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PERSPECTIVE

Tailoring the Shape and Size of Artificial Cells

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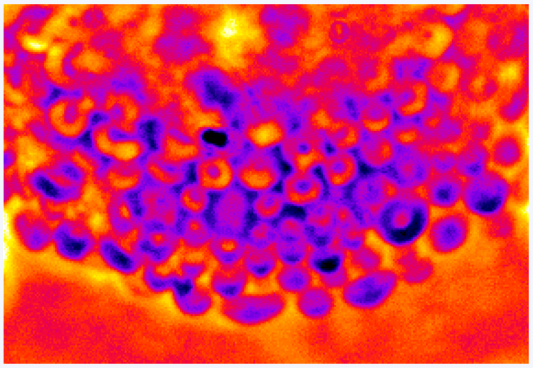
ABSTRACT: **Living cells achieve precise control of shape and size through sophisticated biochemical machinery. However, such precision is extremely challenging to emulate in artificial cellular compartments. So far, various physicochemical and mechanical interventions have been employed to tailor the dimensions of model systems such as liposomes, emulsions, coacervates, and polymer capsules. In this Perspective, we discuss the state of the art in artificial cell research in controlling shape and size and the challenges that need to be addressed.**



Appreciation for shapes and sizes of living cells began as early as the invention of the microscope by Anton von Leeuwenhoek and perhaps preceded any understanding of their function. Over time, scientists realized that there is a strong correlation between the structure and function of living cells.[1] So, it comes as no surprise that scientists have similar appreciation for shape and form when designing artificial cells. The pursuit of designing an artificial cell can be thought to have two broad objectives: (i) to understand the origins and evolution of cellular life by building a model system from minimal building blocks and (ii) to develop emergent assemblies and properties for novel applications. How does the aim of building artificial cells with specific shapes and sizes fit into these objectives? Let us consider a dichotomy first. Some objects have a given shape and size because they take the shape of the container in which they were molded, whereas other objects are inherently programmed to have a set form. The question is which of these two comes first, particularly in the context of artificial cell design. The first approach is to build artificial cellular compartments through mechanical manipulation of the building blocks in a manner analogous to casting, forging, or milling objects in the manufacturing industry. This method can be thought of as a "container first" strategy. The alternate and perhaps less explored approach is based on the informational code inherent in the building blocks that enables them to be self-assembled in a predisposed manner. This approach can be thought of as a "code first" strategy. In this Perspective, we elaborate on the topic of shape and size control of artificial cells based on these two lines with a special emphasis on the paper by Fanalista *et al.* that was recently published in *ACS Nano*.[2]

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Why Artificial Cells? Over the past few decades, there has been tremendous interest and coordinated effort to build artificial cells from basic building blocks.[3−5] This newly emerging discipline called bottom-up synthetic biology is somewhat analogous to synthetic chemistry, where chemists take elemental and molecular building blocks and turn them into substances with entirely new properties. Synthetic biologists are taking biological building blocks such as genes, proteins, macromolecular assemblies, and even intact organelles from various organisms and putting them together to create new biological functionalities.[6] New interfaces are being created between purely biological and chemically derived building blocks. Finally, there is an overarching ambition to understand how living cells originated on Earth in the first place from nonliving matter and, thus, shed light on the design principles of life.[7] Understanding the importance of shape and size for living cells is a key piece of this giant puzzle. In the following sections, we discuss some of the significant work done in controlling shapes and sizes of a few commonly used model systems for artificial cells, namely, liposomes, droplets, and polymer capsules.

