Artificial Cells Hot Paper

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Evolved Colloidosomes Undergoing Cell-like Autonomous Shape Oscillations with Buckling

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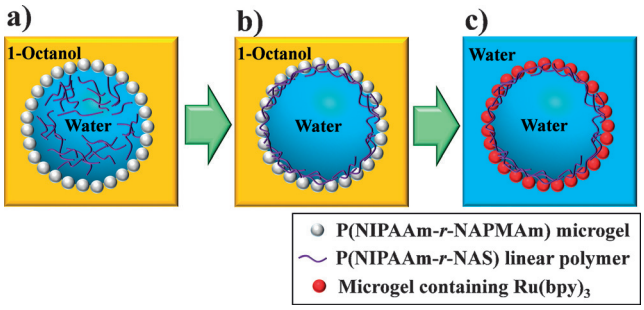
Abstract: *In living systems, there are many autonomous and oscillatory phenomena to sustain life, such as heart contractions and breathing. At the microscopic level, oscillatory shape deformations of cells are often observed in dynamic behaviors during cell migration and morphogenesis. In many cases, oscillatory behaviors of cells are not simplistic but complex with diverse deformations. So far, we have succeeded in developing self-oscillating polymers and gels, but complex oscillatory behaviors mimicking those of living cells have yet to be reproduced. Herein, we report a cell-like hollow sphere composed of self-oscillating microgels, that is, a colloidosome, that exhibits drastic shape oscillation in addition to swelling/deswelling oscillations driven by an oscillatory reaction. The resulting oscillatory profile waveform becomes markedly more complex than a conventional one. Especially for larger colloidosomes, multiple buckling and moving buckling points are observed to be analogous to cells.*

One exciting challenge in materials science is to mimic living systems that undergo autonomous and dynamic changes under conditions far from equilibrium, by only using synthetic materials. Autonomous phenomena involving oscillatory dynamics can easily be observed as significant characteristics of living systems, such as heart contractions, breathing, and circadian rhythms.[1] At the microscopic level, cells act dynamically, and they often change their structures and shapes in pulsatile or oscillatory manners.[2] Recent image analysis developments with high spatio-temporal resolution have revealed the existence of oscillatory shape deformations during cellular dynamics such as cytokinesis,[3] cell migration,[4–6] and morphogenesis.[7,8]

To realize autonomous oscillatory behaviors that exist in living systems by using synthetic materials, we have developed "self-oscillating" gels which exhibit autonomous periodic swelling/deswelling changes driven by an oscillatory chemical reaction.[9] Recently, self-oscillating material systems are evolving, and we have reported several types of self-oscillating polymeric materials such as tubular self-oscillating gels,[10,11] self-oscillating polymer brushes,[12] and self-oscillating block copolymers.[13–17] In addition to our studies, other groups have also reported self-oscillating polymer materials.[18–22] In these material systems, the observed oscillatory profiles are rather simple, reflecting monotonic swelling/deswelling or hydration/dehydration; however, biological systems not only have simple oscillation, but also more complex oscillatory behaviors. For example, in the nervous systems, many oscillatory profiles of brain activity accompany complex waveforms with several maxima and minima of varying amplitudes.[23] Also, complex shape oscillations of cells are observed in vitro[24] as well as in vivo.

To reconcile the differences between materials and biological systems, we have fabricated self-oscillating cell-like hollow spheres that exhibit complex oscillatory behaviors with shape deformations. The observed oscillatory behaviors are significantly different from conventional oscillating behaviors. The outer layer of the hollow sphere is composed of self-oscillating microgel building blocks. Such microparticle-based capsules, or "colloidosomes," can be formed by utilizing self-assembly of microparticles on emulsion droplets.[25–30] As a first step, water-in-oil emulsions were prepared by using P(*N*-isopropylacrylamide-*r*-(*N*-(3-aminopropyl)-methacrylamide)) (P(NIPAAm-*r*-NAPMAm)) microgels as emulsifiers (Scheme 1a). Figure 1a shows an optical micro-



