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New opportunities for creating man-made bioarchitectures utilizing microfluidics

Samar Damiati[1]

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Abstract
Cells are the basic units of life, and can be mimicked to create artificial analogs enabling the investigation of cellular mechanisms under controlled conditions. Building biomimetic systems ranging from proto-cells to cell-like objects such as compartment membranes can be achieved by collecting biobricks that self-assemble to build simplified models performing specific functions. Hence, scientists can develop and optimize new synthetic cells with biological functions by taking inspiration from nature and exploiting the advantages of synthetic biology. However, the bottom-down approach is not restricted to the basic principles of biological cells, and new mimicry systems can be designed starting with a combination of living and non-living simple molecules to focus on a cellular machinery function. In recent years, microfluidic devices have been well established to engineer bioarchitecture models resembling cell-like structures involving vesicles, compartmentalization, synthetic membranes, and the chip itself as a synthetic cell. This review aims to highlight the role of biological cells and their impact on inspiring the development of biomimetic models. The combination of the principles of synthetic biology with microfluidic technology represents the newly-introduced field of synthetic cells and synthetic membranes that can be further exploited in diagnostic and therapeutic applications.

Keywords Synthetic bioarchitectures · Synthetic biology · Microfluidic technology · Protocells

1 Introduction

Synthetic biology is a growing technical field that engineers bridges between biological and artificial systems or between several biological communities to build up mechanical systems. The generated biomimicking systems are either designed to perform a specific known function or to perform a new function that does not exist in nature. However, synthetic biology draws knowledge from several disciplines, including molecular biology, chemistry, physics, mathematics, engineering, biotechnology, and nanotechnology (Fritz et al. 2010; Damiati et al. 2018a). The starting point of synthetic biology is exploiting comparably simple building blocks, which are usually biological materials, to end up with a functional system that meets pressing social needs. The assembly of bioparts such as DNA, RNA, minimal genomes, lipids, and proteins enables the generation of artificial systems mimicking the traditional models of natural dimensions (Chiarabelli et al. 2009; Malinova et al. 2012; Xu et al. 2016; Deplazes 2009). Hence, to engineer biology, there is a research cycle involving four integrated steps: (1) designing a system performing a desired function; (2) building a system exploiting biological and non-biological blocks; (3) examining the generated system to check its functional performance; and (4) analyzing the degrees of complexity, mimicry, and functionality of the created model compared to natural hierarchies (Linshiz et al. 2016).

A main goal of synthetic biology is the construction of synthetic cells, since the cell is the smallest unit of natural living systems. There have been many attempts to mimic biological cells, ranging from unicellular prokaryotic to multicellular organisms, to construct artificial cells exhibiting structural and functional properties of life but with less complexity (Malinova et al. 2012; Siontorou et al. 2017; Damiati 2009). The engineering of a synthetic cell borrows several basic characteristics of synthetic biology that focus on modularity, minimality, and controllability (Xu et al. 2016; Ding et al. 2014). The design of synthetic cells has several purposes, including improving our understanding of cellular life,

✉ Samar Damiati
samar.damiati@gmx.us

[1] Department of Biochemistry, Faculty of Science, King Abdulaziz University (KAU), Jeddah, Saudi Arabia

making biological cells perform new functions that do not exist naturally in order to promote new applications, linking living and non-living worlds, and finding a suitable theory to explain the origin of life (Xu et al. 2016; Yoo et al. 2011).

Synthetic biology is a diverse field that can be divided into two main fundamental approaches to build artificial cells: top-down and bottom-up (Fig. 1). Although these two approaches are different, they are complementary to each other. The top-down approach is based on a living organism and eliminating non-essential cellular components, subsequently exploiting it as a host cell that engineers new functions. This developed minimal cell strips down the genome to the lowest number of genes that is still sufficient to maintain the cellular properties. Indeed, the bioengineering of existing cells allows the total replacement of a genome with a synthetic one (Luisi et al. 1999; Gibson et al. 2010). In contrast, bottom-up approaches are based on single molecular assemblies. This strategy constructs a biomimetic cell by assembling living and non-living molecules that are built on biological and synthetic systems (Mann 2012). The developed design can be considered as a cell prototype that can be functionalized as a simple cell or a simpler bioarchitectural subject such as an artificial membrane. Hence, the two approaches are able to fabricate a broad range of artificial cells, ranging from a simple protocell to an engineered living system.

This review article represents different biomimetic models that have been developed, and focuses on the synergy between bottom-up synthetic biology and microfluidics in the construction of synthetic cells. Furthermore, it highlights the importance of microfluidics manipulation to control and engineer synthetic cell systems either to help investigations of natural cellular mechanisms or to create new biotechnological applications. Successful attempts to create biomimetic models using microfluidic platforms are also summarized.

