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Review

Recent advances of droplet-based microfluidics for engineering artificial cells

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ABSTRACT

Artificial cells, synthetic cells, or minimal cells are microengineered cell-like structures that mimic the biological functions of cells. Artificial cells are typically biological or polymeric membranes where biologically active components, including proteins, genes, and enzymes, are encapsulated. The goal of engineering artificial cells is to build a living cell with the least amount of parts and complexity. Artificial cells hold great potential for several applications, including membrane protein interactions, gene expression, biomaterials, and drug development. It is critical to generate robust, stable artificial cells using high throughput, easy-to-control, and flexible techniques. Recently, droplet-based microfluidic techniques have shown great potential for the synthesis of vesicles and artificial cells. Here, we summarized the recent advances in droplet-based microfluidic techniques for the fabrication of vesicles and artificial cells. We first reviewed the different types of droplet-based microfluidic devices, including flow-focusing, T-junction, and coflowing. Next, we discussed the formation of multi-compartmental vesicles and artificial cells based on droplet-based microfluidics. The applications of artificial cells for studying gene expression dynamics, artificial cell-cell communications, and mechanobiology are highlighted and discussed. Finally, the current challenges and future outlook of droplet-based microfluidic methods for engineering artificial cells are discussed. This review will provide insights into scientific research in synthetic biology, microfluidic devices, membrane interactions, and mechanobiology.

1. Introduction

A cell is the fundamental unit of life. There are two major classifications of cells, known as prokaryotes and eukaryotes. Eukaryotic cells have a nucleus bound by a membrane, membrane-bound organelles, and a cytoskeleton while prokaryotes do not have [1]. To understand the biological components of cells and how a cell behaves in ideal and non-ideal situations, it is essential to understand the fundamentals that is related to disease and development. Cells are a vital component and have been used in a wide variety of fields, including biology, biomedical, synthetic biology, cancer research, space health, and development [2,3]. Cells are complex with dozens of processes occurring within one cell at any given time. Cells are also recognized as the smartest factories where biochemical reactions occur. With the development of modern cell biology, there is an increasing interest in artificial cells that could potentially substitute nature cells for basic cell biology research for the establishment of artificial cell factory. Artificial cells provide a means of simplifying cell studies and are produced to mimic natural cells in a simplified and controlled manner. For successful biomimicry, artificial cells must be encompassed by a cell membrane with any internal organelles also being surrounded by a membrane. Artificial cell should also be able to perform cell-cell communication and be able to use metabolites for biological functions [4]. Compared to nature cells, the advantage of artificial cells is the ability to produce a high throughput of easily manipulated cells. The shape and size of an artificial cell can be easily controlled and can have any components present within the cells [5]. This allows researchers to focus on one aspect of cellular function without unrelated processes complicating the study.

There are several fabrication technologies that have been developed to generate artificial cells for different studies [6–8]. Two mainstream approaches to fabricate artificial cells are the top-down and bottom-up methods. The top-down approach begins with an already existing cell that is simplified by removing portions of the cell and reprogramming the cell to perform certain functions [9]. The bottom-up approach begins with fabricating a compartment similar to the membrane of a natural cell and introducing specific biomolecular components [10]. The bottom-up approach has become the widely preferred method of the fabrication of artificial cells. One method for fabricating an artificial cell is through the use of phase transfer, in which the droplets produced by droplet microfluidic methods are placed in an oil-water column. The

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first layer of the lipid bilayer forms around the droplet from the lipids present in the oil phase. The second layer of the lipid bilayer forms around the droplet as it moves toward the lower water phase [11]. Another approach for fabricating artificial cells is through hydration methods which rely on hydrating a dry, thin layer of lipids in order to produce a vesicle. These methods produce giant unilamellar vesicles (GUVs) because they are large compartments that share similar properties to natural cells [12]. Two forms of hydration methods are electroformation and gentle hydration. Of these two methods, electroformation has typically been preferred because it can easily be replicated and displays greater efficiency, though studies have shown that introducing osmosis to increase the repulsion between the thin membranes of lipids in its production of GUVs may allow for gentle hydration to be a viable means of producing GUVs to mimic natural cells [12]. Once these GUVs are produced, electro-injection can then be used to insert desired materials, resulting in a simple artificial cell for analysis [13]. Thus, the development of high-throughput, easily controllable, and manipulatable approaches to fabricate robust artificial cells is of great significance. While a variety of methods exist for fabricating vesicles and artificial cells, droplet microfluidic-based methods have become prevalent due to the great potential of generating easily controlled, high-throughput artificial cells.

