger than centimeters has not been reported. By contrast, soap bubbles,^{29,30} that is, air-in-air confinement with a lipid membrane, can be easily produced with diameters ranging from several centimeters to several tens of centimeters. In this paper, we report a simple methodology for obtaining ultra-giant lipid vesicles (UGLVs) with diameters of several centimeters. The aqueous solution was dripped from the upper air phase onto a thin interfacial layer with the phospholipids and oleic acid floating on the water surface. Then, the aqueous drop situated on the air–liquid interface spontaneously detached from the thin lipid layer, driven by gravity. Through this procedure, a centimeter-sized stable lipid vesicle, the UGLV, was generated spontaneously.

Experimental

Oleic acid (Product No. 31028-01, Extra pure; Kanto Chemical, Tokyo, Japan) and lecithin paste from soybeans (Product No. 24092-01, Extra pure; Kanto Chemical; a naturally occurring mixture of phospholipids (primarily phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol)) were used without further purification. The Belousov–Zhabotinsky (BZ) reaction was employed as a chemical oscillation reaction.

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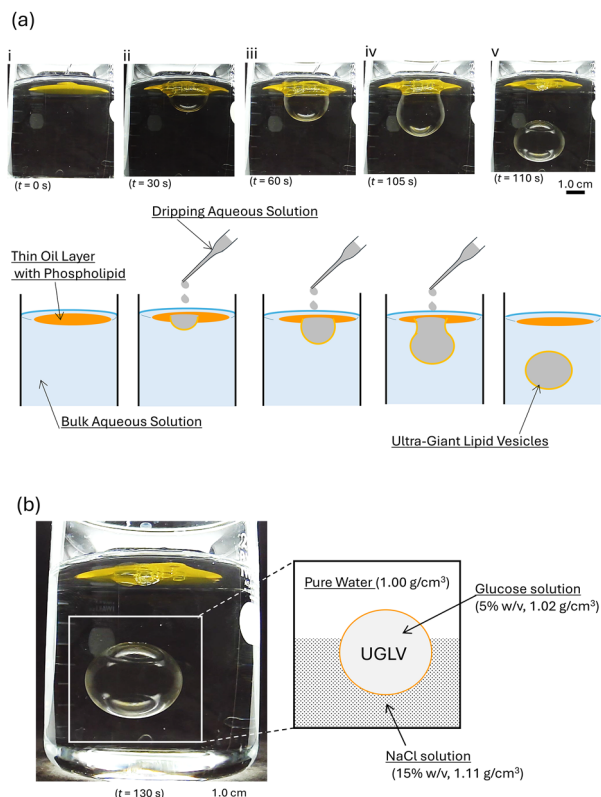


Fig. 1 Preparation of ultra-giant lipid vesicles (UGLVs). (a) Photos (top images) and corresponding schematics (bottom images) of the experimental procedure for UGLV generation. (i) A thin lipid layer of the mixture of oleic acid and soybean phospholipid with a weight ratio of 8 : 1 is formed on the top of the aqueous solution in a beaker. (ii) Dripping an aqueous solution of 5% w/v glucose (density, $\rho = 1.02 \text{ g cm}^{-3}$) from above. (iii) Growth of an aqueous droplet accompanied by successive dripping. (iv) Further growth of the droplet causes gradual sinking into the aqueous phase. (v) The UGLV is generated in the aqueous phase *via* detachment from the thin oil layer. (b) Photograph of a UGLV floating in the middle of the aqueous solution in the beaker. For the aqueous solution in the beaker, the upper and lower layers are pure water ($\rho = 1.00 \text{ g cm}^{-3}$) and a 15% w/v NaCl solution ($\rho = 1.11 \text{ g cm}^{-3}$), respectively. See the original movie in the SI, Video S1.

The final concentrations of the reaction medium were as follows: 0.4 M malonic acid, 0.4 M NaBrO₃, 0.6 M H₂SO₄, 0.01 M NaBr, and 1 mM ferroin (prepared by mixing ferrous sulfate and 1,10-phenanthroline in a molar ratio of 1 : 3). All reagents for the BZ reaction were of high purity and obtained from Tokyo Chemical Industry (Tokyo, Japan). Experiments were performed at room temperature ($\sim 20 \text{ }^\circ\text{C}$). After preparing the oil layer, the inner aqueous phase was deposited onto the thin oil layer, as shown in Fig. 1(a). The inner aqueous phase consisted of a 5% w/v glucose solution with a density of 1.02 g cm^{-3} . Further addition of the inner aqueous phase led to detachment of the inner aqueous droplet, generating a stable UGLV.