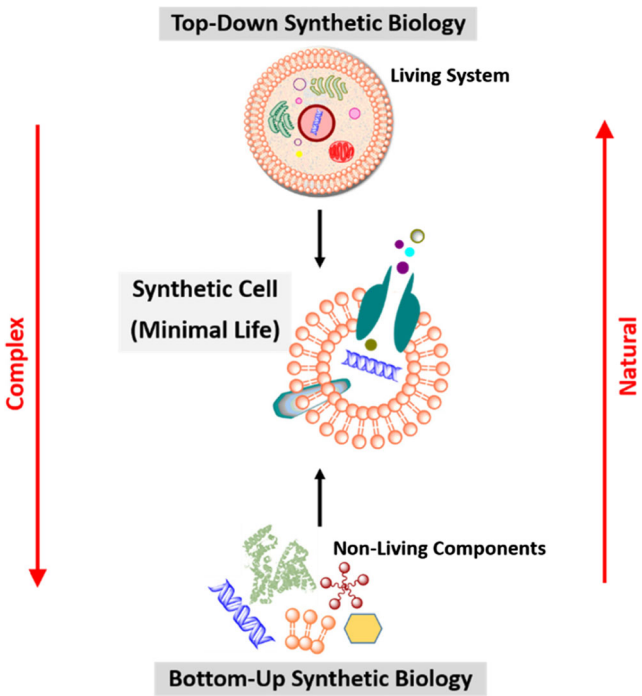

Fig. 1 Two main approaches of synthetic biology: top-down and bottom-up to construct minimal living system

2 Man-made bioarchitectures

The cell, the basic unit in life, is composed of lipids, proteins, and carbohydrates. The main structures and functions of biological cells are guided by lipids and proteins, respectively. Besides the complicated structure of biological cells, lipid–lipid, protein–protein, and lipid–protein interactions make the investigation of cellular mechanisms a challenging task. Hence, the development of biomimetic cellular models attracts many scientists who get their inspiration from nature. Several synthetic cell models can be designed, acting as cell-like counterparts exploiting different chemical and physical controlled mechanisms. The developed mimetic systems can be used in many applications, including DNA sequencing, biosensing, studying the mechanical properties of the lipid membrane and the functions of integrated membrane proteins, and understanding cellular functionality under health and diseases (Chan and Boxer 2007; Osaki and Takeuchi 2016; Bayley 2006). Biomimetic systems can be categorized into typical and non-typical models (Xu et al. 2016). The typical man-made cell has a cell-like structure and is able to perform some essential key features of living natural cells, such as metabolism, self-reproduction, self-maintenance, and dying. This type of bioarchitectural model can be considered as a living system if it can be said to possess: (1) a stable, semi-permeable boundary membrane that separates internal cell constituents from the external environment but is selectively permeable to ions and some substances and allows energy exchange; (2) biomacromolecules (RNA and DNA) that carry the genetic information and allow gene expression; (3) the ability to perform metabolic pathways to provide energy to cells; (4) the abilities of growth, self-reproduction, and self-maintenance to survive besides death; (5) the ability of adaptation in a dynamic environment; and (6) the ability to communicate with the environment and with neighboring cells. Furthermore, these models may exhibit more complex behavior or perform new functions that are not achievable by biological cells (Sole 2009; Pohorille and Deamer 2002; Saraniti 2008; Szostak et al. 2001). On the other hand, the non-typical human-made cell is not restricted in structure and performs few features of natural cells. This category is based on engineered materials mimicking one or more criteria of biological cells, such as functions, morphology, shape, and surface characteristics (Yoo et al. 2011). These cell-mimic particles combine the characteristics of natural cells with non-living materials to represent an advanced bioengineering

approach. In the following section, common models of synthetic bioarchitecture are summarized.

2.1 Protocells

To design and construct a synthetic protocell from the bottom-up using a minimal and defined number of basic building bioblocks, three steps are involved: the generation of genetic circuits (the information), the development of cell-free expression systems (the engine), and the development of cellular compartments (i.e., the shell) (Fig. 2) (Ding et al. 2014; Wu and Tan 2014). The first step of creating protocells focuses on the creation of genetic circuits ex vivo to mimic the genetic activities of biological cells, but outside cells. Genetic circuits are based on biochemical interactions within living cells (Watson and Cockroft 2016). Designed circuits depend on host resources to function—for example, transcription/translation machinery (e.g., promoters, logic gates, and ribosomes), DNA replication tools (e.g., enzymes), and metabolites (e.g., amino acids) (Ding et al. 2014; Brophy and Voigt 2014; Nandagopal and Elowitz 2011). In the second step of protocell creation, the designed genetic circuits are examined in a cell-free system (i.e., the engine) to engineer synthetic metabolic and protein machineries. The designed genetic circuits aim to perform a particular function which may be functional *in vivo* but not *in vitro* due to variations between natural and synthetic environments such as molecular crowding (Tan et al. 2013). Hence, the testing of developed circuits occurs in cycles between *in vivo* and *in vitro* systems. Complex and unspecific transcription/translation machinery makes the implementation of protein synthesis outside of natural cells a difficult approach. Therefore, scientists developed cell-free systems involving all essential components, which can be either whole cell extracts or protein synthesis using recombinant elements (PURE) systems (Shimizu et al. 2001; Zubay 1973). The first type of cell-free system is directly derived from the cytoplasmic components of prokaryotic or eukaryotic cells after eliminating the cellular membranes and native genomic materials. Synthetic genetic circuits are then added to the system to express target proteins. Although this system is commonly available for the commercial production of target proteins, the exact components of the cell extract are unknown (Shin and Noireaux 2012; Carlson et al. 2012). The second type, PURE systems, are reconstituted from purified components, with each one at a tightly controlled concentration. Free access to PURE systems allows the manipulation and engineering of the system, and thus make it a good choice for circuits and synthetic machinery to be functional inside of synthetic cells (Wu and Tan 2014; Jewett and Forster 2010; Ohashi et al. 2010). Tan et al. showed that molecular crowding has a significant role in regulating gene expression dynamics, it increases robustness of gene expression and can be exploited to control the basic genetic construction in synthetic cells. Therefore, to mimic crowding environments, cell-free systems can be supplemented with inert macromolecules of various sizes (Tan et al. 2013).