Controlling Shapes and Sizes of Liposomes. Liposomes or vesicles are arguably the most popular model systems for mimicking living cells thanks to their lipid-membrane-bound architecture. Liposomes can be formed in

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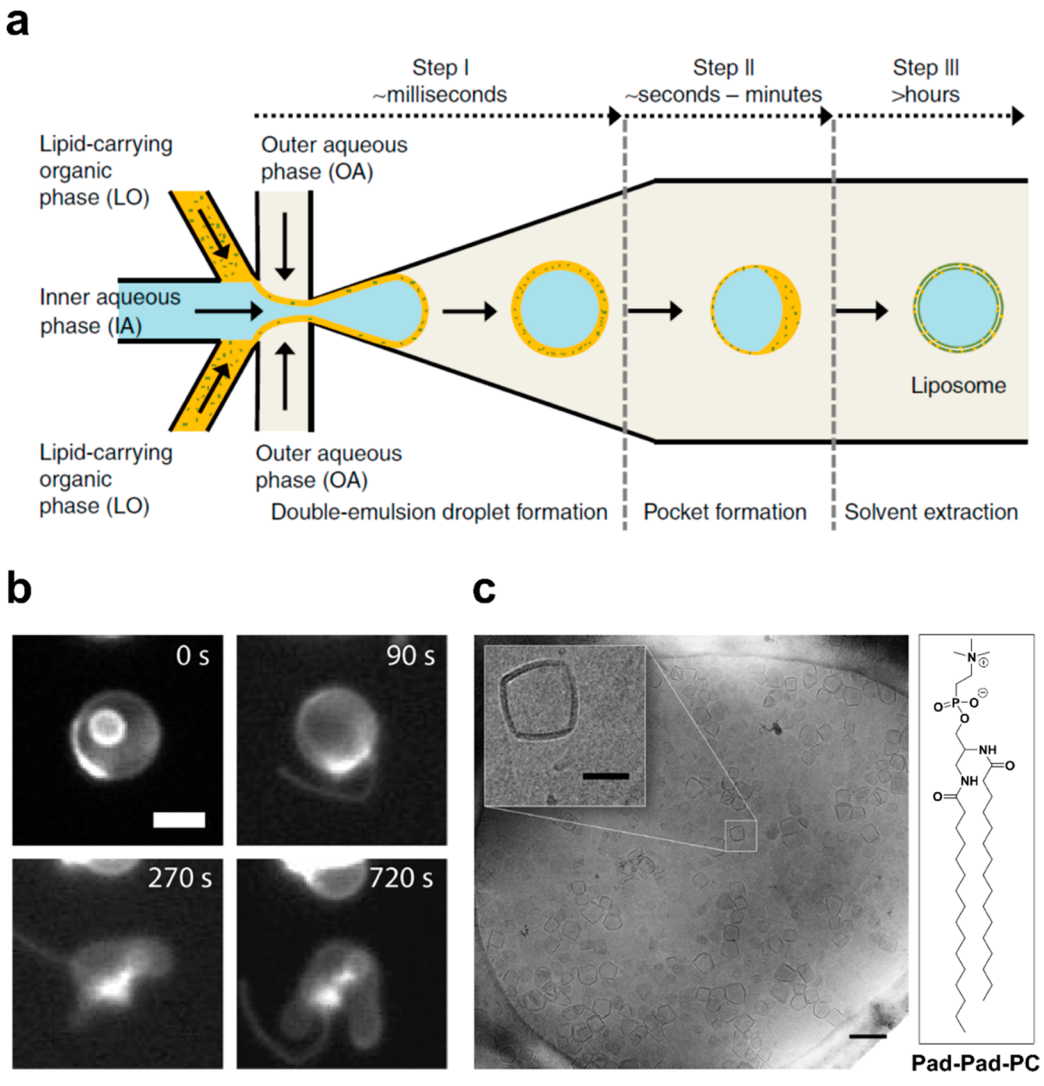

Figure 1. Controlling shapes and sizes of liposomes. (a) Generation of monodisperse giant unilamellar vesicles by octanol-assisted liposome assembly (OLA) method.[12] (b) Shape deformation in a phospholipid liposome in response to SNAP-tag-mediated protein binding to the membrane.[19] (c) Generation of cuboid liposomes from the diamido-phospholipid Pad-Pad-PC. Reprinted with permission from ref 22. Copyright 2017 John Wiley and Sons.