In recent years, microfluidic-based methods have become more prevalent in synthesizing artificial cells, particularly droplet-based microfluidics. Droplet-based microfluidic techniques include active and passive methods that produce droplets to be used for a variety of applications [14]. Active methods of droplet-based microfluidics include electrowetting on dielectric and dielectrophoresis. Electrowetting on dielectric consists of two plates with arrayed electrodes pressed against liquid droplets and an electrode potential which leads to the ability to create, cut, transport, and merge droplets [15,16]. Dielectrophoresis tends to be used as a method of separating and sorting droplets [17]. On the other hand, passive methods of droplet-based microfluidics consist of using devices with differing geometries, including T-junction, flow-focusing, and co-flowing, to produce droplets. T-junction devices consist of a channel carrying oil and an inlet carrying an aqueous phase intersecting it at a perpendicular angle, producing droplets [18]. Flow-focusing devices are also based on the intersection of multiple channels. Co-flowing devices consist of one chamber being encompassed by another chamber. A chamber containing an aqueous solution is present within a chamber containing oil and droplets are produced when the aqueous phase comes into contact with the oil phase [19]. While the geometries of each of the passive droplet-microfluidic techniques differ from one another, each one of these devices brings together two immiscible fluids which produce droplets when they come into contact. Many factors impact the size and shape of the droplets produced, including the flow rate [20], the interfacial tension [21], and the height and width of the channels [22]. The droplets produced by each of these approaches can then be used as artificial cells. This is done through the production of a lipid membrane and the insertion of the necessary materials to conduct functions critical to cell life.

This review will first discuss the shapes and mechanisms of the passive droplet-based microfluidic techniques employed to create droplets, vesicles, and artificial cells. Next, we will discuss the membranes and components of artificial cells and how droplet microfluidics is used to produce them. Finally, we will discuss further uses of artificial cells in gene expression dynamics, membrane interactions, and mechanobiology.

2. Microfluidic technologies for the fabrication of droplets/vesicles/artificial cells

Droplet-based microfluidic devices are advantageous in biomedical research as they can be used to easily produce a large number of droplets of a controlled size, structure, and compartmentalization [23]. In the generated droplets, the droplets are provided with additional protection as well as a stable microenvironment; both of these features allow for the cell to be easily tested and analyzed in comparison to using other methods [24]. These microfluidic devices consist of channels that contain two immiscible phases which do not mix with one another; one is a continuous phase, while the other is a dispersed phase. Due to the ability of microfluidics to manipulate fluids [25], the aqueous phase is encapsulated and turned into hundreds and thousands of droplets of a uniform size [26]. Currently, droplet-based microfluidic devices are most successful in creating droplets of a spherical shape. However, other studies are being performed to use microfluidics to produce droplets of different shapes, such as a rod, which could mimic some types of bacteria [27].

Droplet-based microfluidic devices can be used in various biological and chemical experimental studies, such as those that relate to drug delivery, micro-reactors, and in point-of-care diagnostic chips [28]. Beyond these applications, droplet-based microfluidic devices have become exceptionally useful in relation to synthetic biology. Fabricating and studying artificial cells are one of the main applications [29], largely because using these cells reduces labor and is less restrictive than using natural cells. Artificial cells maintain both artificial and natural components of cells and can mimic how typical cells function [30]. In order for this mimicry to be successful, artificial cells must be able to perform similar functions as natural cells. This includes the synthesis of biomolecules, signaling within the cell and communication between other cells, locomotion to take cells from one location to another, and reproduction to form new cells, and other features [31]. Currently, some of these features are demonstrated better than others in artificial cells. It is also important that each artificial cell produced contains multiple inner compartments that maintain the sizes, contents, and composition similar to that of a natural cell [32].

2.1. T-junction

There are several different types of microfluidic devices that can be used for droplet generation. One of these is the T-junction. In T-junction devices, the width of both channels and the height can be altered in order to manipulate the droplets produced depending on the needs of the droplets [22]. This is considered to be the most popular type of cross-flow droplet generation [24,33]. The layout of a T-junction consists of two channels intersecting one another at a 90-degree angle [34], Fig. 1A. These two inlets are injected with two separate fluids that are immiscible with one another and each move solely in one direction. The continuous phase travels through one inlet and consists of the carrier fluid that the droplets will travel through. The dispersed phase travels through a perpendicular inlet and consists of the fluid that will be broken up into droplets [33]. The droplets form at the intersection of the two channels where the dispersed phase runs into the continuous phase due to different factors like surface tension and shear force [35]. The size of the droplets that a T-junction system can create is impacted by factors including the velocity of the flow, the thickness of the fluid, and the interfacial tension [31]. They are also impacted by the channels the fluids that are flowing through, as well. The height and width of the main channels can also determine the size of droplets [33]. Baxani et al. developed bilayer networks within a hydrogel shell using a T-junction microfluidic device [36], Fig. 1B.

