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Multicompartment Synthetic Vesicles for Artificial Cell Applications

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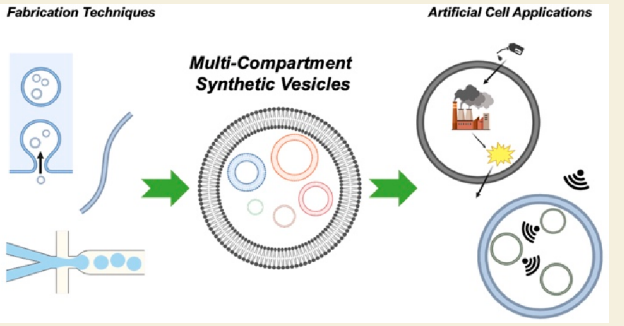
ABSTRACT: This review explores the cutting-edge development of multicompartment synthetic vesicles designed for artificial cell applications, drawing inspiration from the complex compartmentalization inherent in living cells. It delves into the recent advancements in engineering vesicles equipped with both membranous and membraneless organelles (vesicles-in-vesicles and coacervates-in-vesicles), offering a detailed examination of the methodologies and materials employed. This paper highlights the critical role of these multicompartment vesicles in simulating cellular microenvironments and functions, facilitating the spatial and temporal segregation of biochemical processes, such as signal transduction, gene expression, ATP synthesis, and energy production. Moreover, this review outlines potential future directions, emphasizing the importance of these vesicles in the evolution of artificial cells with a focus on their application in creating more sophisticated biomimetic systems.



KEYWORDS: *Multicompartment Synthetic Vesicles, Self-Assembly, Microfluidics, Emulsion Transfer, Artificial Cells*

1. INTRODUCTION

Cells, the fundamental units of life, present intricate structures and multifaceted functions. The exploration of cellular biology, spanning centuries, has profoundly impacted our understanding of living organisms, which enables the development of various biological applications while also inspiring researchers to develop innovative materials that mimic the sophisticated functions of cells.[1,2] Despite significant advances, the complexity and fragility of integrated cellular processes pose ongoing challenges.[3] To overcome these complexities, scientists have developed artificial cell-engineered constructs that replicate specific cellular structures and functions.[4] These models offer simplified systems for dissecting and understanding the intricate workings of biological cells.[5]

Artificial cells serve as functional platforms that deconstruct complex biological processes into more manageable components. Among the various models for artificial cells, synthetic vesicles that bear structural resemblance to living cells (e.g., bilayer membrane) have garnered significant attention.[6,7] Synthetic vesicles that mimic the structure and function of biological vesicles found in cells (e.g., cytoplasm, nucleus, peroxisomes, etc.) can be constructed using diverse building blocks: lipid vesicles (liposomes), polymer vesicles (polymersomes), or proteinosomes. Liposomes offer high permeability and membrane fluidity, facilitating efficient molecule transport, yet they exhibit lower stability and limited versatility due to the short chain length of lipid molecules. Conversely, polymersomes have high stability and versatility, capable of encapsulating a wide range of molecules and equipping membranes with functional domains, although they are less biocompatible and less fluid compared to liposomes.[8] Proteinosomes, vesicle structures made from protein−polymer conjugates, are capable of presenting functionally folded proteins on their membrane.[9] Their high biocompatibility and specific functions derived from proteins make them a promising platform for artificial cells; however, their potential susceptibility to denaturation under certain conditions remains a challenge.[10] As liposomes, polymersomes, and proteinosomes each offer unique advantages and challenges for the development of artificial cells, ongoing research aims to leverage these benefits, overcome the limitations, and emulate crucial membrane characteristics. These vesicles, serving as simplified yet effective analogues of natural cellular structures, provide valuable insights into cellular mechanisms, such as sensing, transport, fusion and fission, and growth.

A defining feature of living cells is the compartmentalization of organelles, each tasked with specialized functions, including but not limited to transcription/translation,[11] energy harvesting,[12] or metabolism.[13] The orchestrated interaction of these organelles facilitates the overall cellular functions.[14,15] Multicompartmentalization, therefore, is not just a characteristic of

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living cells but a fundamental principle guiding the development of artificial cells. In constructing artificial cell models, researchers have focused on synthetic vesicles with multiple internal compartments, effectively creating artificial organelles within these vesicles.[16] Various compartment types have been employed as artificial organelles, including droplets,[17,18] hydrogels,[19] coacervates,[20] and vesicles.[21] These compartments replicate various cellular microenvironments, enabling the spatial segregation of incompatible enzymes and substrates, thereby mimicking the compartmentalized nature of cellular organelles.[22,23]

In cellular biology, organelles are categorized as membranous and membraneless structures. Membranous organelles, typically enclosed by phospholipid bilayers, include structures such as the nucleus, mitochondria, and lysosomes, each housing specific enzymes and playing crucial roles in cellular functions. In contrast, membraneless organelles are biomolecular condensates formed through liquid−liquid phase separation, which are also referred to as coacervates.[24] They are integral to various cellular processes, including gene expression, regulation, and RNA processing.[25,26] Moreover, leveraging the dynamic assembly and partitioning tendencies of coacervates enables the engineering of local concentrations of enzymes and substrates, facilitating precise modulation of biochemical processes.[27−29] Recent research in multicompartment vesicles encapsulating synthetic analogues of membranous and/or membraneless organelles have been extensively investigated and considered as promising artificial cells platforms.[30,31]

Herein, we provide a comprehensive overview of the latest advancements in multicompartment synthetic vesicles for artificial cell applications. These vesicles, resembling both membranous and membraneless organelles, offer a versatile platform for emulating complex cellular functions. It is worth noting the existence of tissue-like liposome networks, which are often categorized as multicompartment vesicles due to their complex structure.[32,33] However, in this review, we define multicompartment vesicles more narrowly as structures that encapsulate distinct internal compartments within a singular outer membrane. Additionally, artificial cell platforms featuring coacervates-in-coacervates structures have also been actively investigated.[28,34,35] While multiple liposome networks and coacervates-in-coacervates fall within the broader category of complex artificial cellular structures, our discussion concentrates on synthetic vesicles that serve as artificial cell membranes that load internal compartments of membrane-bound and membraneless organelles (vesicles and coacervates, respectively). We delve into the state-of-the-art techniques employed in constructing these vesicles, highlighting their potential applications in replicating and studying complex cellular mechanisms. The pursuit of developing artificial cells is not just an academic endeavor; it also holds immense potential for practical applications. By creating models that closely mimic cellular functions, researchers can gain deeper insights into biological processes, paving the way for innovations in materials science and engineering, medicine, biotechnology, and beyond. This review aims to bridge the gap between theoretical knowledge and practical applications, offering a perspective on how multicompartment synthetic vesicles can revolutionize our approach to understanding and utilizing cell mimetics and artificial materials design in various fields.