Scheme 1. Fabrication of self-oscillating colloidosomes. a) Water-in-oil emulsions stabilized by microgels. b) Microgels fixed by cross-linkers. c) Colloidosomes conjugated with $Ru(bpy)_3$-NHS in water.

scopic image of the water-in-oil emulsions. 1-Octanol was chosen as an oil phase, as previous reports demonstrated that water-in-oil emulsions stabilized by PNIPAAm microgels appeared when fatty alcohols were used as the oil phase.[31] The aqueous phase contains P(NIPAAm-*r*-(*N*-acryloxysuccinimide)) (P(NIPAAm-*r*-NAS)) linear polymers as cross-linkers. After the emulsions were formed, succinimidyl esters of the cross-linkers react with amino groups of the microgels in

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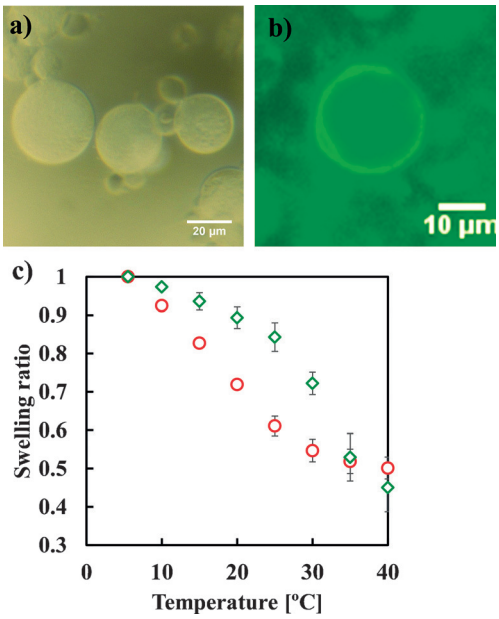

Figure 1. a) Optical microscopic image of water-in-oil emulsions stabilized by P(NIPAAM-*r*-NAPMAm) microgels. b) Fluorescent image of the colloidosome in pure water after conjugation with $Ru(bpy)_3$-NHS. c) Temperature dependence of equilibrium swelling ratio of colloidosomes in the reduced state (red circles, $[HNO_3] = 0.7$ M and $Ce_2(SO_4)_3 = 2.1$ mM) and the oxidized state (green diamonds, $[HNO_3] = 0.7$ M and $Ce(SO_4)_2 = 2.1$ mM).

an aqueous phase (Scheme 1 b). Then, the supernatant layer containing the unreacted microgels and cross-linkers was discarded, and the obtained colloidosomes were washed with ethanol, which is a co-solvent for water and 1-octanol. Next, $Ru(bpy)_3$ moieties that catalyze the oscillatory Belousov–Zhabotinsky (BZ) reaction were introduced into the colloidosomes by a coupling reaction between succinimidyl ester groups of $Ru(bpy)_3$ ($Ru(bpy)_3$-NHS) and residual amino groups of P(NIPAAm-*r*-NAPMAm) microgels. Finally, the solvent was gradually replaced by dialysis, thus leaving behind the colloidosomes in pure water (Scheme 1 c, Figure 1 b). The fluorescent signal of $Ru(bpy)_3^{2+}$ in the membrane of the colloidosome clearly indicates that $Ru(bpy)_3$ was successfully introduced into the surface of the colloidosome. Figure S2 shows the diameter distribution of obtained colloidosomes. The average diameter was 26.0 μm.