The third step involves the self-assembly of amphiphilic building blocks and the encapsulation of the genetic circuits (i.e., the information) and cell-free systems (i.e., the engine) to generate an active synthetic cellular shell. Several factors can affect the encapsulation of genetic circuits and cell-free systems into shells, such as pH, membrane compositions, and the viscosity of the cell-free system. Different materials composed of hydrophilic and hydrophobic monomers can be used as building blocks to construct the shell, such as phospholipids, fatty acids, and polymers. For example, the spontaneous assembly of fatty acids allows the generation of spherical micelles or bilayer vesicles in aqueous solution. Changing

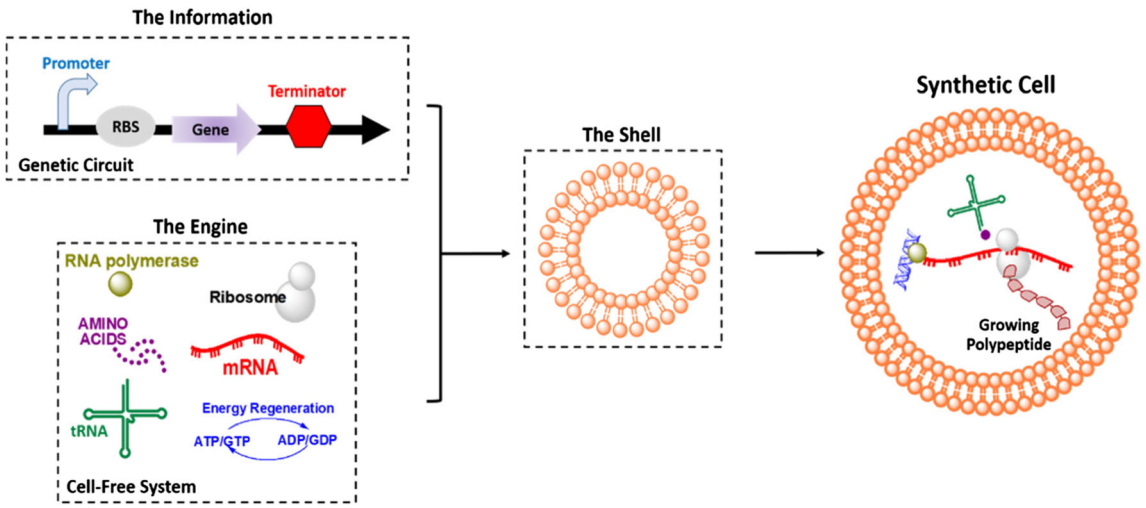

Fig. 2 Construction of biomimetic systems in three steps. First step, designed genetic circuit is constructed *in vivo*. Second step, cell-free system is used to test the genetic circuit and synthetic machineries for protein synthesis. Third step, gene circuits (the information) and cell-free expression systems (the engine) are encapsulated inside synthetic membrane (the shell)

amphiphile concentrations, ionic contents, and pH enables the conversion between lipid micelles and vesicles. However, membrane shell stability can be affected by several environmental factors, such as osmotic pressure, pH changes, and ionic contents. Hence, improving stability can be achieved by changing shell compositions (Monnard and Deamer 2002; Sunami et al. 2010; Apel et al. 2002; Harada and Discher 2011). Moreover, many techniques can be used to construct the shell, such as extrusion and lyophilization methods and microfluidic devices. The extrusion method generates uniform-sized synthetic cells (usually <1 μm in diameter) while lyophilization generates large synthetic cells with a heterogeneous size and lamellarity (Oberholzer et al. 1999; Lentini et al. 2014; Ishikawa et al. 2004). The microfluidic method generates controlled-size synthetic cells.