2. COMPARTMENTALIZATION IN SYNTHETIC VESICLES: EMULATING CELLULAR COMPLEXITY THROUGH MEMBRANE-BOUND AND MEMBRANELESS ORGANELLES

Artificial cells, by design, aim to replicate the complex functionalities of natural cells, which are inherently compartmentalized into various organelles that serve distinct and crucial biological processes.[5] The creation of separate compartments within synthetic vesicles, including both membrane-bound and membraneless structures, allows for the spatial and temporal segregation of biochemical reactions. This segregation is pivotal for reconstituting the orchestrated cellular processes in a synthetic setup, enabling the study and manipulation of fundamental living mechanisms in a controlled environment.[15]

Membrane-bound compartments in synthetic vesicles play a critical role in replicating the complex functionalities of natural cellular organelles, such as selective permeability,[36,37] active transport,[38] and environmental responsiveness. By manipulating lipid compositions or incorporating specific proteins, these compartmentalized vesicles with tailored properties achieve precise control over material exchange and regulated interaction with external stimuli.[39] This modular approach not only facilitates the spatial organization and efficiency of biochemical reactions but also offers a systematic platform essential for exploring cellular processes such as signal transduction,[40] gene expression,[41] and adenosine triphosphate (ATP) synthesis.[42] Through such sophisticated design, membrane-bound vesicles within synthetic cells can exemplify the convergence of biology and engineering, promising innovative applications in biotechnology and medicine.[43,44]

Membraneless compartments, formed through liquid−liquid phase separation or coacervation, mimic cellular microenvironments through their ability to selectively concentrate and organize biomolecules like enzymes and nucleic acids.[45] The strategy exploits the dynamic behavior of coacervates to precisely modulate and facilitate efficient biochemical processes, akin to nonmembranous organelles in cells.[46] Recent advancements allow for the engineering of coacervates to control the spatial and temporal organization of reactions by adjusting assembly parameters, such as ionic strength,[47] pH,[48] and molecular composition.[49] This enables the mimicry of regulated cellular processes, advancing the development of synthetic cells that replicate the complexity of living organisms. Hence, incorporating coacervates into synthetic vesicles represents a significant step in synthetic biology and artificial cell development.

To further illustrate the development and functionalities of these cell-mimetic microcompartments in vesicle structures, we discuss various methods for fabricating synthetic vesicles that encapsulate both membranous and membraneless organelles. In this article, the term "vesicles" refers to both outer membranes that mimic cell membranes and membrane-bound compartments. Thus, "vesicles-in-vesicles" and "coacervates-in-vesicles" specifically denote systems of membranous compartments within synthetic cells and membraneless compartments within synthetic cells, respectively. By summarizing recent advancements in creating these structures and their cell-mimetic functionalities, we aim to highlight the interplay between the structural and functional aspects of compartmentalization in artificial cell models.

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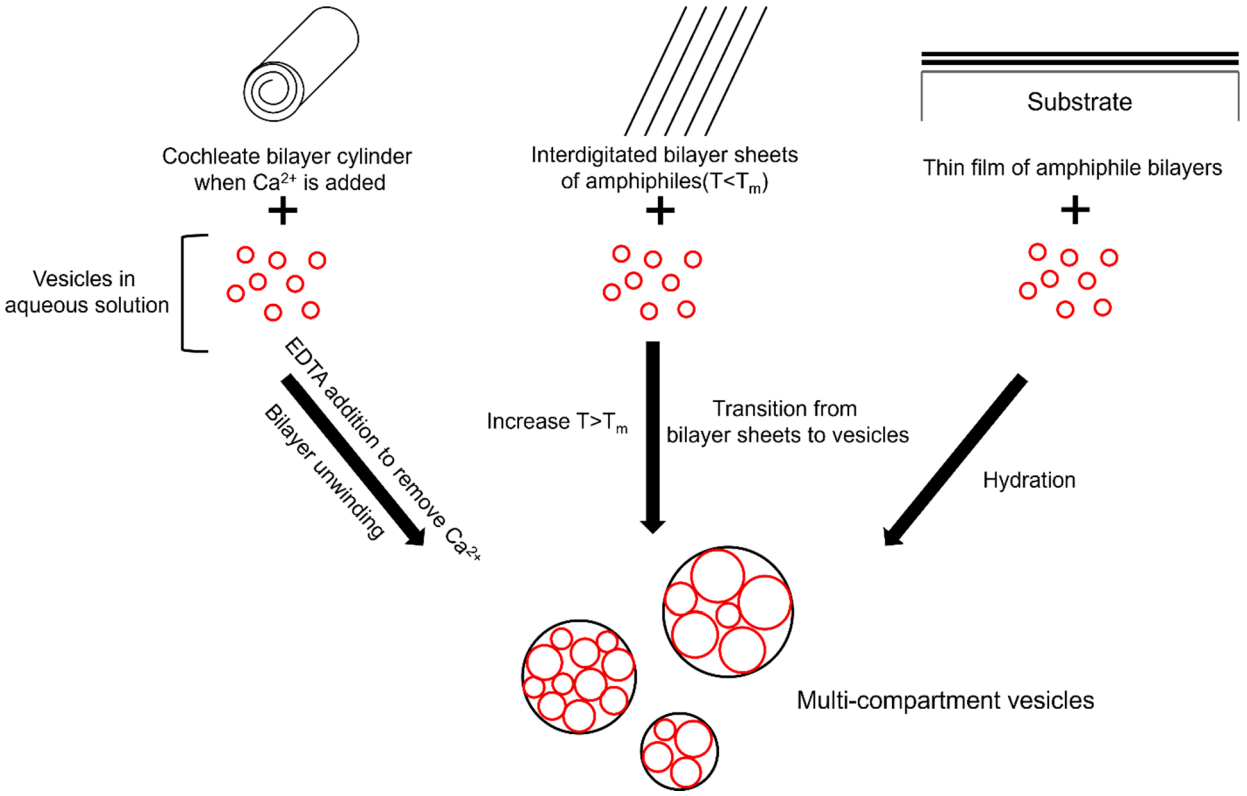

Figure 1. Schematic illustrations of synthesis techniques for multicompartment vesicles (i.e., vesicles-in-vesicles, called vesosomes) based on engineering an amphiphile bilayer. Reproduced with permission from ref 51. Copyright 2003 American Chemical Society.

3. FABRICATION OF MULTICOMPARTMENT SYNTHETIC VESICLES

3.1. Vesicles-in-Vesicles

The encapsulation of vesicular compartments within synthetic vesicles has been a key focus in creating multicompartment synthetic vesicles, commonly referred to vesosomes.[50] These vesosomes, characterized by smaller vesicles embedded within an outer membrane, function as artificial membranous organelles. They are distinctive for their ability to encapsulate various cargos in each compartment. Owing to these unique features, vesosomes are increasingly explored as platforms for artificial cells. To date, numerous innovative methods have been developed for constructing synthetic vesosomes, reflecting their growing significance in the field. Herein, we introduce the fabrication of multicompartment synthetic vesicles, particularly vesicles-in-vesicles, highlighting three main methods: bilayer sheet-based, emulsion-based, and microfluidic-based techniques. These techniques collectively advance the field of artificial cell development, each contributing unique capabilities, and can be applied to various building blocks such as lipids, polymers, and proteins.