2.2. Flow-focusing

Flow-focusing devices are another type of microfluidic device that can be used for the fabrication of droplets. The layout of this type of device differs slightly from the T-junction devices. Two immiscible liquids are inserted into the device, Fig. 1C. An aqueous fluid flows through one inlet while oil enters from above and below, resulting in the aqueous solution being separated into droplets as it continues through the channel [37]. The dispersed phase travels down one inlet until it reaches an intersection where it meets the continuous phase liquid that has entered through two other inlets above and below the dispersed phase

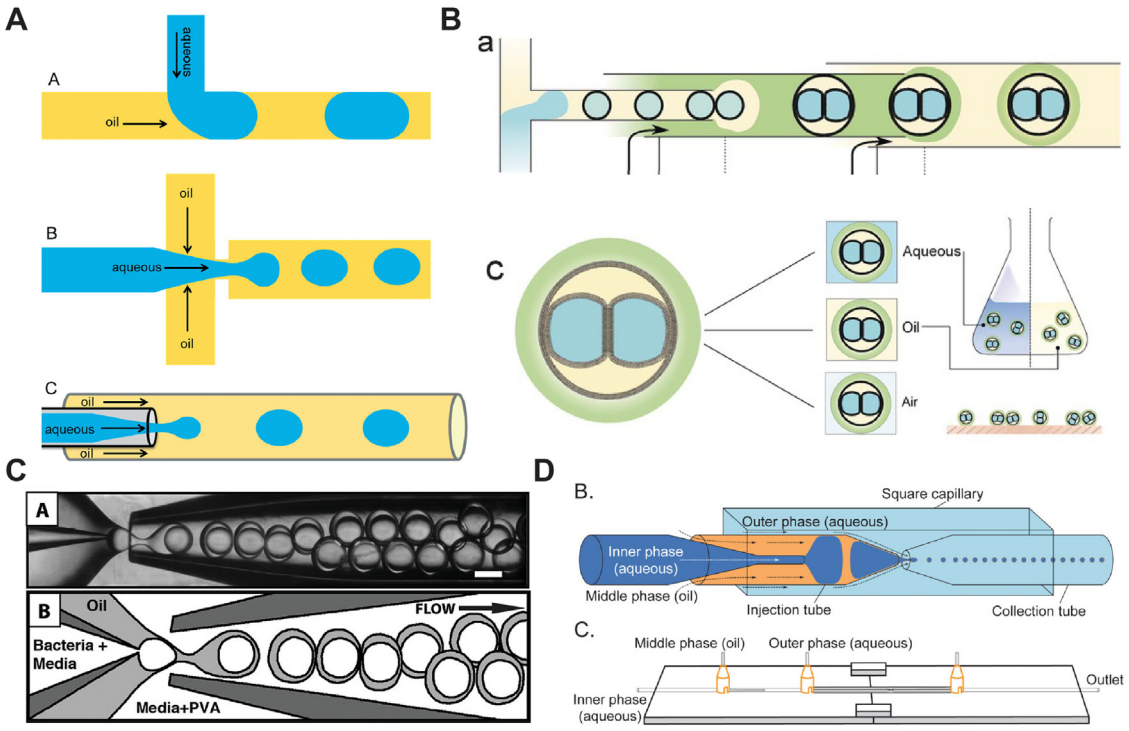

Fig. 1. (A) Different microfluidic drop maker geometries, T-junction, flow-focusing, and co-flowing. (B) Illustration of the double coaxial microfluidic concept employed for the formation of droplet interface bilayers (DIB) and encapsulation of a droplet of oil containing DIBs. (C) Microscale double emulsions created using a flow-focusing glass-capillary microfluidic device and schematic illustration of the flow-focusing region with liquid composition labeled. (D) Schematic illustration of a co-flowing microfluidic device for generating double emulsions. Adapted from [34,36,38,39].

liquid [38]. The droplets are formed due to the shearing force between the two materials [31], which, when symmetrical, form stable droplets. The droplets are also formed due to the hydrodynamic focusing exerted by the continuous phase [24]. The size of these droplets is influenced by the angle created between the channels carrying the dispersed and continuous phased as well as changing the flow rates of the continuous phase [26]. This type of device can be used to form hydrogel droplets of exceptionally small sizes [24].