Although protocells have a relatively low complexity, their design and construction require deep knowledge about their assembly and cellular machineries. Engineered protocells can be exploited to mimic one or multiple biological structures/functions while sharing many fundamental characteristics of synthetic biology.

2.2 Cell-like membranes

Generating synthetic membranes individually appears as a promising platform for man-made bioarchitectures. The unique structures and functions of biological membranes have been admired by many scientists, and that motivated them to design many synthetic membrane models such as vesicles, lipid monolayers, and supported lipid bilayer membranes (Damiati 2009). However, building synthetic membranes involves the reconstitution of membrane proteins into self-assembled lipid membranes to mimic natural pores or channels and allow the exchange of ions/metabolites (Simeonov et al. 2013). The self-assembly of fatty acids, phospholipids, or polymers dispersed in aqueous solution results in the generation of versatile fluid-filled spherical monolayer vesicles such as micelles, or bilayer vesicles such as liposomes, nanodiscs, or polymersomes, depending on the compositions used to generate the vesicles and the temperature conditions (Fig. 3) (Gupta and von Recum 2014). These small enclosed compartment membrane models are usually used to investigate membrane phase behavior, membrane fusion, and molecular recognition. Indeed, vesicles can be exploited for membrane protein reconstitution and electro-physical characterization (Damiati 2009; Simeonov et al. 2013).

A lipid monolayer (i.e., Langmuir monolayer) is a two-dimensional synthetic system mimicking biological membranes in a very simple form (Fig. 4a). These insoluble films are created by continuously adding amphiphilic molecules to an air–water interface of constant area based on the self-assembly of the used molecules in a specific orientation. Monolayer models enable the evaluation of lipid–lipid and drug–lipid interactions (Eeman and Deleu 2010; Maget-Dana 1999). In contrast to the biological membranes which are composed of lipid bilayers, the presence of half the bilayer membrane makes the Langmuir monolayer a limited system.

There are several membrane models composed of lipid bilayer structures used as model systems to investigate membrane proteins. A free-standing lipid bilayer is a biomimetic model allows fusion of the transmembrane protein into the lipid membrane (Fig. 4b). The lipid bilayer is formed over a small aperture (<1 mm in diameter) in a hydrophobic substrate. This model has a limited lifetime and suffer from the poor mechanical stability (Damiati 2009; Köper et al. 2006). To overcome these limitations, a supportive substrate can be used. Supported lipid bilayers are one of the most popular biomimetic models, comprised of a planar structure of lipid molecules placed on top of a solid substrate. These special bioarchitectures allow the evaluation of chemical interaction between two monolayers, and the presence of the solid supports enables surface characterization using many tools, such as optical, electrical, and acoustic properties (Fig. 4c) (Damiati 2009). Solid-supported membranes have some drawbacks—mainly the space limitation between the lipid membrane and the solid substrate, which restricts the integration of membrane proteins and membrane components' mobility. The direct contact between membrane and substrate may cause protein denaturation, and thus leads to a loss of functionality. Furthermore, it is difficult to use this model for some biosensing applications due to low stability and lack of robustness (Sackmann and Tanaka 2000). Therefore, various architectures have been developed based on decoupling the lipid membrane from the solid substrate by introducing a spacer layer (typically ≤100 nm thick) that minimizes the interaction between the lipid membrane or integrated protein and the solid support, and reduces the non-specific adsorption of protein from the solution (Fig. 4d). Polymer and tethered solid-supported lipid membranes are the modified forms of these bioarchitectures (Damiati 2009; Sackmann and Tanaka 2000; Tanaka and Sackmann 2005). Several studies have reported the successful reconstitution of several proteins in their functional forms into these biomimetic models due to their higher stability (Damiati et al. 2015a; Damiati et al. 2015b).

3 Microfluidic technology

Over the past few decades, microfluidic technology has offered promising research tools to investigate life processes (Damiati et al. 2018a; Damiati et al. 2018b; Schulze et al. 2017; Saliba et al. 2018). Microfluidic chips enable the integration of biological and chemical processes on a single platform, while dealing easily with the control and manipulation of the flow behavior of small volumes of fluids in channels. Moreover, these microscale chips usually comprised of micro-

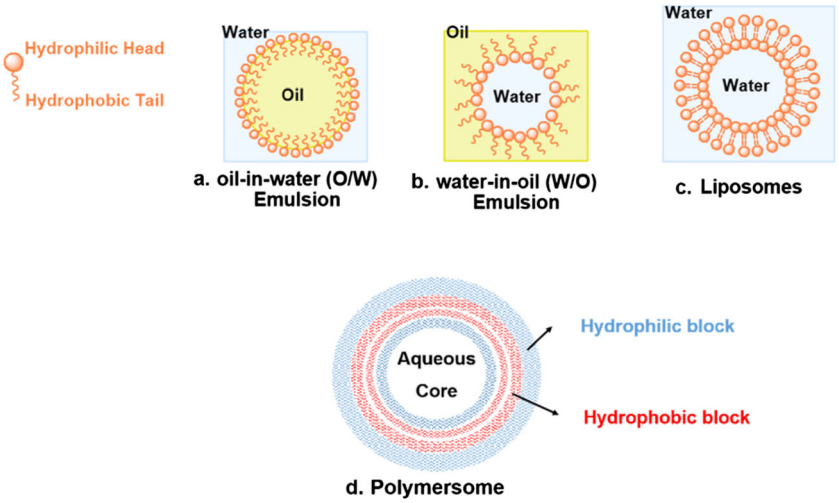

Fig. 3 Self-assembled lipid (emulsion and liposome) and amphiphilic polymeric (polymersome) vesicles