Figure 1 c visualizes the temperature dependence of the equilibrium swelling ratio of the colloidosome for both the reduced and the oxidized states of $Ru(bpy)_3$. In both redox states, the colloidosome showed thermosensitive behavior, decreasing in size with increasing temperature, owing to the lower critical solution temperature (LCST)-type phase transition of PNIPAAm. In contrast to the reduced state, the swelling ratio in the oxidized state was larger throughout the most of the temperature range. These behaviors indicate that the oxidized colloidosomes became more hydrophilic than when in the reduced state, which is consistent with our previous studies for self-oscillating gels and polymer systems. The thermosensitivity of the colloidosome is affected not only by the thermosensitivity of the microgels, but also by the thermosensitivity of the cross-linker used to fix the hollow structure. When non-thermosensitive P(*N*,*N*-dimethyl-acrylamide-*r*-NAS) (P(DMAAm-*r*-NAS)) was used as a cross-linker instead of P(NIPAAm-*r*-NAS), the thermosensitivity of the colloidosome before $Ru(bpy)_3$ conjugation was less pronounced than with P(NIPAAm-*r*-NAS) (Figure S3).

The colloidosomes exhibited a unique buckling instability with a stepwise temperature decrease. Figure 2 a shows the changes in circularity (upper) and projected area and circumference (middle) for the observed colloidosome in response to

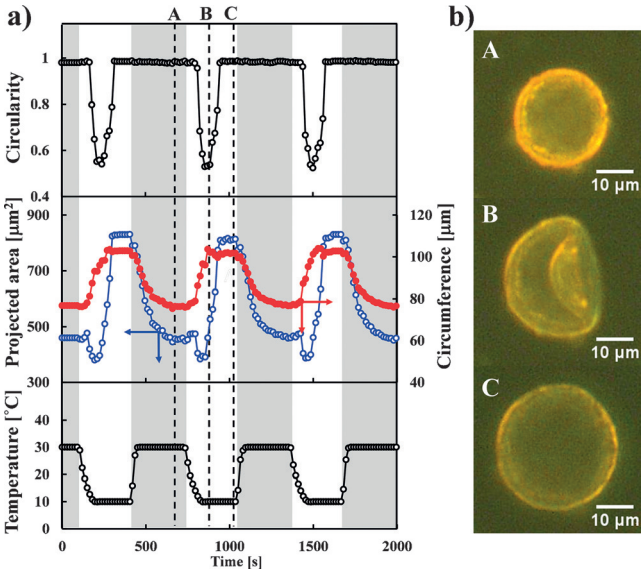

Figure 2. a) Time-evolution of temperature (lower), projected area (middle, open blue circles), circumference (middle, closed red circles), and circularity (upper). b) Snapshots by optical microscope at the times corresponding to dotted lines in Figure 2 a. The solution contained $[HNO_3] = 0.7$ M and $Ce_2(SO_4)_3 = 2.1$ mM.

repeated stepwise temperature changes (lower). Snapshots of the colloidosome at times A, B, and C (indicated as dotted lines in Figure 2 a) are shown in Figure 2 b. The colloidosome was in the deswelling state at 30 °C (A). When temperature was decreased from 30 °C to 10 °C, the colloidosome spontaneously buckled inward (B) accompanying a drastic decrease in circularity as shown in Figure 2 a. After the buckling, the colloidosome spontaneously recovered the original unbuckled round shape as the diameter increased (C). Here, the buckling deformation can be attributed to tangential expansion of the membrane of the colloidosome by hydration. In contrast, the colloidosome deswelled with stable circularity when temperature was increased from 10 °C to 30 °C. These results indicate that there is a bistable region, where the buckling deformation occurs with decreasing temperature, and the unbuckled round shape is maintained with increasing temperature. The buckling/unbuckling behavior repeated with cyclic changes of temperature without any degradation.