3.1.1. Bilayer Sheet-Based Synthesis. Traditional strategies for the construction of vesicles are relatively straightforward. During giant vesicle formation, an aqueous solution containing smaller vesicles is used, leading to their encapsulation within the outer giant vesicles. This process involves the formation of amphiphilic bilayer sheets. Zasadzinski et al. demonstrated vesosome construction using cochleate bilayer cylinders, which unwind upon adding ethylenediaminetetraacetic acid (EDTA)[50,51] (Figure 1, left column). Anionic lipids form these cochleate bilayer cylinders in the presence of Ca^{2+}. The addition of EDTA, which makes water-soluble complexes with Ca^{2+}, induces the unwinding of these cylinders, thereby forming vesicles. If an aqueous solution with smaller vesicles is mixed with these cochleate bilayer cylinders before adding EDTA, the smaller vesicles are encapsulated during the unwinding process, resulting in the formation of vesosomes. Although this technique is simple and straightforward, a limitation that only negatively charged amphiphiles such as dioleoyl-phosphatidylserine (DOPC) are feasible to form cochleate bilayer cylinders remains as a challenge.

The same group reported another technique using interdigitated bilayer sheets to create vesosomes[52] (Figure 1, middle column). At high ethanol concentrations, rigid and interdigitated bilayer sheets are formed below the phase transition temperature.[53,54] Ethanol intercalates within the polar head groups of the amphiphiles, causing the hydrophobic tails to interdigitate.[55] As the temperature increases to above the melting point (T_m), the hydrophobic chains melt, leading to the formation of less rigid bilayers and the closure of bilayer sheets to form vesicles.[56] During vesicle formation, surrounding smaller vesicles can be entrapped to form vesosomes.[57] Unlike the cochleate bilayer method, this method does not limit the electrostatic charge of building blocks. By varying the concentration of ethanol added to the interdigitated bilayer sheets, the size of the vesicles can also be controlled.[58] However, the necessity to increase the temperature above the T_m may limit the type of cargo based on their thermal stability.

Another widely used method for vesosome construction is thin-film rehydration[59] (Figure 1, right column). Here, a solution of dissolved amphiphiles is placed on a substrate; this is followed by solvent evaporation to form a thin film. Smaller

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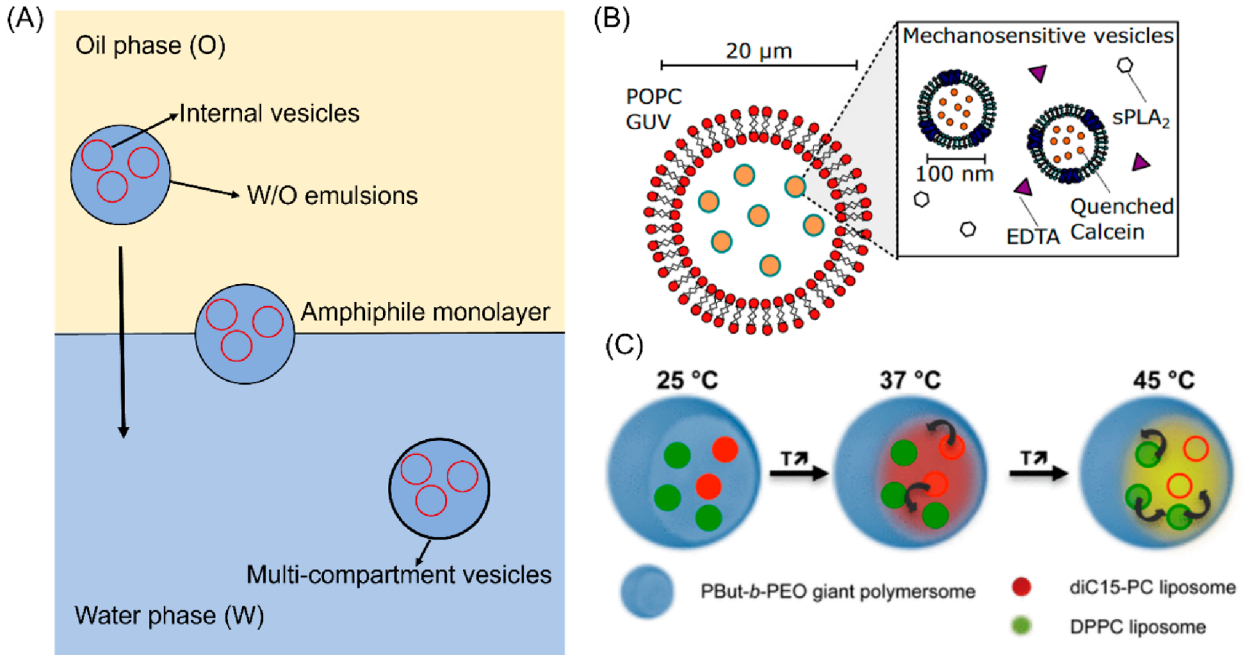

Figure 2. (A) Schematic illustration of emulsion transfer method for the fabrication of multicompartment vesicles. (B) Schematic illustration of giant liposomes containing mechanosensitive liposomes within the membrane. Reproduced with permission from ref 39. Copyright 2019 National Academy of Sciences. (C) Schematic illustration of multicompartment vesicles (liposomes-in-polymersomes), enabling the sequential release of cargo at different temperatures. Reproduced with permission from ref 68. Copyright 2017 American Chemical Society.

vesicles present in the aqueous solution are then encapsulated inside the giant vesicles during rehydration, resulting in vesosome formation.[40,60] In addition, hydrophilic gels or conductive materials (e.g., indium tin oxide (ITO)) can be coated on the substrate to enhance the hydration and swelling efficiency, known as gel-assisted thin-film rehydration[61] or electroformation.[62] These techniques are also applied to construct multicompartment vesicles.[63,64] The most powerful benefit of this thin-film rehydration method is its simplicity and scalability. This method is relatively straightforward and suitable for large-scale production; however, less precise control over vesicle structure, such as size, may remain as a limitation.

Having explored bilayer sheet-based synthesis in vesosome construction, we recognize the pivotal role of these methodologies in the broader context of artificial cell development. Each method, from cochleate cylinder unwinding to thin-film rehydration, offers a unique perspective on the intricate process of engineering multicompartment synthetic vesicles. It is crucial to select each method judiciously, considering the specific materials and structures relevant to the targeted applications. This strategic selection is key to optimizing vesicle functionality and applicability in diverse research areas. The upcoming discussion delves into how emulsion-based techniques further sophisticated vesosome fabrication, broadening the horizons for research in artificial cellular systems and emphasizing the importance of methodological alignment with application-specific requirements.