2.3. Co-flowing

A third type of microfluidic device used to generate droplets is co-flowing device. This type of device differs further from the other two types previously discussed because the continuous and dispersed phases do not intersect with one another. These devices operate by precisely controlling the flow of two or more streams of fluids through microscale channels, allowing for the generation of complex structures with high spatial resolution [39]. In the context of artificial cell fabrication, co-flowing microfluidic devices can be used to encapsulate genetic material, proteins, and other biomolecules within a lipid bilayer membrane. Dewandre et al. developed a co-flow focusing microfluidic device allowing for the generation of droplets in an axisymmetric flow-focusing using a 3D printed nozzle [40]. Kalantarifard et al. developed a universal approach for the generation of high monodispersity droplets using flow-focusing and co-flowing microfluidic devices [41]. Ho et al. developed a co-flow glass microfluidic device to engineer artificial cells [39].

To summarize, all these three types of microfluidic devices are widely utilized for fabrication of droplets. For the application of generating artificial cells, both flow-focusing and co-flowing microfluidic devices offer advantages over T-junction microfluidic devices. Flow-focusing devices can easily generate uniform artificial cells with precise size control, complex structures, and muticompartments, which are difficult to produce with T-junction devices. Similarly, co-flowing devices can encapsulate particles and other components within droplets, i.e., triple-core W/O/W droplets [42]. Additionally, both flow-focusing and co-flowing devices have the ability to encapsulate multiple compartements within a single droplet, which is essential to create artificial cells with multiple organelles. In contrast, T-junction microfluidic devices have limited ability to encapsulate complex components, making them less suitable for creating artificial cells with membrane properties.

3. Fabrication of artificial cells

Artificial cells, also referred to as synthetic cells, minimum cells, or protocells, are microengineered entities that mimic the functions and features of biological cells by recapitulating cellular behaviors and properties. It has been reported that artificial cells can be utilized to investigate the properties of biological cells, cell dynamics, and fundamental biological processes [43], Fig. 2. Thus, it is essential to develop high-throughput, easy manipulation, and size-controllable approaches to fabricate artificial cells. Microfluidic devices for synthetic biology can be categorized into two main types: channel-based and droplet-based. Channel-based microfluidics enable long-term, real-time observation of cell behavior, making them useful for detecting subtle changes. In contrast, droplet-based microfluidics can produce droplets with precise size control and high throughput, making them well-suited for fabricating artificial cells. In this section, we will first discuss the fabrication of artificial cells membranes using microfluidics. Next, we will discuss the recent progress of droplet-based microfluidics for the fabrication of droplet-based artificial cells.

3.1. Synthesizing artificial cell membranes by microfluidics

Eukaryotic cells contain a plasma membrane and membrane-bound organelles, which perform functions for complex processes. In order for artificial cells to successfully mimic natural cells, the artificial cells produced through microfluidic-based methods must be encompassed by a membrane and contain multiple compartments. In naturally-occurring