Results

Stability of the UGLVs

The formation process of UGLVs is shown schematically in Fig. 1(a). Fig. 1(b) shows an example of a UGLV floating in the

middle of an aqueous solution in a beaker. Here, 50 mL of 15% w/v NaCl solution ($\rho = 1.11 \text{ g cm}^{-3}$) and 150 mL of pure water ($\rho = 1.00 \text{ g cm}^{-3}$) were placed as the lower and upper phases, respectively, in a beaker to keep the UGLV floating in the middle of the outer aqueous solution. We dripped an aqueous solution of 5% w/v glucose ($\rho = 1.02 \text{ g cm}^{-3}$) above the mixed lipid layer consisting of oleic acid and phospholipids at a weight ratio of 8 : 1. The density of oleic acid is $\rho = 0.89 \text{ g cm}^{-3}$, making it lighter than that of the neighboring aqueous phase ($\rho = 1.00 \text{ g cm}^{-3}$). A thin oil layer with a volume of 0.5 mL was situated at the upper surface of the aqueous phase, forming a thin convex shape owing to its low affinity for the hydrophilic glass wall, as shown schematically in Fig. 1(a). After preparing the oil layer, a 5% w/v glucose solution was gradually dripped onto the thin oil layer. Further addition of the glucose solution caused the underwater geometry of the deposited glucose solution to adopt a spherical shape, where a thin lipid layer was formed at the interface between the two different aqueous phases of the bulk solution and the deposited solution. Consequently, a centimeter-sized droplet or UGLV was spontaneously generated (Fig. 1(a)(v)). Fig. 1(b) shows the floating UGLV self-emerge through the procedure illustrated in Fig. 1(a). Thus, centimeter-sized liposomes

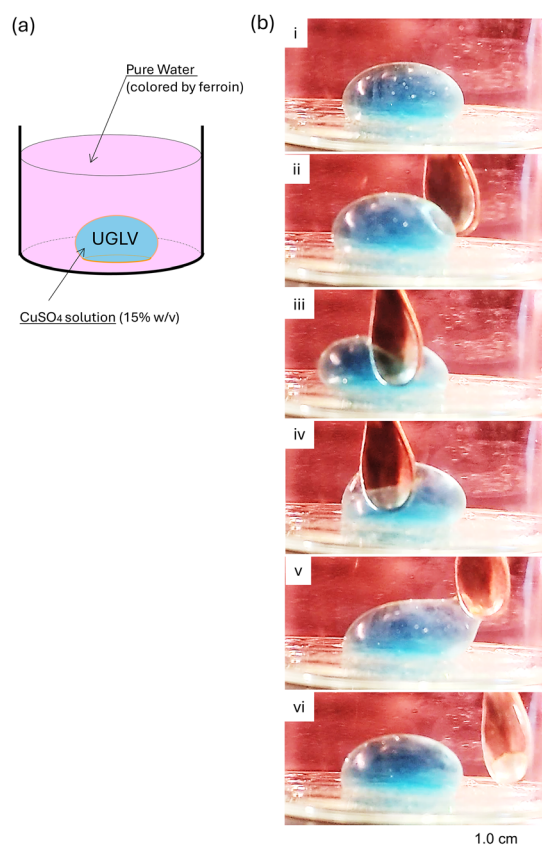


Fig. 2 Stability of UGLV against mechanical stimulus. (a) Schematic of a UGLV containing a 15% w/v CuSO₄ solution (blue color) surrounded by a ferroin-colored aqueous solution. The vesicle is situated in a pure water solution, $\rho = 1.00 \text{ g cm}^{-3}$. (b) Snapshots of the UGLV mechanically perturbed by a spatula. The UGLV retains the closed stable state against mechanical agitation. The interval for the images between (i) and (vi) is ca. 2 s. See the original movie in the SI, Video S2.



(UGLVs) can be easily produced through a simple experimental procedure, that is, dipping an aqueous solution onto a thin lipid layer above a bulk aqueous solution. Next, we examined the stability of the UGLV against mechanical agitation.