3.1.2. Emulsion-Based Synthesis. While bilayer-based vesosome fabrication offers significant advantages, its limitations include a narrow range of suitable amphiphiles and low encapsulation efficiency.[65] Emulsions, formed by the self-assembly of amphiphilic molecules at the water−oil interface, provide a robust alternative for constructing vesosomes due to their stable structure and higher encapsulation efficiency.[66,67] These emulsions facilitate the creation of vesosomes using a diverse array of amphiphilic molecules, ranging from phospholipids and synthetic block copolymers to protein conjugates.

A commonly employed method in vesosome synthesis is the emulsion transfer technique (Figure 2A). This process initiates with the generation of a primary water-in-oil (W/O) emulsion, where an aqueous solution containing smaller vesicles to be encapsulated is emulsified by an amphiphile in an oil phase. An amphiphile monolayer assembled at the oil−water interface stabilizes the oil and water phases separately into droplet forms. The primary W/O emulsions are then transferred to the interface, either by gravitational settling or centrifugation, resulting in the formation of vesosomes within the aqueous phase.[69] Hindley et al. utilized this method to synthesize multicompartment liposomes for studying protein−membrane−protein (P1-M-P2) signal pathways in the synthetic platforms (Figure 2B).[39] They demonstrated how these microscale nested vesicles can respond to external calcium signals, activating interactions between phospholipase A2 and a mechanosensitive channel within the internal membranes. The research highlights the effectiveness of emulsion-based approaches in designing and testing new signaling networks, including the incorporation of synthetic molecules, and lays the foundation for engineering multicompartment pathways that can sense and respond to environmental changes.

Furthermore, the emulsion transfer method has proven to be adaptable with various molecule types. For example, Peyret et al. demonstrated the synthesis of liposomes-in-polymersomes using this approach (Figure 2C).[68] The authors highlighted the temperature-dependent release of encapsulated cargo from internal liposomes, leveraging differences in thermostability. This work indicates that the use of different types of molecules broadens the applications of multicompartment vesicles in the field of artificial cells.

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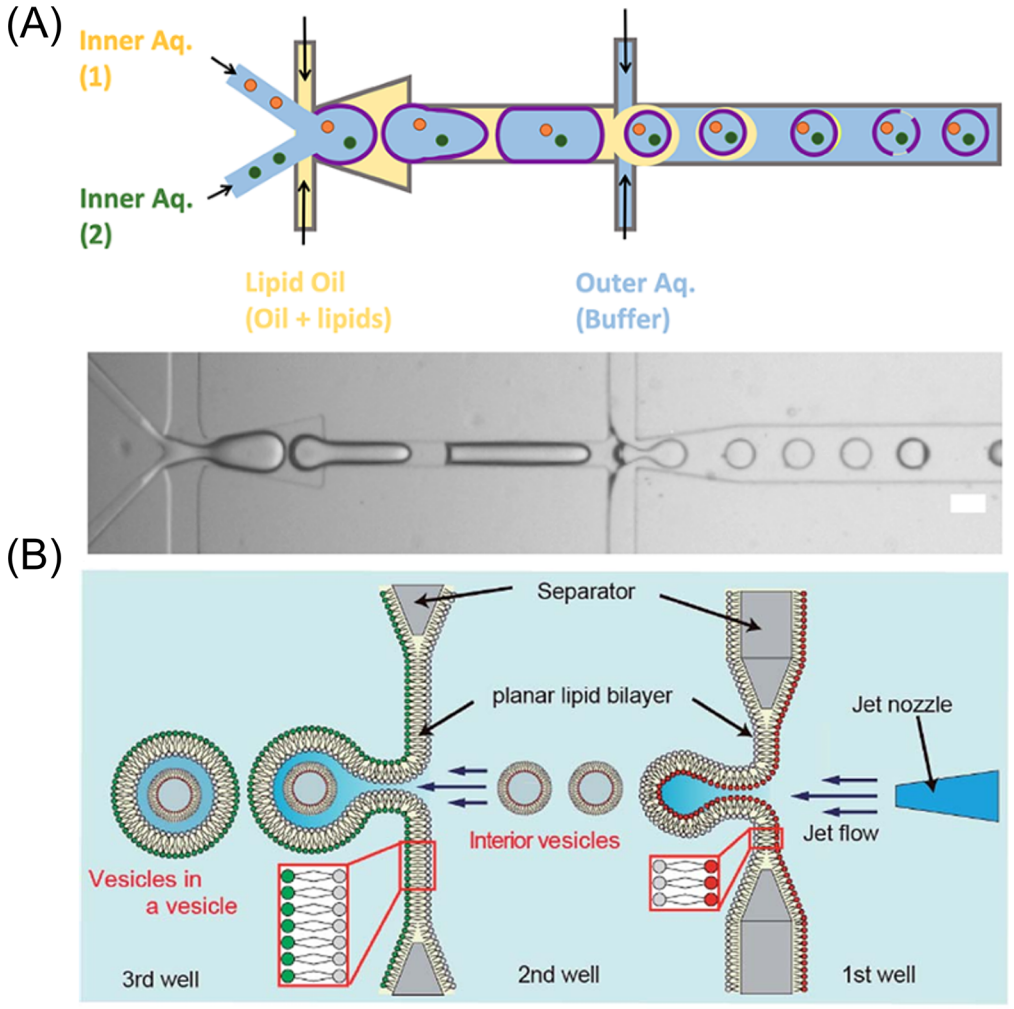

Figure 3. Synthesis of multicompartment vesicles through microfluidic devices. (A) Schematic diagram and bright field image of multicompartment liposome synthesis by leveraging W/O/W double emulsion. Reproduced with permission from ref 80. Copyright 2021 American Chemical Society. (B) Schematic illustration of the synthesis of multicompartment vesicles via a pulsed jet-flow method toward two parallel lipid bilayers. Reproduced with permission from ref 81. Copyright 2019 Royal Society of Chemistry.

In this section, we explored the utility and versatility of emulsion-based fabrication of multicompartment vesicles. This method, exemplified by the emulsion transfer technique, has significantly contributed to the field of artificial cell construction, enabling the study of complex biological phenomena, such as protein signaling pathways and stimuli-regulated cargo release. Moreover, the emulsion method enables the preparation of heterogeneous synthetic vesicles such as asymmetric synthetic vesicles, which have different compositions between two leaflets.[70] These heterogeneous synthetic vesicles have strong potential for building multicompartment vesicles structures with complex and additional functions for artificial cell development.[71] As we progress to discussing microfluidic-based synthesis in the next section, it is important to note the potential this emerging technique holds for further refining and expanding the capabilities in artificial cell engineering.