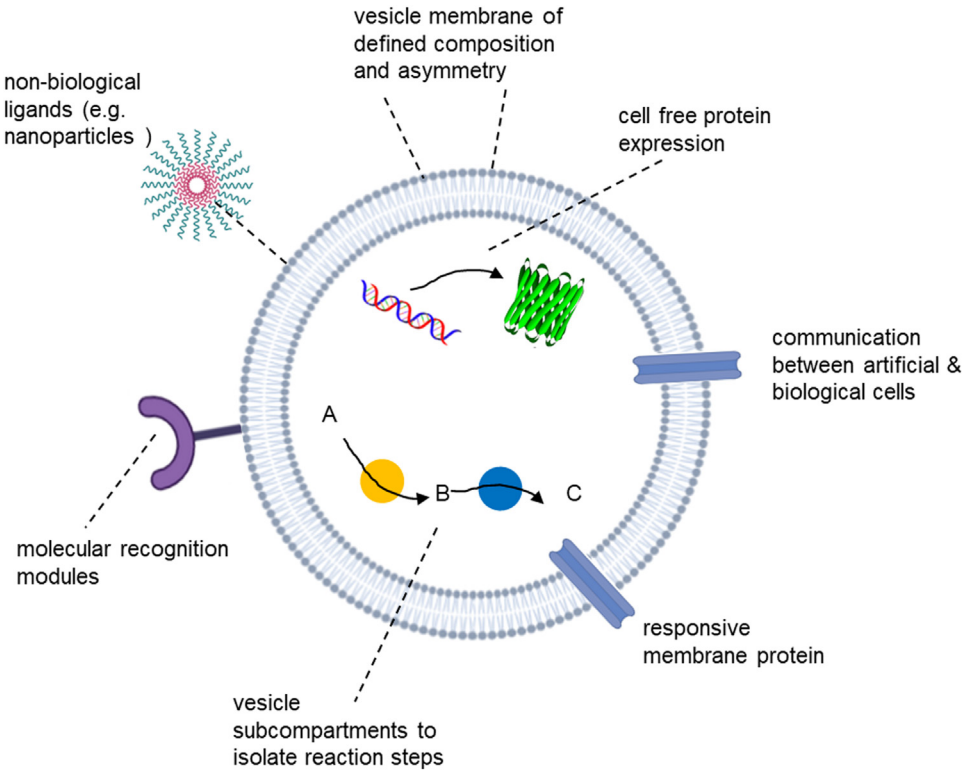

Fig. 2. Schematic illustration of a bottom-up based artificial cell which contains some key cellular components and features, including responsive membrane protein, cell-free expression of protein, non-biological ligands, and molecular recognition modules. Redraw from ref [43].

cells, the membrane is unilamellar and exhibits asymmetry [44]. These features allow for imperative cell functions such as cell-cell communication, the uptake of nutrients, and the removal of waste. Separate compartments within the cell are necessary to allow for biological processes to occur simultaneously in different regions [45]. Therefore, artificial cells must display the same characteristics. The synthetic membrane is typically derived from an assembly of molecules, including lipids, copolymers, and protein-polymer conjugates. Thus, the formed membrane-enclosed entities are referred to as lipid vesicles, polymersomes, and proteoliposomes, respectively.

One lipid vesicle, or liposome, that acts as an artificial membrane is a vesosome. A vesosome is a liposome that contains other liposomes to act as subcompartments [46]. Droplet microfluidic methods have allowed vesosomes to be a viable option for producing artificial cells with membrane-enclosed inner compartments. Deng et al. produced single liposome vesicles and used double or multiple emulsions to insert these liposomes into a larger liposome, creating a vesosome. Multiple lipid vesicles were successfully encompassed within another liposome, mimicking the organelles within a eukaryotic cell [47]. Polymersomes of varying sizes have been produced using T-junction and double emulsions. Compartments have also been produced within other polymersomes to act as organelles in a natural cell [48]. Polymersomes have not always been considered a preferred material for artificial cell membranes due to a lack of stability and a thicker, less permeable membrane than liposomes and do not allow for interactions with membrane-bound proteins [49]. As these features do not closely correlate with the features of natural cells, artificial cells based on polymersomes cannot mimic natural cells as accurately. However, Seo and Lee found that pluronic-based polymersomes can be stable for up to one week and be semi-permeable to allow smaller molecules to be transported across the membrane [50].

In artificial cells, both the number of compartments and the function of each of the compartments can be controlled. To aid in communication within the cell, each compartment contains transmembrane pores to allow materials to enter and exit [51]. These pores can be specific for a desired function which reduces the complexity that would occur in a natural cell. It remains a challenge to control the spatial organization of the compartments within an artificial cell. Spatial organization in natural cells is a dynamic feature and changes over time in order to reflect the needed functions of the compartments; however, it remains static in artificial cells [52]. This feature could then be manipulated in order to place membrane-bound compartments in optimal locations within an artificial cell.

3.2. *Synthesizing droplet-based artificial cells by microfluidics*

Droplet microfluidic techniques have been revolutionary in the field of synthetic biology by providing a means for fabricating a high-throughput of artificial cells with a controllable size and membrane composition [43]. Droplet microfluidics can construct droplets using methods such as T-junction, flow-focusing, co-flowing, and double emulsions. These microfluidic devices can often be produced using photolithography. Other studies are determining other means of creating these devices. Sasami and Sugenami have developed a way to use consumer-grade laser cutters in their production of these devices to allow for a simpler and more affordable means of construction [53]. By doing so, the production of artificial cells is more accessible to a wider array of researchers.

The droplets formed are then encapsulated by a membrane, ideally, one that is made up of lipids, in order to mimic cells in nature. Studies have shown that both single and double emulsion techniques can be used at the same time in order to first produce a monodisperse water droplet and then produce a lipid vesicle [54]. A means of producing the outer membrane of an artificial cell include water/oil/water (w/o/w) double emulsions. In double emulsions, lipids have been dissolved in the intermediate oil phase. When the oil phase is extracted, a vesicle with a lipid bilayer remains [55]. Droplet-interface-bilayers (DIBs) are bilayers formed between two droplets when surrounded by oil and have been coupled with droplet microfluidics in order to produce a artificial cell with a bilayer membrane. Droplet microfluidic techniques, such as the use of one or more T-junctions, have been used to produce water-in-oil droplets with a monolayer surrounding the droplet [56,57]. DIBs are formed when two of these water-in-oil droplets make contact and