sized channels and the integration of nano/micro-fluidic components enable a minimal consumption of reagents/samples and high-speed analytical performance (Wang et al. 2018; Whitesides 2006). Several parameters should be taken into account in the design of a microfluidic device, such as channel geometries, materials used to construct the device, and the compatibility of these materials with various solvents. Due to natural fluid streams such as laminar flow in micro-channels, component mixing is mainly based on molecule diffusion across the interface of converging fluid streams. The rate of mixing characterizes the rate of reaction. Moreover, most microfluidic systems usually consist of inlets, valve, mixer, separator, micropump, and concentrator (Squires and Quake 2005; Kodzius et al. 2012; Siegel et al. 2010). Among the different fluid dynamics of geometries in microfluidic channels; two classes based on flow type are presented: continuous (single phase) and segmented (two-phase) (Fig. 5). The continuous flow strategy is an easy approach due to the high level of controllability and less sensitivity to protein fouling issues. Liquid flow is actuated in channels by external mechanical pumps, external pressure sources, integrated mechanical micro-pumps, or by combining capillary forces and electrokinetic mechanisms. Continuous-flow microfluidics are suitable for simple biochemical applications and for certain extra post-synthesis tasks, but these devices are less suitable for applications needing a high degree of flexibility and a high degree of fluid manipulation. In contrast, the segmented flow strategy (also known as discrete, multiphase, droplet-based microfluidic systems) involves the manipulation and creation of thousands of discrete droplets while focusing on continuous flowing streams of miscible fluids inside microfluidic channels. Generated uniform-sized droplets allow for fast mixing within nano-liter and femto-liter volumes due to the short diffusion distance and chaotic mixing within droplets (Hung and Lee 2008; Badilescu and Packirisamy 2012). The simplest continuous flow microfluidic devices are microcapillary devices for droplet generation. Three important microfluidic configurations are considered for droplet generation: T-junction, co-flow, and flow-focusing geometries. The T-junction is the simplest type, and enables the generation of oil-in-water (O/W) and water-in-oil (W/O) droplets. Droplets are generated at the channel intersection, where the main channel is filled with the continuous phase while the orthogonal channel is filled with the dispersed phase. Co-flow microfluidic devices depend on a 3D co-axial flow. A dispersed phase is injected into a circular capillary centered inside a larger-diameter

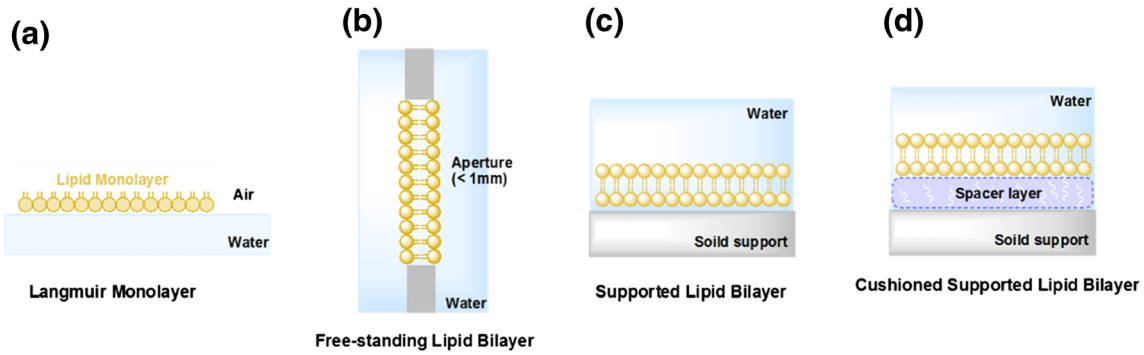

Fig. 4 Different models of lipid membranes systems

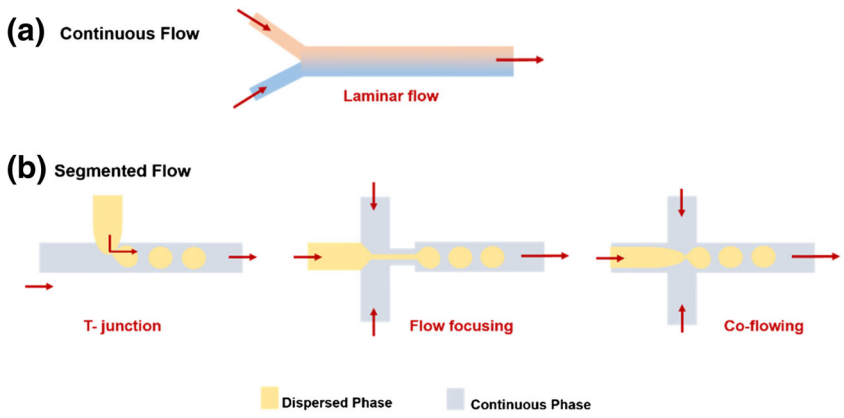

Fig. 5 Schematic of continuous and segmented flow microfluidics. Droplets microfluidics address several regimes for droplets generation: T- junction, flow focusing, and co-flowing