3.1.3. Microfluidic-Based Synthesis. While emulsion-based techniques are efficient and versatile for various types of molecules, achieving monodispersity in the size of multicompartment vesicles remains challenging.[72,73] To overcome this challenge, microfluidic techniques have been developed, offering enhanced precision in the construction of multicompartment vesicles.[74,75] Microfluidic devices enable the generation of single vesicles through the precise control of fluid flow within microchannels. Briefly, an oil phase and an aqueous phase are initially introduced into separate inlets, flowing through the microchannels, where they meet at a designated junction. At this interface, building blocks dissolved in the oil phase self-assemble into a bilayer structure, facilitated by their amphiphilic nature.[76] This controlled process allows one to produce single vesicles with uniform size and high encapsulation efficiency, making microfluidic devices promising tools in constructing vesosome structures.[77−79] Therefore, microfluidic approaches have been widely developed for customization in vesicle assembly, marking a pivotal step forward in the advancement of synthetic cell development.

Deng et al. employed a water-in-oil-in-water (W/O/W) double emulsification process using a microfluidic device to produce multicompartment vesicles.[77] In this method, small liposomes are localized within the inner droplet of W/O/W double emulsions, with lipid molecules dissolved in the oil phase. The oil phase of the double emulsions is then spontaneously evaporated, resulting in the formation of multicompartment liposomes with a narrow size distribution. Additionally, Shetty et al. constructed multicompartment

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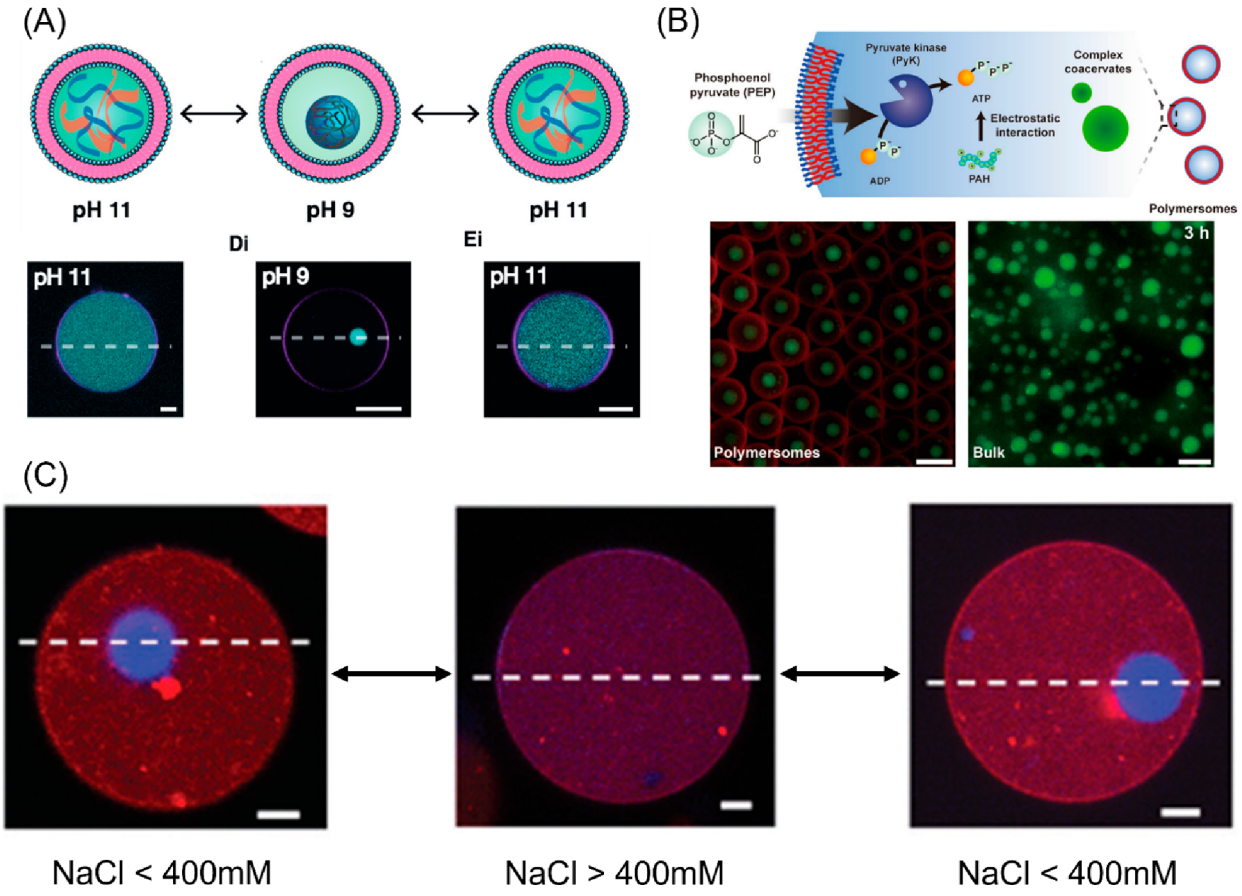

Figure 4. Engineering strategies to induce coacervation within synthetic vesicles. (A) Schematic and confocal fluorescence images of reversible coacervation at different pH. Reproduced with permission from ref 48. Copyright 2020 Love et al. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (B) Schematic illustration of signal-driven coacervation within polymersomes (upper row) and fluorescence microscopy images showing coacervation after signal molecules are added (lower row). Reproduced with permission from ref 49. Copyright 2022 Seo et al. Springer Nature. (C) Confocal fluorescence microscopy images showing reversible coacervation within proteinosomes through control of ionic strength. Reproduced with permission from ref 47. Copyright 2021 Royal Society of Chemistry.

liposomes containing two internal liposomes, which were made using PEGylated lipids to prevent the fusion of internal compartments (Figure 3A).[80] This structure allowed for the confinement of three different enzymes within separate spaces in a single giant liposome, facilitating the study of enzyme cascade reactions.

In addition to the W/O/W double emulsion technique, Kamiya et al. introduced a novel approach for synthesizing asymmetric multicompartment vesicles using a pulsed jet-flow method (Figure 3B).[81] This process involved utilizing a triple-well device with two asymmetric planar lipid bilayers. By applying pulsed jet flow across the bilayers, they successfully generated multicompartment liposomes. Furthermore, they showcased the fusion between internal and outer asymmetric liposomes, providing a complex model to study intracellular traffic behaviors such as those exhibited by exosomes.

This section on microfluidic-based synthesis has highlighted the technological advancements in creating multicompartment vesicles with enhanced precision and controlled size distribution. Techniques like W/O/W double emulsification and pulsed jet-flow methods exemplify innovative approaches in synthesizing complex vesicular structures. These methods not only address the challenges of monodispersity but also enable the study of intricate biological phenomena such as enzyme cascades and intracellular trafficking. These developments in microfluidic technology set the stage for the further exploration of sophisticated synthetic systems in artificial cell research.