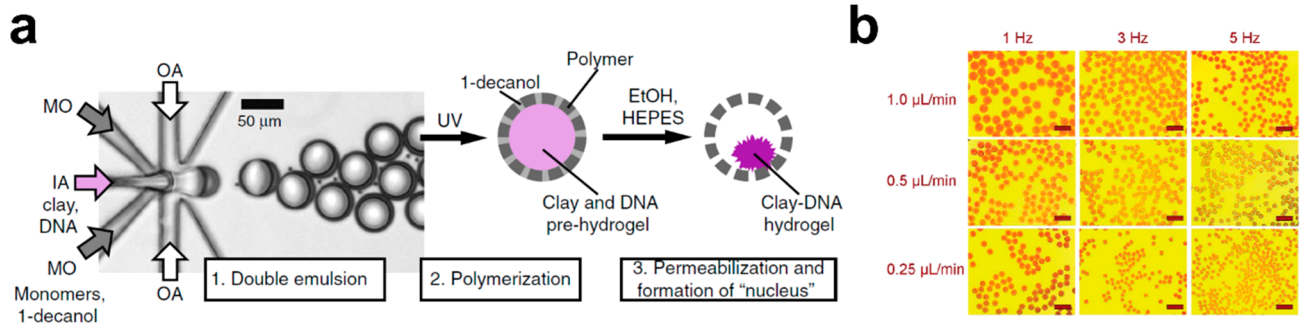

Figure 2. Generation of monodisperse microcapsule cell mimics. (a) Microfluidic generation of double emulsion droplets that encapsulates a prehydrogel in a photocurable middle layer and schematic of subsequent processing steps. IA, inner aqueous; MO, middle organic; OA, outer aqueous phase. Reprinted with permission from ref 25. Copyright 2018 Springer Nature. (b) Optical microscopy images of representative capsules generated at different flow rates and frequencies. All scale bars represent 500 μm. Reprinted with permission from ref 2017. The Royal Society of Chemistry.

media by a variety of bulk techniques such as thin film hydration,[8] inverse emulsion methods,[9] electroformation,[10] and *in situ* chemical reactions.[11] These methods are rapid and technically straightforward but suffer from high polydispersity and low encapsulation efficiency.[12] Various efforts have been made to generate monodisperse liposomal preparations. Extruding a multilamellar dispersion of vesicles through polycarbonate membranes supported in a hand-held device is