channel while a continuous phase flows in a square capillary in the same direction. Since the continuous phase surrounds the dispersed phase, the viscous shear force becomes stronger with increasing the diameter of the dispersed phase. In flow-focusing devices, the middle channel contains the dispersed phase, while the continuous phase is introduced from two outside channels into the same square capillary but in opposite directions (Chou et al. 2015; Anna et al. 2003; Basova and Foret 2015).

4 Microfluidic toolbox for constructing minimal biomimetic models

Combining the bottom-up cell-free systems with microfluidic technology allows engineering of semi-natural cells that can perform functions that exist in nature and provides high-throughput applications. Convenient strategies of *de novo* gene synthesis usually produce a pool of short DNA fragments that need further ligation reactions to generate longer fragments (Stemmer et al. 1995). This limitation can be overcome by using microfluidic platforms which allow the rapid mixing of gene fragments resulting in generation of combinatorial libraries. Moreover, microfluidic chips enable synthesis, purification, and assembly of DNA on a single device (Damiati et al. 2018a). At protein level, the cell-free protein synthesis system offers several advantages compared with the cell-based protein machinery, such as high protein yield; production of soluble and functional proteins; and the possibility of generating protein population in a single reaction due to the expressing of multiple templates (Jackson et al. 2004). This can be exploited by microfluidic technology to enable the protein synthesis, characterization, screening and direct evaluation of desired molecules on same platform and in a short time and utilizing minimum quantities of materials (Contreras-Llano and Tan 2018).

The wealth of microfluidic formats has contributed significantly to the design of a variety of synthetic biology applications, such as the construction of protocells and biomimetic membranes. The following sections describe successful attempts combining microfluidic devices with various interdisciplinary fields to engineer man-made bioarchitectures.

4.1 Droplet-based microfluidics

Most of protocell design relies on vesicles that are usually formed from fatty acids and phospholipids. Polymers and coacervates can also be used. Several approaches can be presented to choose the molecular entities to construct cell-like systems involving prebiotic, semi-synthetic, and fully synthetic species, and hybridization among these approaches is also possible (Stano and Mavelli 2015). On the contrary to conventional methods which usually generate poly-disperse droplets, microfluidic technology is one of the most popular vesicle production methods that enables generation of monodispersed and size-controlled nano- and micro-particles (500 nm–500 μm in diameter), which enables the encapsulation of biomolecules into discrete droplets. The two approaches of the synthetic biology can be exploited in vesicle construction. In the top-down approach, droplet microfluidics enable the encapsulation of DNA strands or single cells in single droplets, which allows direct evolution for gene expression profiling and whole-cell analysis (Fig. 6) (Szita et al. 2010; Theberge et al. 2010). In the bottom-up approach, droplets are used as precursors to lipid vesicles by assembling a bilayer around the droplet exterior while the contents of droplets become the interior of the vesicle-based cells. This strategy enables the encapsulation of large charged biomolecules such as DNA, enzymes, and proteins, and thus enables biochemical reactions to occur in cell interior (An Swaay 2013).

A wide range of models for generating vesicles using droplet microfluidics has been reported. Two common types of droplets are widely constructed using microfluidic devices: oil-in-water (O/W) are normal-phase droplets which are generated in hydrophilic channels, and water-in-oil (W/O) are inverse droplets which are generated in hydrophobic channels (Fig. 7). These models are also known as emulsions, and their generation depends on the target applications. However, the