A similar buckling phenomenon was observed in our previous report on cross-linked polymersomes.[15] However, relaxation kinetics from the buckled shape to the swelled spherical shape is much faster for the colloidosomes than for

the polymersomes, leading to the new behaviors. In living systems, buckling of biological membranes (called invagination) occurs especially during the development of living organisms, such as in the ventral furrow invagination of the *Drosophila* embryo,[32,33] sea urchin primary invagination,[34,35] and formation of optic cups.[36,37] Interestingly, several hypotheses for the mechanism of invaginations are analogous to our system: One hypothesis for sea urchin primary invagination is that the swelling of the membrane is regulated by secretion of hygroscopic molecules.[34] More recently, Eiraku et al. proposed a relaxation–expansion model for the optic cup invagination, in which tangential expansion of the membrane caused by cell proliferation played an important role.[36,37] These mechanisms can be compared with the buckling mechanism of the colloidosomes observed in our system, in which the tangential expansion of the polymeric membrane is essential for the unique buckling deformation of the hollow particles.

To investigate the self-oscillatory behaviors of the colloidosomes coupled with the BZ reaction, the dispersion of colloidosomes in a catalyst-free BZ solution was observed by optical microscope (a detailed mechanism for the BZ reaction is described in the Supporting Information). Figure 3 shows two distinctive types of observed self-oscillatory behaviors. One behavior is swelling/deswelling oscillation while maintaining a spherical shape (Figure 3a and c), which is similar to the behavior of conventional self-oscillating gels. As shown in the lower graph of Figure 3a, the projected area and the circumference synchronously oscillated without a phase difference. The circularity was kept constant at approximately 1, regardless of whether in a swelling or deswelling state. This indicates that the shape of the colloidosome remained a near-perfect sphere throughout oscillations in which the volume alone changed periodically. The spatio-temporal patterns constructed by extracting one-line images along the cross-section clearly shows simple swelling/deswelling behavior of the membrane (Figure 3c).

On the other hand, in the other behavior, the circularity was not constant, owing to buckling deformation and unbuckling relaxation to spherical shape (Figure 3b). The circularity repeatedly had sharp decreases and increases in a pulsatile manner. In this case, the oscillating waveform of the projected area became more complex, corresponding to shape deformations, and a phase difference between the oscillations of the circumference and the projected area appeared. The spatio-temporal patterns along different directions 1 and 2 (Figure 3d) showed buckling/unbuckling deformations (line 2) and swelling/deswelling oscillations (line 1) occur simultaneously. Figure 3e shows a magnified view of one cycle for the oscillatory profile in Figure 3b and illustrations of the deformation of the colloidosome in each step. When $Ru(bpy)_3$ changed from the oxidized state to the reduced state, the colloidosome was deswollen with stable circularity (from A to B in Figure 3e). Conversely, when the $Ru(bpy)_3$ changed from the reduced state to the oxidized state, tangential expansion is induced by hydration of the membrane. If the degree of expansion exceeded the threshold, buckling deformation occurred (from B to C). After deformation, the colloidosome relaxes from the buckled