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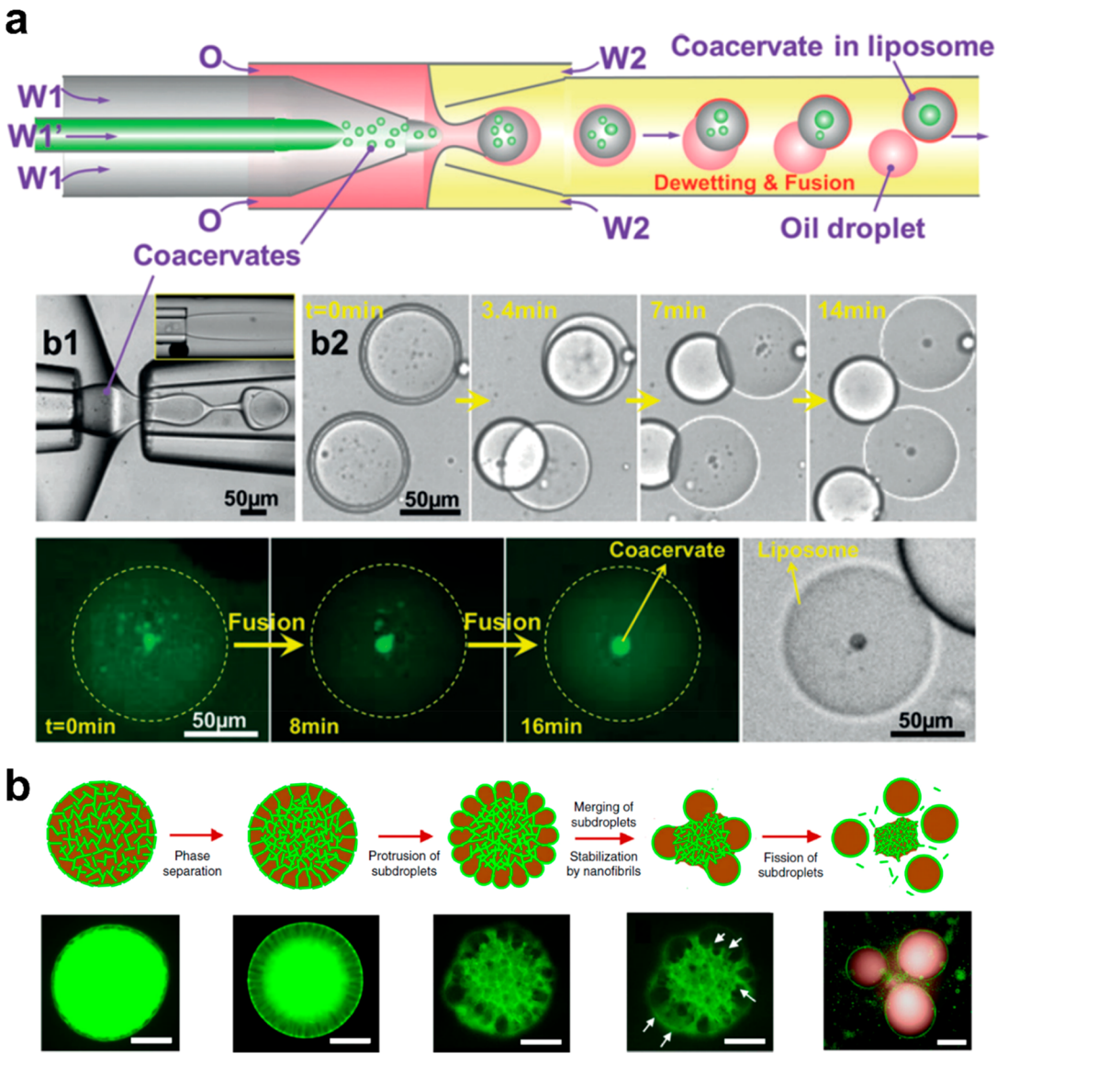

Figure 3. Control of droplet size and various shape transformations. (a) Generation of monodisperse coacervate droplets within liposomes using microfluidic methods. Reprinted with permission from ref 33. Copyright 2017 John Wiley & Sons, Inc. (b) Lysozyme fibril-induced shape transformation in water-in-water PEG/dextran droplets. Reprinted with permission from ref 36. Copyright 2018 Springer Nature.

In the *ACS Nano* publication by Fanalista *et al.*, an octanol-assisted liposome assembly method is employed and elaborated upon to create rod shapes, reminiscent of *E. coli* bacteria.

perhaps the most adaptable method[13] and has been utilized in numerous studies. However, extrusion is best suited for producing unilamellar vesicles of sizes less than 200 nm, which is an order of magnitude smaller than the dimensions of cells. Microfluidic-based methods offer several benefits, such as the generation of monodisperse giant unilamellar vesicles (>1 μm), high encapsulation efficiency, and adaptability to small working volumes (~10 μL).[14−16] In the *ACS Nano* publication by Fanalista *et al.*, an octanol-assisted liposome assembly (OLA) method introduced by the Dekker lab was employed (Figure 1a)[12] and elaborated upon to create rod shapes, reminiscent of *E. coli* bacteria. The authors further showed that filaments of the cytoskeletal protein FtsZ could be assembled within the rod-shaped liposomes. In the future, it will be interesting to test if such mechanical interventions could enable synthesis of complex membranous structures such as those resembling brush border membranes of intestinal microvilli, T-tubules of muscle cells, or neuronal axons.