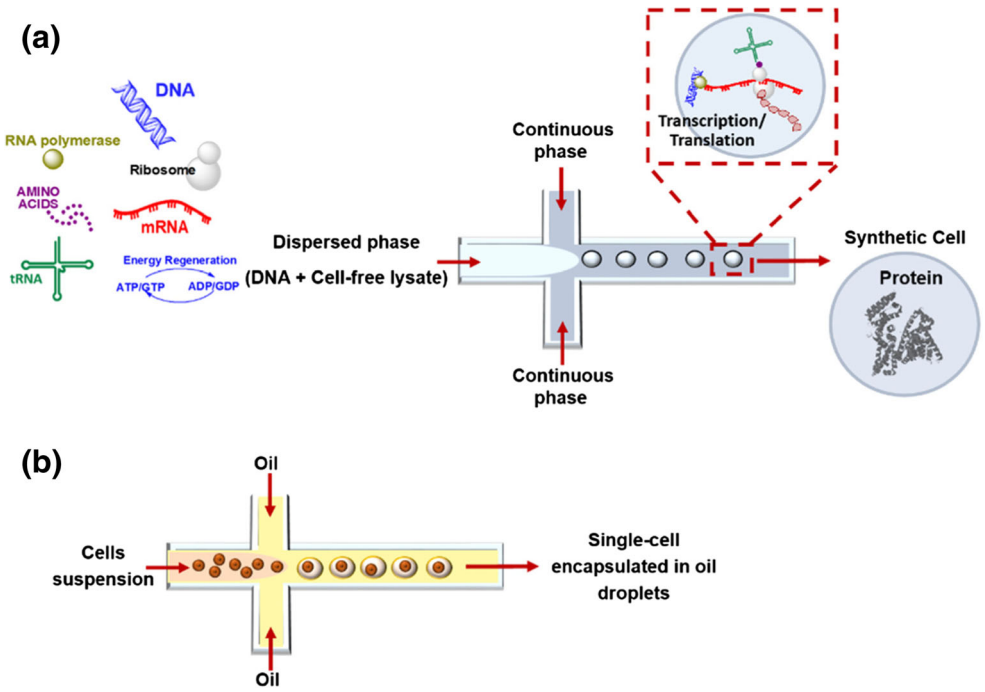

Fig. 6 Droplet-based microfluidic devices for **a** cell-free protein expression, and **b** single-cell encapsulation in micro-droplets

preparation of O/W or W/O droplets is controlled by choosing a suitable surfactant that is added at a sufficient ratio to the continuous phase. Piccin et al. developed a polyester-toner T-junction microfluidic device that enabled the fast generation of highly monodisperse O/W and W/O droplets in the same chip by adding appropriate surfactant and without any requirement of surface treatment of the microfluidic channels (Piccin et al. 2014). This is attributed to the polyester surface, which has partial hydrophilicity properties. Another vesicle model is that of double emulsions (Shum 2008). Similar to single-layer emulsions, double emulsions are comprised of two types: oil-in-water-in-oil (O/W/O) and water-in-oil-in-water (W/O/W) emulsions. They are produced by sequential droplet generation and subsequent encapsulation in larger vesicles in a single microfluidic platform. Yan et al. reported on a straightforward method exploiting a single microfluidic device to form monodisperse W/O/W double emulsions that act as *in vitro* compartmentalization validated by fluorescence microscopy and flow cytometry. The produced uniform compartments droplets showed high stability that did not leak loaded dye—neither in the external carrier phase, nor cross-contamination between vesicles. Moreover, correlations between dye concentration and fluorescence signals were clearly observed (Yan et al. 2013).

Liposomes are another cell-like model that can be assembled on a microfluidic chip. Liposome structures are closer in mimicry to the biological cell membrane due to their lipid bilayer arrangement (Fig. 3c). Microfluidic technology allows for the generation of monodispersed liposomes in shorter time and with fewer steps compared to conventional methods. Jahn et al. developed a microfluidic platform allowing for control of liposome size and size distribution for specific applications without any post-processing steps (e.g., sonication, extrusion). The designed microfluidic device had five-inlet micro-channels and three-outlet micro-channels, and constructed stable liposomes in nano-scale ranging from 50 to 150 nm based on laminar flow in the channels which enabled controlled diffusive mixing at the liquid interfaces (Jahn et al. 2007). Another strategy developed by Kuhn et al. relies on the immobilization of liposomes onto a strip pattern formed on a glass surface via

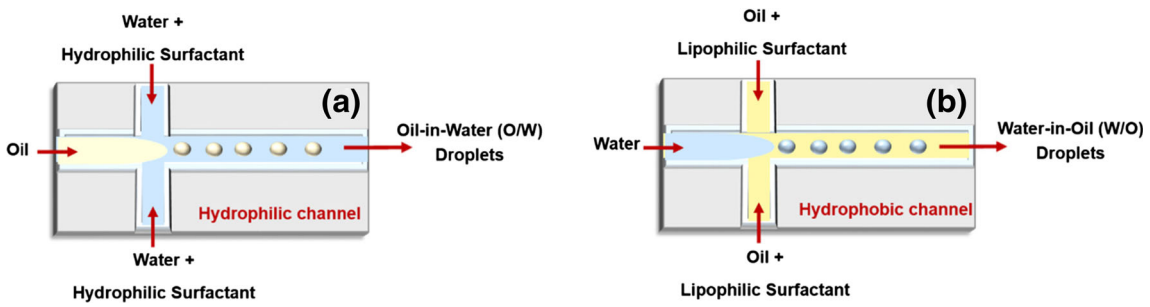

Fig. 7 Schematic diagram showing formation of oil-in-water (O/W) and water-in-oil (W/O) droplets in microfluidic devices

avidin–biotin bonding (Kuhn et al. 2011). This platform is integrated into a microfluidic device allowing continuous delivery of antibiotic – tetracyclines - at a constant concentration to mimic drug transportation via the blood in the human body. The developed system was used to investigate the kinetics of the permeation of tetracyclines across liposomes and to monitor the lipid membrane permeability and lipophilicity.