3.2. Coacervates-in-Vesicles

Transitioning from the sophisticated methods of fabricating multicompartment synthetic vesicles through bilayer sheet-based, emulsion-based, and microfluidic-based techniques, we now advance to exploring the realm of coacervates within vesicles. While many cellular organelles are membrane-bound, some exist as membraneless compartments, preventing their components from mixing with their surroundings.[82] These membraneless organelles are crucial for cell function, contributing to vital processes such as RNA processing,[35] gene expression and regulation,[83] and enzyme activity. Recent discoveries have shown that these membraneless organelles are formed via coacervation or liquid−liquid phase separation (LLPS).[45]

Consequently, various coacervates have been explored as models for artificial membraneless organelles,[46,84−86] and the construction of coacervate-in-vesicle structures has become a key area of research in advancing artificial cell models.[87,88] A pioneering work that utilized coacervate-in-vesicle structures for an artificial cell model can be found from the report demonstrating the transformation of polyelectrolyte/ribonucleotide-enriched coacervate droplets into membrane-bound vesicles.[89] Exploiting the ability of coacervate droplets to

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concentrate organic dyes, proteins, and nanoparticles within an aqueous lumen, the authors demonstrated the coupling of enzyme cascade reactions that are generated within single vesicles. In this regard, this section highlights recent methodologies in creating coacervate-in-vesicle systems and examines how coacervate inclusion within vesicular structures opens new frontiers in the mimicry and functionality of artificial cells.

The construction of coacervates-in-vesicles often utilizes the spontaneous coacervation process within vesicle membranes, leveraging the stimuli-responsive and dynamic nature of coacervates.[34,88] Changes in external factors such as temperature,[34] pH,[90] or ionic strength[91] trigger the coacervation of macromolecules within synthetic vesicle lumens, making this approach versatile for various applications.

Complex coacervation, involving the electrostatic attraction of oppositely charged macromolecules, with pH plays a crucial role in phase separation.[92,93] For example, Love et al. utilized this principle in their study, demonstrating pH-responsive coacervation in liposomes containing polylysine and adenosine triphosphate (ATP) for artificial cell development (Figure 4A).[48] The charge of polyelectrolytes depends on the pH of the solution. Lowering the pH below the pK_a value of polylysine leads to protonation of the amine group, resulting in complex coacervation with negatively charged ATP. To induce coacervation within the vesicle membranes, the high pH buffer (pH = 11) was replaced with a trigger solution (pH = 7.3), adjusting the overall solution to pH = 9. This pH adjustment was possible because the liposome membrane is permeable for small molecules such as protons. The authors co-encapsulated fluorescein isothiocyanate (FITC)-labeled formate dehydrogenase and observed its partitioning into coacervate droplets. As coacervation proceeds, solvent molecules are excluded from the coacervation phase, leading to an increase in the local concentration of enzymes. They demonstrated the potential for tuning enzymatic activity based on the coacervation process.

Seo and Lee further extended this concept by showing small molecule-driven coacervation, facilitated by enzymatic reactions within polymersomes. They incorporated pyruvate kinase (PyK), an enzyme that catalyzes the conversion of adenosine diphosphate (ADP) to ATP in response to an external signal (Figure 4B), leading to complex coacervation of negatively charged ATP with cationic poly(allyl amine hydrochloride) (PAH).[49] Phosphoenol pyruvate, used as an external signal molecule to induce complex coacervation, has a low molecular weight that allows it to permeate through the membrane to reach the interior. Small molecule-driven coacervation enables the creation of synthetic compartments exhibiting biomimetic behavior, paving the way for the construction of life-like artificial cellular systems. Li et al. explored a different approach, developing a reversible coacervation method within protein vesicles (i.e., proteinosomes) by altering ionic strength (Figure 4C).[47] They achieved this by co-encapsulating positively charged poly(diallyldimethylammonium chloride) (PDDA) and negatively charged succinylated dextran (Su-Dex), where high salt concentrations reduce the attractive force between the charged molecules, resulting in coacervate disassembly. The authors demonstrated the reversible phase transition of coacervate droplets by exchanging phosphate-buffered saline (PBS) solutions of high and low salt concentrations, as the membrane is permeable to both cations and anions. These studies, along with the adaptability of traditional vesosome construction methods like emulsion transfer and microfluidics, demonstrate the versatility of coacervate-in-vesicle structures in artificial cell applications.[41,94] For example, a microfluidic-based method is employed to create coacervates inside cell-sized liposomes, enabling the controlled study of LLPS dynamics crucial in cell biology.[95] The platform facilitates the sequestration of proteins and metabolic reactions within coacervates, offering insights into the intracellular phase behavior and aiding in the synthetic reconstitution of cellular functions.

This section elaborated on coacervates-in-vesicles, focusing on their environmental sensitivity and applications in artificial cells. It included detailed studies demonstrating pH-responsive, signal-driven, and reversible coacervation techniques, highlighting the versatility of these systems in mimicking cellular processes and enhancing artificial cell models.

In section 3, we explored the fabrication methods of multicompartment synthetic vesicles, underscoring their potential as fundamental units in artificial cell construction. Each approach uniquely contributes to our understanding and capabilities in mimicking cellular complexity. The section also delved into the role of coacervates-in-vesicles as membraneless organelles, emphasizing their dynamic nature and responsiveness to environmental stimuli. The choice between designing subcellular compartments using vesicles and coacervates depends on factors such as the desired control over material exchange and compartmentalization. Vesicles offer selective permeability and membrane-associated processes,[6] while coacervates provide dynamic molecular interactions without a membrane barrier.[96] These foundational concepts in vesicle fabrication pave the way for discussing their practical applications in artificial cells, promising exciting advancements in synthetic biology, which are discussed in the following section.

4. ARTIFICIAL CELL APPLICATIONS

Multicompartment vesicles hold significant potential for artificial cell applications due to their structural similarity with eukaryotic cells.[15] In this section, we focus on cell-mimetic functions achieved by multicompartment vesicle systems, focusing on signal transduction, artificial nuclei and gene expression, and ATP synthesis and energy production. Although these systems have been widely investigated and current research involves recreating well-understood biological systems in synthetic contexts, the significance of artificial cells lies in their potential to revolutionize various applications. They offer opportunities to engineer cellular functions for therapeutic purposes, drug delivery, and biotechnological applications. Moreover, artificial cells serve as powerful tools for testing hypotheses and exploring possibilities that are difficult to manipulate in natural systems.[97] We explore how multicompartment vesicles facilitate intricate signal transduction pathways, a cornerstone in cellular communication and response mechanisms. We also examine the role of multicompartment vesicles in mimicking and reconstructing artificial organelles by highlighting recent works, which have demonstrated the versatility in replicating essential cellular functions and processes through compartmentalized artificial organelles.