Fig. 2 shows a UGLV containing a 15% w/v CuSO_4 solution (blue) situated at the bottom of a beaker with ferroin-colored water (red) (Fig. 2(a)), produced using a procedure similar to that shown in Fig. 1. Fig. 2(b) shows the response of the UGLV to mechanical perturbation applied with a spatula. Interestingly, even after the spatula was inserted inside the UGLV, it spontaneously recovered its spherical shape. As demonstrated in Fig. 1 and 2, mechanically stable centimeter-sized UGLVs were successfully produced using a simple experimental procedure involving dripping an aqueous solution onto a thin lipid layer containing phospholipids and oleic acid. In relation to the effect of oleic acid on lipid membranes, oleic acid and its mixture with phospholipid have been reported to form stable giant vesicles.^{31,32} Another study claims that fatty acids, including oleic acid, are better suited as components of vesicle-based protocells in relation to the possible scenario of the origin of life.³³ The actual role of oleic acid in generating stable UGLVs may be important to clarify in a future study. Next, we

attempted to extend our findings on the formation of UGLVs to possible application in smart chemical reactors.

Chemical oscillation inside the UGLV

Fig. 3(a) shows the UGLV containing the BZ solution, situated between pure water and a 15% w/v NaCl solution. The left panel of Fig. 3(b) shows that the UGLV undergoes a rhythmic chemical reaction. It is noteworthy that a chemical wave was generated inside the UGLV. The spatiotemporal diagram in the right panel of Fig. 3(b) shows the rhythmic appearance of the oxidized state (Fe^{3+} ; blue color) at a periodicity of ~ 30 s.

Fig. 4 shows an example of the interaction between the oscillatory chemical reactions of two UGLVs situated at the bottom of the vessel, where they have minimal contact. As shown in the spatiotemporal diagram (right panel of Fig. 4), during the early stage, the chemical oscillations exhibited a phase difference of ~ 1 s, as indicated by the difference in the appearance of the blue color (Fe^{3+}). Gradually, the oscillations began to synchronize with each other, maintaining identical timing. The inner aqueous phase in the UGLV separated from the lipid layer is not perfectly isolated from the outer aqueous phase, as has been reported for vesicles separated from the environmental solution with a lipid membrane.^{1–28,31–33} Some of the relatively small chemical species

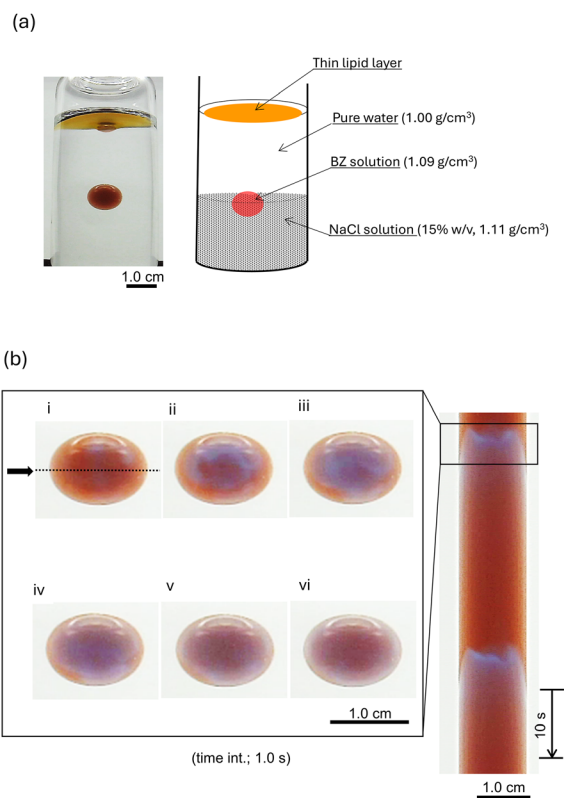


Fig. 3 Formation of a UGLV containing the medium of the oscillatory BZ reaction. (a) Photograph and schematic of a UGLV containing the solution of the BZ reaction ($\rho = 1.09 \text{ g cm}^{-3}$) situated around the interface between the aqueous solutions with different densities. (b) Left side: Oscillatory reaction inside the UGLV at a 1.0 s time interval. Right side: Spatiotemporal plot for the cross-section indicated by the dashed line in (i). The box with black lines indicates the time span between (i) and (vi) in the left photos. See the original movie in the SI, Video S3.