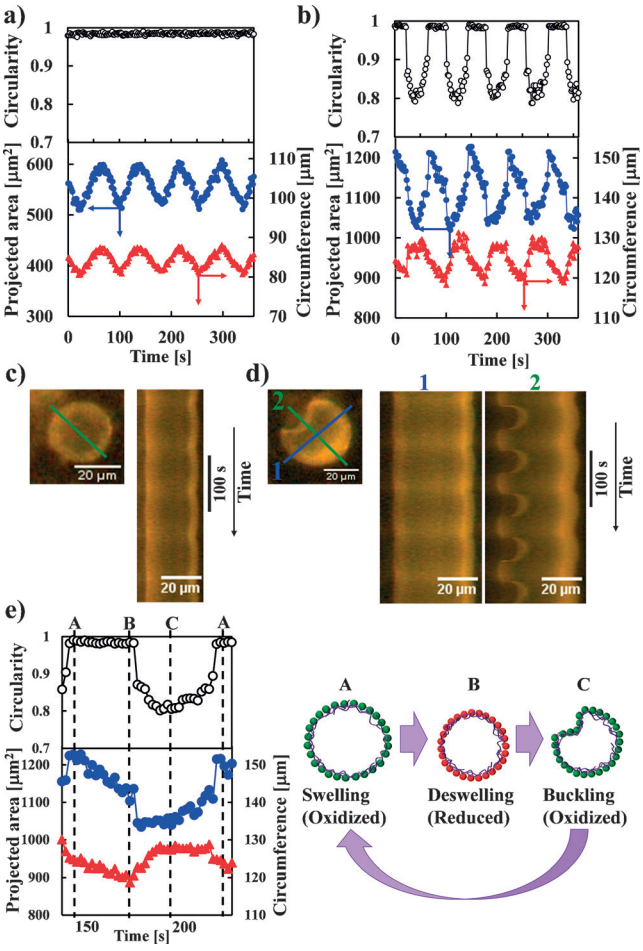

Figure 3. a, b) Time-evolution of projected area (lower, blue circles), circumference (lower, red triangles), and circularity (upper) for a) simple swelling/deswelling oscillations and b) oscillations with shape deformations. c, d) Snapshots of colloidosomes and the spatio-temporal patterns constructed by sequentially lining up one line images along the cross-section (indicated by green and blue lines) for each oscillatory profiles shown in (a) and (b). e) Magnified view of one cycle in the oscillatory profile shown in (b), and illustrations corresponding to states at times A, B, and C. Conditions of the BZ reaction were $[HNO_3] = 0.3$ M, $[NaBrO_3] = 0.4$ M, $[CH_2(COOH)_2] = 0.025$ M, 20 °C.

shape to a fully swollen spherical shape (from C to A). Consequently, the oscillating profile of the projected area shows a waveform with the following changes: i) decrease due to deswelling of the colloidosome by membrane dehydration; ii) further decrease owing to an abrupt buckling deformation by membrane hydration; and iii) increase owing to relaxation from the buckled shape to the swollen spherical shape. This complex oscillation would be expected when bistability is observed under the same temperature, that is, buckling deformation during temperature decrease and stable circularity during temperature increase. In previous results regarding shape oscillations in polymersomes,[15] oscillation occurred only between the deswelled state and the buckled state, which may be attributed to the slow kinetics of relaxation from the buckled state to the unbuckled state. In contrast, here, colloidosomes produce faster kinetics that can be explained by the porous nature of colloidosomes,[38] resulting in a new

mode of oscillation. Movies corresponding to Figure 3 are also available in the Supporting Information (Movies S1 and S2).

Figure 4 shows the occurrence rate of oscillations with shape deformations for each diameter range of colloidosomes. The occurrence rate is defined by the rate of the number of colloidosomes that cause shape deformations to all

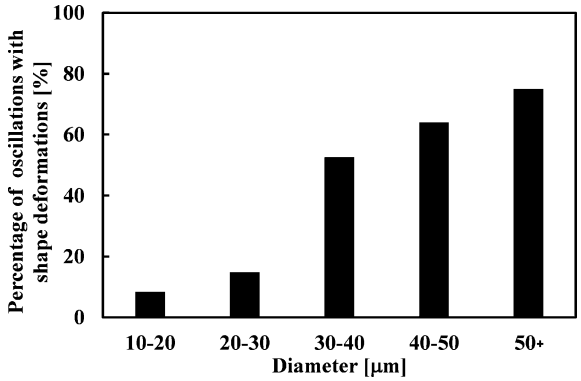

Figure 4. Occurrence rate of oscillations with shape deformations for each colloidosome diameter range. The diameter is the minimum diameter as measured in the fully deswelled state during oscillation.