4.1. Signal Transduction

Signal transduction, the process by which cells interpret and respond to external signals, is a fundamental aspect of cellular function. This complex mechanism involves a series of reactions across various cellular compartments, ensuring

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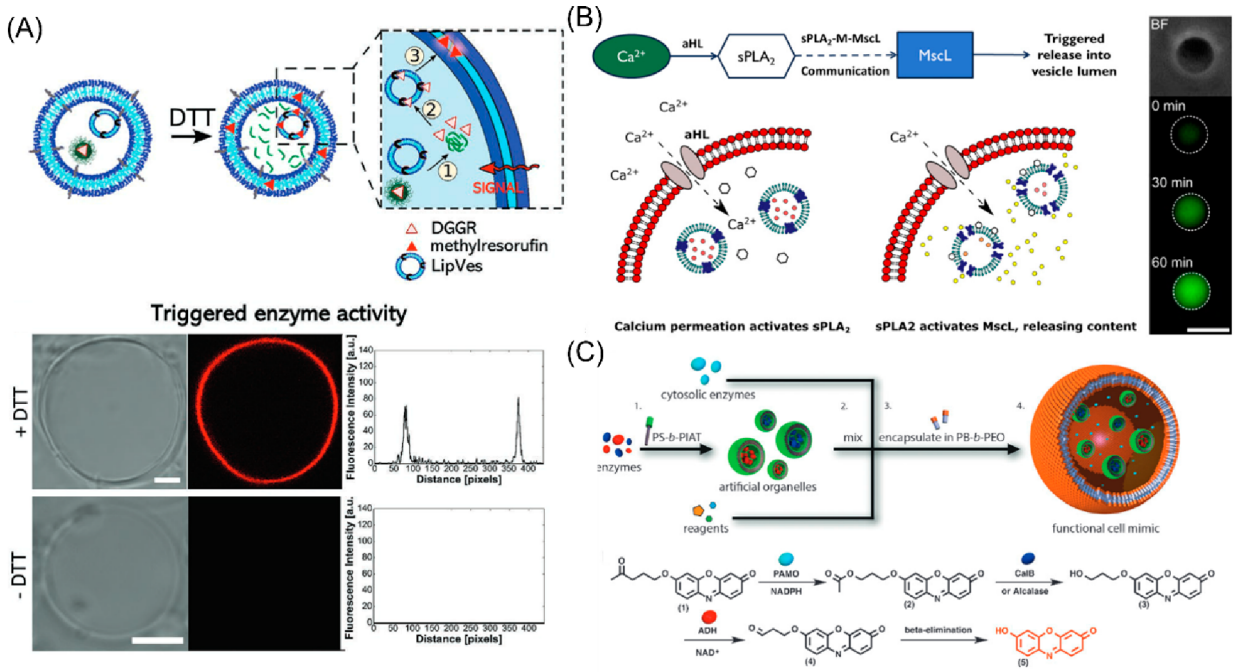

Figure 5. Signal transduction within multicompartment vesicles for the development of artificial cells. (A) Schematic image of signaling transduction within a giant polymersome, confirmed by bright field and fluorescent microscopy images in the presence and absence of signal molecules. Reproduced with permission from ref 40. Copyright 2020 Meier et al. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (B) Schematic image of signal pathway, confirmed by fluorescence microscopy images, when Ca^{2+} ions are introduced into the membrane at t = 0, 30, and 60 min, respectively. Reproduced with permission from ref 39. Copyright 2019 National Academy of Sciences. (C) Schematic representations of the design of multicompartment vesicles, encapsulating smaller vesicles containing different enzymes in their lumens, for a series of enzymatic reactions. Reproduced with permission from ref 104. Copyright 2014 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

accurate signal communication to target organelles or enzymes. The significance of recreating signal transduction pathways lies in their ability to replicate essential cellular functions in synthetic systems. By recreating these pathways, researchers can engineer artificial cells capable of responding to external stimuli,[43] communicating with each other,[98] and executing complex tasks. This capability opens up numerous applications, including targeted drug delivery systems[99] that respond to specific signals within the body and biosensors capable of imaging.[44] In the pursuit of mimicking these intricate biological processes, artificial cell research has made significant strides, particularly in the development of multicompartment vesicles.[100,101] Compared to single compartmentalization, multicompartmentalization allows for spatiotemporal control over complex reactions, such as signal amplification[102] or noise filtering.[103] Therefore, understanding and replicating the spatiotemporal aspects of signal transduction in multicompartmentalized artificial cells not only advances our knowledge of basic biological processes but also facilitates the development of more sophisticated synthetic systems in diverse biomedical and biotechnological applications.

An exemplary study by Belluati et al. showcased this innovation. They developed polymersomes with two distinct compartments and strategically positioned enzymes within the membrane (Figure 5A).[40] This design becomes activated upon the introduction of signal molecules, such as dithiothreitol (DTT), which penetrate the polymersome membrane. This penetration triggers the release of lipase substrates, leading to a cascade of interactions with pre-encapsulated enzymes and culminating in the production of detectable fluorescent molecules within the polymersome. Similarly, Hindley et al. developed multicompartment vesicles capable of sensing calcium signal through intracellular communications (Figure 5B).[39] In their system, the presence of calcium ions (Ca^{2+}) instigates a transformative process: $sPLA_2$ converts phosphatidylcholine lipids (DOPC) to lysophosphatidylcholine (LPC), altering the symmetry of the internal vesicle surface to the asymmetric membrane and creating lateral pressure throughout the membrane. The lateral pressure, in turn, activates the mechanosensitive channel of large conductance (MscL), leading to the release of previously encapsulated substances (e.g., calcein). This work exemplifies a sophisticated method of simulating intracellular communication.

In another study, Peters et al. ventured into the realm of enzyme cascade reactions through a singular giant polymersome multicompartmentalized with small polymersomes (Figure 5C).[104] This intricate setup involved three different enzymes, each residing within specific compartments: two polymersomes and the intervesicular space. Upon the introduction of signal molecules, these enzymes engage in a sequential reaction, producing fluorescent resorufin as the end product. The fluorescence intensity of resorufin serves not only as a measure of the efficiency of this system but also as a window into understanding cellular communication mechanisms.

These studies collectively contribute to a deeper understanding of artificial cell applications, illustrating the potential of multicompartment vesicles in replicating complex cellular processes. As we continue to unveil the intricacies of these systems, these research efforts offer a promising avenue for advancing synthetic biology and biotechnological applications.