combine to form one droplet with a bilayer membrane, mimicking a natural cell [57]. Hu et al. successfully used droplet microfluidics to produce giant unilamellar vesicles (GUVs) with an asymmetric lipid bilayer [58]. GUVs are spherical vesicles that range in size from 10-100 um, similar to the size of eukaryotic cells [58,59]. They have been used as membranes for artificial cells. Additionally, droplet-stabilized giant unilamellar vesicles have been produced using droplet microfluidics to act as compartments within an artificial cell. By further stabilizing these vesicles, biomolecules can be inserted into the compartments [59]. Eukaryotic cells contain a wide array of biomolecules. Typically, the internal compartments contain specific internal contents which is related to the function of the organelle. Studies such as the one performed by Lu et al. have used a method of droplet microfluidics that uses water and either air or nitrogen rather than the typical immiscible aqueous and oil phases [32]. In this study, each droplet became a capsule, the size of which depended on the flow of the liquid and the pulsing frequency of the gas. These droplets were resuspended in alginate and then put through the same droplet microfluidic device so that a new capsule formed around a designated number of the previously produced droplets. The desired proteins, nucleic acids, and other materials intended for an internal capsule need only be included in the alginate to enter into specific compartments [32]. This study has allowed for a means of controlling the number of internal contents within an artificial cell as well as the size of each compartment and the biomolecules present within. By using this droplet microfluidic process, biomolecules can be inserted into specific compartments in an artificial cell to mimic that organelle's functions in a natural cell.

In addition to the biomolecules within a compartment, the shape of a cell is closely related to the function of the cell. Droplet microfluidic techniques have been able to successfully produce spherical artificial cells, partially due to the capillary action of the droplets [25]. However, many cells in nature, particularly bacteria, are not spherical. Fanalista et al. produced droplets using double emulsions and deformed the droplets into shapes like rods and discs [27]. Providing a means to manipulate the artificial cells into different shapes allows for artificially mimicking a variety of cells that are not exclusively spherical.

4. Applications of artificial cells

Artificial cells have a variety of potential applications in biotechnology, medicine and drug delivery. Artificial cells can be valuable tools for studying fundamental biological processes, such as membrane interactions and cell signaling, and have the potential to lead to the development of new therapies for diseases. In this section, we first discuss the applications of artificial cells to study fundamental biological processes, transcription and translation, and membrane interactions. Following, we discuss the application of artificial cells in understanding mechanobiology.

4.1. Transcription and translation (TXTL)t

Transcription and translation are two imperative processes in cells. Artificial cells used in cell-free transcription and translation (TXTL) models have allowed for quick and rigorous genetic studies in a controlled environment [60]. TXTL studies can be performed on an expansive scale, particularly if a microfluidic chip is produced which allows for thousands of TXTL reactions to occur within phospholipid membranes [61]. By using artificial cells, it has been possible to examine switches used to turn transcription on and off. Light is considered an ideal molecular switch due to its non-toxic nature and has been studied in *E. coli* cell-free expression systems [62]. By using the YF1 fusion protein and the FixJ regulator, a two-component system was produced which can turn TXTL on and off in cells based on the presence or absence of blue light [62]. In addition to using artificial cells for studying the effect of light on TXTL systems, artificial cells can also be produced to respond to temperature changes in the cell. Artificial cells can be produced to contain temperature-sensitive non-coding RNA sequences which will perform protein synthesis at specific temperatures [63]. This is beneficial in studies of bacteria that rely on temperature sensitivity as well as studies in humans to perform temperature-specific drug delivery. While cell-free TXTL systems have often been studied in relation to prokaryotes, particularly E. coli [64], recent studies have produced mammalian cell-free systems. A locked nucleic acid (LNA) probe was produced and used to determine that protein synthesis dynamics in a HeLa system differ in bulk reactions in comparison with cell-sized single-emulsion droplets produced by droplet microfluidic techniques [65], Fig. 3A. Similar studies have performed synthetic studies containing cell-free expression systems compartmentalized within lipid vesicles. Despite being simpler than natural cells, these cells have still displayed different protein synthesis dynamics with bulk cell-free expression, suggesting that this is due to the semi-permeable nature of the lipid membrane [66]. Together, these studies provide a human-based system that can be combined with artificial cells to gain a greater understanding of gene circuits.