Biological membranes are not monolithic structures. They can be thought of as "alloys" of lipids and proteins which impart emergent mechanical properties. For example, biological membranes are laterally incompressible while being highly amenable to bending and shearing at the same time.[17] The shape of a liposome is determined by bending elasticity and curvature. Bending energy can be reduced drastically by introduction of a spontaneous curvature, which, in turn, can be induced by binding of specific proteins or through incorporation of specific lipids. Significant work has been done on biomimetic shape transformations of liposome membranes in

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response to various external factors. Membrane deformations in response to microtubule filament adsorption,[18] SNAP-tag-mediated protein binding (Figure 1b),[19] and electrostatic interaction-mediated protein binding[20,21] have previously been reported.

Liposomes typically assume spherical geometries to minimize surface energy. Nevertheless, alternate configurations can be achieved by tweaking the structural parameters of the membrane-forming lipid molecules. Noncovalent interactions, such as hydrogen bonding and dipolar interactions between lipid molecules, can be tuned by introducing specific functionalities that can have significant effects on the self-assembly. We can think of these parameters as structure-based informational code inherent in a lipid molecule that manifests into self-assembly of a well-defined shape. Remarkably, this kind of "bilayer code" approach was taken by Neuhaus *et al.* to generate cuboid phospholipid vesicles from diamido-phospholipids (Figure 1c).[22] Our group recently demonstrated that small differences in the molecular structures of single-chain amphiphiles can lead to a dramatic change in self-assembly properties.[23] For example, β-galactopyranosylamides of unsaturated fatty acids self-assemble into vesicles, but the corresponding β-glucopyranosylamides self-assemble into nanotubes. In the future, further investigation into the interplay of molecular structure and supramolecular organization should be explored to discover such emergent properties. The impact of chiral centers in the lipid headgroups, permutations of various fatty acid chain lengths, and bioisosteric functional groups on self-assembly are some directions likely to yield interesting results.

A relatively unexplored method to control liposome sizes is to constrain the number of nucleation sites for membrane formation in a manner that is analogous to crystal growth. Budin *et al.* reported that size and dispersity of vesicle populations could be influenced through the number of nucleation sites.[24] They demonstrated that fatty acid vesicles seeded from fewer nucleation sites inside a microcapillary grew by continuous incorporation of new monomers to form monodisperse populations. In contrast, vesicles formed in a bulk medium were smaller and polydisperse, likely due to a larger number of nucleation sites. In this regard, we believe that approaches such as *in situ* lipid synthesis,[11] where the generation of a new phospholipid can be carefully controlled, may find application in generating monodisperse vesicle populations.

Controlling Shapes and Sizes of Polymer Capsules. Porous polymer capsules can be thought of as mimics of cell walls. Usually, these capsules comprise a shell that is permeable to small solutes and ions; however, the pore sizes can be altered to allow the passage of proteins and other macromolecules. Monodisperse cell-mimic populations are desirable in almost any experiment because they make quantitative analyses easier and reduce variation that derives from container size. Many different microfluidic methods have been reported to produce monodisperse cell-sized polymer or gel microcapsules. Diameters of spherical microcapsules derived from double emulsion droplets produced *via* microfluidics can be controlled in the range of ten to a few hundred micrometers by varying flow rates and channel dimensions. Our group has recently developed porous polymer cell mimics containing nucleus-like DNA hydrogel compartments that are capable of gene expression and communication (Figure 2a).[25] Diameters of porous cell mimics could be adjusted by varying the respective flow rates of inner aqueous, middle organic, and outer aqueous phases. The size of the hydrogel nucleus was determined by the container size and measured approximately half of the cell-mimic diameter. If nonspherical containers are desired, rigid containers such as polymer or gel microcapsules could be polymerized into rod or disk (permanent) shapes that do not depend on a trap after polymerization. In this regard, it will be interesting to see if polymer capsules of various shapes could be fabricated using the devices developed by Fanalista *et al.*

Methods typically employed for producing polymer capsules suffer from a drawback in that they require the use of an oil phase, traces of which may interfere with downstream applications. Lu *et al.* described a method of preparing chitosan- and alginate-based monodisperse microcapsules that does not require the use of oil droplets.[26] Using this method, the authors demonstrated that the size range of the capsules can be controlled faithfully by varying the gas pulse frequency and liquid flow rate in the droplet generator (Figure 2b). They further showed that subcompartments resembling artificial organelles can be produced controllably inside the capsules.