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4.2. Artificial Nuclei and Gene Expression

The construction of artificial nuclei within multicompartment vesicles is a burgeoning research area in the realm of artificial cell development. This spatial organization enhances the efficiency, specificity, and control of gene and protein expression within synthetic systems.[105] Moreover, multicompartmentalization enables the integration of multiple signaling pathways with expressed genes and proteins, facilitating the construction of complex and biomimetic synthetic cellular systems.[106] This research aims to replicate the complex gene expression processes found in eukaryotic cells.[107]

A notable contribution in this field comes from the work of Deng et al., who employed an in vitro transcription (IVTx) mixture within liposomes.[77] This approach is instrumental in visualizing gene expression by co-encapsulating an RNA detector alongside the IVTx mixture. They encapsulated a single liposome, containing the IVTx mixture as artificial nuclei, into larger liposomes, thereby constructing an artificial cell model. The larger vesicle, encapsulating an in vitro transcription and translation (IVTT) mixture, simulates a cytoplasm-like environment, evident through the expression of a model red fluorescent protein. This methodology not only highlights the functional mimicry of eukaryotic cell nuclei but also demonstrates the potential of artificial cells in gene expression studies.

Furthermore, the same research group expanded this concept by using coacervates as artificial nucleoids (Figure 6A).[41] They co-encapsulated the IVTx mixture within a complex coacervate composed of spermidine and polyuridylic acid. This approach allowed for the observation of RNA production within the coacervate via fluorescence microscopy by co-encapsulating an RNA detector. These artificial cell models, based on the design of coacervate-in-vesicle systems, offer a novel way to study gene expression dynamics in a controlled environment.

In addition to the remarkable works detailed in the previous sections, ongoing research continues to expand the horizon of artificial cell applications in gene expression and regulation. This area of study is instrumental in bridging synthetic biology with applied materials engineering. Promising approaches that we can further employ include light-activated gene expression systems, which utilize photosensitive mechanisms for gene regulation.[108] RNA-based regulatory systems are also gaining traction, employing riboswitches and aptamers for precise control within multicompartment vesicles.[109] The integration of metabolic pathways into synthetic vesicles and the exploration of enzyme encapsulation are pivotal for simulating cellular metabolism.[110] Protein synthesis and folding within artificial organelles are being studied to mimic and understand complex biological processes.[111] Furthermore, the investigation of synthetic organelle interactions is crucial for replicating the communication and material exchange seen in natural cells.

4.3. ATP Synthesis and Energy Production

In this section, we focus on research efforts that have successfully recapitulated ATP synthesis and energy production within artificial cell systems.[42,112,113] ATP is a crucial molecule in cellular metabolism, serving as the vital energy currency of cells.[114] The construction of ATP synthesis in artificial cells holds significant potential across multiple domains. These synthetic systems may serve as versatile platforms for energy production, enabling consecutive

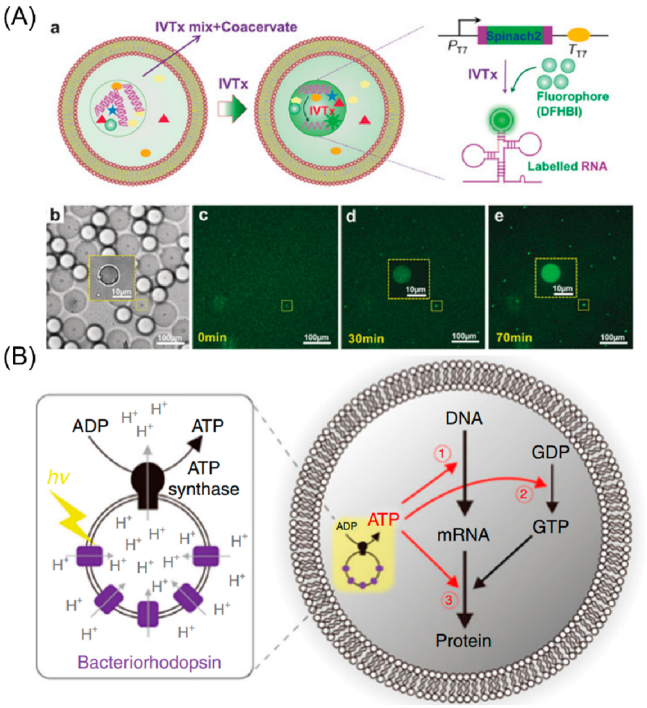

Figure 6. (A) Schematic image of an artificial cell model, a vesicle containing a coacervate that consists of an IVTx mixture (upper row), and optical and fluorescent images showing RNA expression within the coacervate (lower row). Reproduced with permission from ref 41. Copyright 2017 Huck et al. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (B) Schematic image of an artificial organelle that consists of bacteriorhodopsin and ATP synthase encapsulated in a giant liposome. Reproduced with permission from ref 42. Copyright 2019 Berhanu et al. Springer Nature.

biochemical reactions and driving synthetic biological devices.[115] Therefore, several studies have been conducted to develop artificial organelles that can synthesize ATP within artificial cells.[112,113] For example, Berhanu et al. constructed biomimetic artificial organelles capable of synthesizing ATP, thereby enabling artificial cells to produce energy from light (Figure 6B).[42] This breakthrough was achieved by encapsulating proteoliposomes containing bacteriorhodopsin (a protein that functions as a light-driven proton pump) and ATP synthase within giant liposomes. The study demonstrated that ATP production within these liposomes could trigger internal protein expression, leveraging ATP as a substrate in IVTT processes.

This exploration of ATP synthesis and energy production within artificial cells underscores the broader trend in synthetic biology: the creation of multicompartment vesicles that replicate the functions of living organelles. These efforts are vital not only for enhancing our understanding of cellular biology but also for their potential applications in areas such as drug development, disease modeling, and the development of complex biosensors. The integration of these synthetic systems marks a significant milestone in the field, heralding the era of more sophisticated and functional artificial cell models.

5. CONCLUSION

In conclusion, artificial cells represent a significant tool in unraveling complex biological mechanisms and probing the mysteries of the origins of life. The foundational step in artificial cell development lies in constructing cell-like

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structures, with multicompartment vesicles emerging as structures that closely resemble living cells. This review has provided a comprehensive overview of multicompartment vesicles, spanning from fabrication techniques to their applications as artificial cell platforms. We have particularly focused on vesicles-in-vesicles and coacervates-in-vesicles, reflecting the two primary structures of cell organelles. The incorporation of vesicles or coacervates within vesicle designs offers tremendous potential in mimicking cellular complexity and functionality. The sophisticated compartmentalization achieved through these engineering strategies facilitates the replication of natural cellular processes, including signal transduction and the creation of artificial organelles. Therefore, the ability to encapsulate and orchestrate distinct biological reactions within these multicompartment systems will open a new era in the development of advanced artificial cells. Although the journey toward fully realizing multicompartment vesicles for artificial cell applications is still ongoing, the insights and methodologies detailed in this review provide a valuable roadmap for researchers in the field. For example, creating hybrid systems incorporating both vesicles and coacervates within single vesicles can be an intriguing direction for future investigation, potentially unlocking new dimensions in artificial cell research. Each step forward not only enhances our understanding of cellular biology but also opens up new possibilities in biotechnology, medicine, and beyond.

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Notes

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