4.2. Membrane interactions

A major function of natural cells is the interactions between membranes. For example, in eukaryotic cells, collection migration is a process of a cohort of cells versus single-cell locomotion. It is a combination of the exchange of mechanical cues and mechano-sensing. The ability to sense and respond to chemical and mechanical cues relies on the existence of membrane proteins in nature cells. Membrane proteins are critical in signaling cascades for cell-cell and cell-environment interactions. Advances in artificial cells have allowed for a greater understanding of cell-cell interaction. Membrane interactions are involved in important biological events, including membrane protein insertion, membrane fusion, and intercellular communication. Advances in membrane protein reconstitution methods lead to significant progress in intercellular communication among artificial cells [67–69]. Most artificial cells are compartmentalized by phospholipid membranes, which allows for their semi-permeability. For example, Katarzyna et al. engineered gene-mediated communications between artificial cells by encapsulating the non-permeable molecule doxycycline (Dox) in one population and a plasmid encoding firefly luciferase (fluc) under a Tet promoter in the other population [70]. Moreover, Buddingh et al. engineered artificial cells that use adenosine monophosphate (AMP) as the sending signaling molecule [71], Fig. 3C. It was also reported that the membrane was used as part of the signaling cascade, where phospholipid vesicles are sender cells and proteoliposomes ae receivers. These two populations of cells communicate with each other while the sender cells use glucose as a signaling molecule and the receiver cells process glucose via glucose oxidase as a membrane component. In another work, Yang et al. designed an artificial signaling transduction system that can control the influx of environmental ions by triggering the activation of synthetic transmembrane channels immobilized on giant membrane vesicles (GMVs) [72]. In addition to compartmentalized artificial cells, water-in-oil-based artificial cells also communicate with each other by diffusing membrane-permeable molecules and pore-mediated propagation of signaling molecules. Booth et al. designed light-sensitive tissues which were made of droplets-in-oil that communicate only in the presence of external light triggers [73], Fig. 3B. In another work, Strutt et al. engineered artificial cells by inserting MscL in the droplet interface bilayer, where MscL opening is triggered by membrane tension due to membrane asymmetry [74].

4.3. Mechanobiology

Mechanobiology is the study of how physical forces affect the dynamics and functions of cells during complex biological processes. In nature, cells sense mechanical and other biophysical cues of their microenvironment, altering their morphology, migration, and differentia-