colloidosomes. Oscillation with shape deformations clearly occurs more frequently for larger colloidosomes. This result is partially supported by the elastic theory for spherical shells. The order of the stretching energy (E_s) and the bending energy (E_b) per unit area of spherical shells can be expressed as follows:[39]

$$E_s \sim Eh(\zeta/R)^2 \quad (1)$$

$$E_b \sim Eh^3\zeta^2/R^4 \quad (2)$$

where E is the Young modulus of the shell, h is the shell thickness, ζ is the radial displacement, and R is the radius of the shell. Therefore, the ratio is expressed as $E_b/E_s \sim (h/R)^2$. This means that bending deformation occurs more easily than stretching deformation for shells with a small h/R. In this study, since h corresponds to the size of the microgel and is independent of R, it can be concluded that E_b/E_s becomes smaller as the size of the colloidosome increases. Therefore, when stretching deformation occurs due to membrane hydration, larger colloidosomes become more sensitive to buckling deformations. Other reports clarify both theoretically and experimentally that the relative shell thickness (h/R) determines the buckling phenomena for hollow particles,[40–42] although the driving force presented in those studies was not tangential expansion of the membrane, as it is in this study.

More complex buckling behaviors were also observed for some large colloidosomes (Figure 5; Supporting Information, Movies S3 and S4). Figure 5a shows two buckled regions in one colloidosome during oscillation. Previously, multiple buckling behavior was predicted by finite element simulations for lipid bilayer membranes of mitochondria, in which the

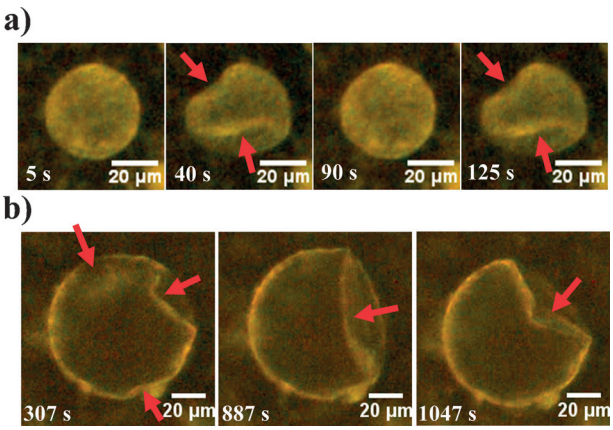

Figure 5. Complex oscillatory behaviors for large colloidosomes. a) Oscillatory behavior with multiple buckling deformations. b) Oscillatory behavior with multiple moving buckling deformations for a larger colloidosome. Red arrows indicate buckling points.

driving force of buckling was an increase in surface area,[43] similar to this study. Figure 5b shows another example of oscillatory behavior with multiple buckling deformations in a larger colloidosome. In this case, the buckling position migrates irregularly during oscillation. Since E_b/E_s decreases as the colloidosome becomes larger, the buckling point can easily move due to thermal fluctuations. These behaviors are seemingly analogous to actual living cells. During shape oscillation of non-adherent fibroblast cells in vitro, relatively complex oscillatory behaviors are observed for larger cells, whereas simple oscillatory behaviors are observed for smaller cells.[24]

In this study, artificial colloidosomes that undergo cell-like autonomous and periodic shape deformations were obtained. The colloidosomes were fabricated based on templating water-in-oil emulsions stabilized by thermosensitive microgels, followed by conjugation with $Ru(bpy)_3$ catalysts. The colloidosomes have thermosensitivity and unique buckling/unbuckling deformations in response to stepwise temperature changes, in addition to swelling/deswelling behaviors. Since the thermosensitivity changes according to the redox states, the colloidosome undergoes self-oscillations following periodic redox changes during the BZ reaction under constant temperature. In addition to simple swelling/deswelling oscillations, complex oscillatory profiles with shape deformations were observed. It was clarified that larger colloidosomes cause shape deformations more frequently during oscillation. This observation can be interpreted theoretically: Compared to smaller colloidosomes, larger colloidosomes have a relatively large stretching energy compared to a small bending energy. More complex oscillatory behaviors, such as multiple buckling deformations and moving buckling points, were observed for larger colloidosomes. The complex oscillating behaviors presented in this study can be a viable starting point for the realization of artificial cells. We believe that, in addition to realizing biomimetic colloidosomes, further understanding their behaviors would be a significant step in closing the gap between artificial and living cells.

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