Controlling Shapes and Sizes of Droplets. In the context of artificial cells, the term "droplets" typically refers to emulsified droplets (such as water-in-oil) and aqueous two-phase droplets (also called coacervates). Similar to liposomes, droplets produced by bulk techniques tend to be highly polydisperse. An additional practical problem when working with droplets is their inherent instability to coalescence and Ostwald ripening. Due to these processes, droplets tend to coarsen over time and phase separate. To produce a monodisperse droplet population, some means of stabilization must be provided. This stabilization often comes in the form of surfactants that coat the surface and prevent coalescence. Doping the droplets with a hydrophobic component has been proposed to minimize Ostwald ripening.[27] Droplets, being liquid, commonly tend to assume spherical geometry to minimize surface energy. However, because of their liquid nature, spherical droplets can conveniently be deformed to assume the shape of the container in which they are present. The most significant finding in the recent work by Fanalista *et al.* is perhaps the ability to prepare cell-sized droplets with various aspect ratios through variations made in the dimensions of the microfluidic devices. The authors also successfully showed that the aspect ratios can be modulated through external factors such as osmotic pressure.

The most significant finding in the recent work by Fanalista *et al.* is perhaps the ability to prepare cell-sized droplets with various aspect ratios through variations made in the dimensions of the microfluidic devices.

In recent years, there has been increasing interest in the study of membrane-free organelles such as prolamellar bodies, Cajal bodies, stress granules, and P-bodies.[28] Various coacervate droplet systems have been described that mimic such organelles.[29−31] Coacervates have been shown to concentrate biomacromolecules to an extent that is comparable to the macromolecularly crowded environment of a cell.[32]

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Polydisperse coacervate droplets are typically formed spontaneously due to electrostatic complexation or hydrogen bonding between two or more macromolecular species. Remarkably, Deng *et al.* showed that monodisperse coacervates can be generated inside liposomes using microfluidic methods, a significant achievement toward a functional synthetic cell (Figure 3a).[33] Likely, future extensions could be the reconstitution of simplified phospholipid synthesis pathways[34] or isothermal DNA amplification[35] within the coacervate phase. Protein nanofibril-mediated shape transformations and budding in coacervate droplets (Figure 3b) were recently described by Song *et al.*[36] We posit that such methods could be further extended to achieve shape transformations in a regular and predictable manner.

FUTURE DIRECTIONS AND OPEN QUESTIONS

Several methods of shape and size control of artificial cell containers have been described. However, methods dealing with both shape and size control simultaneously are relatively few and difficult to develop. In this regard, the work by Fanalista *et al.* deserves special attention and may provide useful future directions. Inspiration may be drawn from other fields, such as nanofabrication, where precise control of particle shape and size is of paramount importance. Several open questions remain to be addressed. For instance, how can shape and size control be achieved simultaneously in an artificial cell undergoing growth and division? Although several artificial systems displaying growth and division-like events have been described,[34,37,38] such proliferation events mostly take place nondeterministically and may be considered highly rudimentary compared to their biological counterparts. Proliferation of an artificial cellular container into two identical copies of itself remains a major goal. We surmise that approaches radically different from those prevalent in nature may need to be considered. Recently, the topic of division of active droplets into identical daughter droplets has been treated theoretically,[39] and it will be interesting to translate those ideas experimentally. Another long-sought goal in artificial cell research is the generation of Turing instabilities, which, coupled to artificial cellular membrane growth, may lead to controlled deformation of membranes and eventual division.[40] We envision that appreciation for shape and size as key design parameters will provide new avenues for synthetic biologists in an integrative approach for building artificial life and providing answers to several fundamental questions in biology.

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Notes

The authors declare no competing financial interest.

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