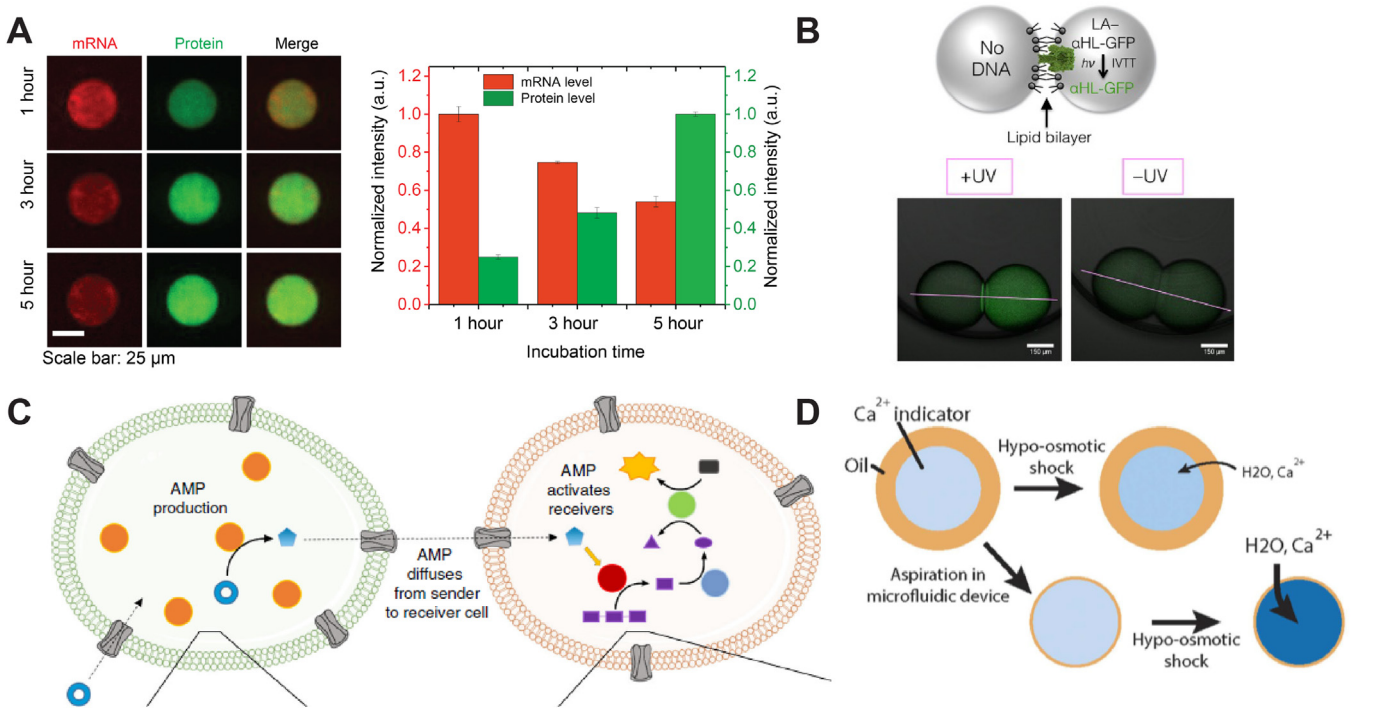

Fig. 3. (A) Transcription and translation dynamics in cell-free expression in single emulsion droplets. (B) Light-activated expression in a pair of artificial cells. One cell contains LA–*α*HL–green fluorescent protein (GFP) DNA, the other contains no DNA. No expression is observed without light activation. (C) Artificial – Artificial cell communication. A signaling molecule (AMP) is produced by the sender cells and diffuses to the receiver cells. Signal amplification permits communication over long distance. (D) Prototyping mechanosensing artificial cells. Double emulsion serves as a model of an artificial cell where the oil middle phase can vary in thickness and acts as a semipermeable barrier for ions to pass through. A microfluidic device can be utilized to compress or aspirate on double emulsions to mechanically activate artificial cells. It shows mechanically activated artificial cell through aspiration and osmotic shock facilitates calcium ion transport through oil. Figures are adapted from ref [65,71,73,79].

tion. Engineering mechanosensitive artificial cells are essential to understand the fundamental mechanisms of signaling transduction. In the last decade, engineered artificial cells have been mainly focused on sensing chemical inputs or physiochemical properties of the membrane. Recently, engineering mechanosensitive artificial cells have attracted researchers' interest. In principle, an engineered mechanosensitive artificial cell could sense and respond to mechanical cues, including shear, tensile, or compression forces. Ho et al. demonstrated the development of a microfluidic device to mechanically activate artificial cells by demonstrating the influx of calcium ions as a response through thinning of oil [75], Fig. 3D. It was the first demonstration that an artificial cell integrated a mechanical input into a chemical output. Majumder et al. reported a mechanosensitive artificial cell with the capability of biosensing by expressing MscL using cell-free expression [10,76]. They generated a DNA-programmed cell-sized artificial cell that can sense osmotic pressure and external calcium concentration. The ability to integrate artificial cells with mechanosensitive functions to sense and respond to external stimuli will open up the possibilities for rapid sensing. Recently, Hindley et al. reported a nested vesicle-in-vesicle artificial cells that can respond to an external Ca^{2+} stimulus by initiating a mechanosensitive sPLA2-M-MscL network, which can control calcein release from MscL into the main compartment of the artificial cells [77]. By utilizing protein communication through inner lipid membranes to control Ca^{2+} behavior of an artificial cell, they designed a multicompartment, synthetic communicative pathway in artificial cells. Moreover, the first synthetic mechanosensitive potassium channel was reported by using a newly developed aromatic fluorinated amphiphilic cyclophane [78]. This new ion channel has both stimuli responsiveness and selective ion transport abilities, which could open new doors for the future engineering of mechanosensitive artificial cells.

5. Summary and outlook

Artificial cells, minimal cells, protocells, and other "cell-like" systems have greatly attracted researchers' interest due to their ability to mimic the key characteristics of living nature cells. Artificial cells not only provide insights into the basic understanding of processes in nature cells but also provide opportunities to develop smart, cell-like materials. Moreover, it is also beginning to have a significant impact in the field of bioscience as novel therapeutic agent. During the last decade, droplet-based microfluidic approaches for synthesizing artificial cells have shown great potential for engineering such systems with high precision and in a manner compatible with relevant biological materials. Future development of artificial cells should focus on mimicking life-like systems with an effective metabolism to sustain the biomimetic processes within the compartment. Furthermore, compartmentalization should not only allow the replication of membrane components or genetic information but also the functional units that execute the biomimetic processes. Additionally, intercellular communication between artificial cells or between artificial and natural cells would open up more opportunities for collective behavior inspired by multicellular organisms. In addition, communication between artificial cells would open up interesting avenues to collective behavior inspired by bacterial colonies or multicellular organisms. Finally, this bottom-up-based fabrication of artificial cells will not only enhance our basic understanding of physical and chemical processes in living systems but also provide opportunities to study the principles of genetic evolution. Ultimately, the study of artificial cells has the potential to unlock new insights into the fundamental principles of life, as well as to enable the development of novel therapies and technologies with broad implications for human health.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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