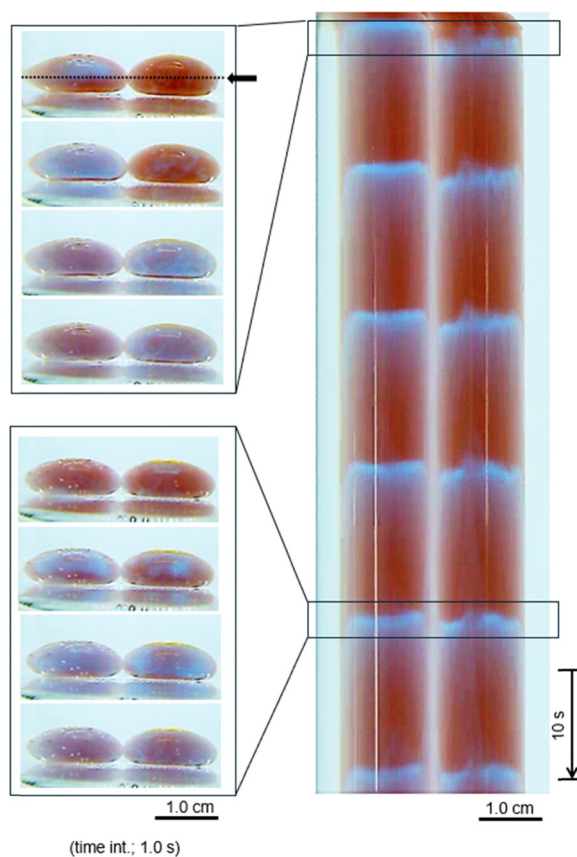


Fig. 4 Coupling of chemical oscillations between neighbouring UGLVs, situated at the bottom of the beaker. The spatiotemporal plot indicates a time delay of ~ 1 s in the initial stage of the contact formation; after ~ 40 s, the oscillations synchronized with each other without time delay. See the original movie in the SI, Sideo S4.



can diffuse through the lipid membrane and contribute to the mutual coupling between the oscillatory chemical reactions encapsulated in different UGLVs. In a future study, it would be interesting to localize the specific pacemakers of the coupled BZ reactions in relation to the neighboring UGLVs and to extend the experiments to include more than two UGLV oscillators.

Discussion

In a past study concerning the size effect of the BZ reaction, it was shown³⁴ that uniform oscillations are generated in a smaller reaction space than the typical diffusion length for the duration of the oscillation period. The critical diameter for achieving uniform oscillation in confined conditions was found to be *ca.* 0.6 mm.³⁴ By contrast, travelling waves occur above the critical length of the BZ reactor, as indicated on the left sides of Fig. 3 and 4. In our experiments, we did not use a pacemaker for the BZ reaction inside the UGLV. Thus, the spatial pattern of the traveling waves was somewhat irregular. It may be interesting to try to generate regular traveling waves inside centimeter-sized vesicles triggered by a pacemaker.

Tomasi *et al.*³⁵ reported an experiment on chemical communication among giant vesicles entrapping the BZ reaction medium. They prepared giant vesicles covered by a phospholipid membrane with a diameter of *ca.* 0.3 mm using microfluidics. Successful transfer of the oscillatory chemical rhythm to neighboring vesicles was observed. In their experimental images, individual vesicles exhibited an almost spatially homogeneous oscillation between oxidized and reduced states inside the vesicle, implying a size effect below the critical diameter, as mentioned above.³⁴ Similar homogeneous oscillations within individual vesicles and emulsions have been reported in the experiments entrapping the BZ reaction medium, where the diameter is less than 0.3 mm.^{36–39} Using water-in-oil droplets and giant liposomes with a diameter of around 1 mm, a travelling wave of a BZ reaction has been generated inside a droplet.^{40–42} With respect to the effect of chemical information transfer between neighboring BZ vesicles, Tomasi *et al.*³⁵ assumed that transfer of the activator, HBrO₂, through the lipid layer should have the most significant effect among the various chemical signals, including mass, charge, and electron transfers. Tomasi *et al.*³⁵ and other researchers^{43–45} stated the necessity for further research to clarify the detailed mechanisms of chemical communication among vesicles. Studies on chemical communication among vesicles with different diameters, ranging from several tens of micrometers to several centimeters, would be promising for introducing new viewpoints on the dynamic effect of chemical communication.

Author contributions

N. M. and K. Y. conceived the experimental design and supervised this study. All experiments were conducted and analyzed by N. M., K. H., and M. H. A spatiotemporal plot of the chemical waves was prepared by Y. S.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this study's findings are included in the article.

Additional datasets with movies are available in the supplementary information (SI), including supplementary Videos S1, S2, S3 and S4. See DOI: <https://doi.org/10.1039/d5sm01226k>.

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