Membrane asymmetry is an important feature that synthetic cells should possess in order to replicate natural cells and membranes. This feature is attributed to differences in lipid compositions between inner and outer leaflet membranes (Elani et al. 2015a; Elani 2016). Matosevic et al. developed a microfluidic device that produced a layer-by-layer phospholipid membrane, with defined asymmetry and lamellarity (Matosevic and Paegel 2013). In this device, W/O droplets are trapped in a static droplet array. Lipid monolayers are then deposited around the W/O droplets as boundaries after the injection of an oil/water interface, resulting in the formation of unilamellar vesicles. Repetition of this process leads to multi-lamellar vesicles.

Polymer-based vesicles (polymersomes) are also developed as an alternative to lipid vesicles and as simple synthetic cells (Fig. 3d). Martino et al. reported on capillary microfluidic devices generating polymersomes encapsulating a DNA plasmid and a bacterial cell-free extract (Martino et al. 2012). In this cell mimicry model, protein expression and triggered release of expressed protein to the extracellular environment are fundamental processes. Both reactions were evaluated in the developed polymersomes by tracking the fluorescent signal and using negative osmotic shock to change polymersomes' membranes permeability.

Non-vesicle protocells have also been generated using microfluidics. For example, coacervates are membrane-less spherical aggregates of colloidal droplets generated using flow-focusing microfluidic chips from a hydrophilic polymer with either adenosine triphosphate or polysaccharide. Both compositions are able to generate droplets with high stability and narrow size distributions. However, coacervate droplets are able to encapsulate genetic information and different biomaterials, which represent these populations as a stable synthetic cell model (an Swaay et al. 2015; Aumiller et al. 2016).

4.2 Multi-compartment synthetic cells

Compartmentalization can be applied by microfluidics to form vesicle-based cells with spatial organization. Multi-compartment vesicles are chassis for synthetic cells that possess lipid bilayers spanning their internal volumes, partitioning them with defined compartmentalization (Trantidou et al. 2018). Thus, the number and size of compartments can be evaluated. Depending on the applications, each compartment and lipid compositions can be controlled (Fig. 8). This strategy has been exploited *in vitro* to express different proteins in each compartment of multi-compartment vesicles (Elani et al. 2015b). A platform fitted with electrodes was designed by Robinson et al. allowed trapping of multiple giant unilamellar vesicles (GUVs) in a microchannel and initiation of electrofusion (Robinson et al. 2018). This model enabled the introduction of new chemical materials into GUVs due to vesicles fusion. The developed device was used to monitor the dynamics of lipid membrane fusion and to measure the reaction kinetics.

4.3 Constructing synthetic lipid bilayer membranes in microfluidics

The basics of microfluidic technology can be used to characterize lipid membranes and membrane proteins as high-throughput applications for drug discovery and drug safety screening. Several studies have revealed the possibility of generating lipid bilayers within microfluidic structures, allowing protein research with high-quality resistivity and overcoming issues related to the low stability and reproducibility of lipid membranes (Fig. 9). Malmstadt et al. reported on a basic mechanism to fabricate a free-standing lipid bilayer structure with 5 nm thickness (Malmstadt et al. 2006). The generation of the lipid bilayer was based on the self-assembly of amphiphilic molecules that are aligned at the fluid-phase interfaces and was driven by selective solvent extraction in a microfluidic channel. The generated membrane was able to host a channel protein—α-hemolysin—at a measurable single-molecule conductance resolution. Another microfluidic system enabling the formation of a lipid bilayer membrane was developed by bringing two monolayers in contact. Phospholipids in the organic phase assembled at the interface between water and organic solvent in a microchannel resulted in a bilayer structure, confirmed by measuring membrane capacitance and ion channel signal detected after the reconstitution of antibiotic peptides into the lipid bilayer (Funakoshi et al. 2006). A microfluidic platform with integrated electrodes enabled the simultaneous formation of an array of lipid bilayer membranes that were developed for proteomics applications. Ion channel recordings were measured at multiple sites, presenting the model as a high-throughput platform for protein–protein and protein–compound interactions (Zagnoni et al. 2009). Dong et al. developed a microfluidic immunosensor to detect Staphylococcus enterotoxin B (SEB) in milk (Dong et al. 2006). In this strategy, reinforced supported bilayer membranes (r-SBMs) with biotinylated anti-SEB IgG antibody were formed by vesicle fusion in microfluidic channels and strengthened with a streptavidin layer to improve membrane stability. This r-SBMs chip had high sensitivity to detect SEB and presents a promising model for the transport and storage of membrane-functionalized commercialized micro-devices.

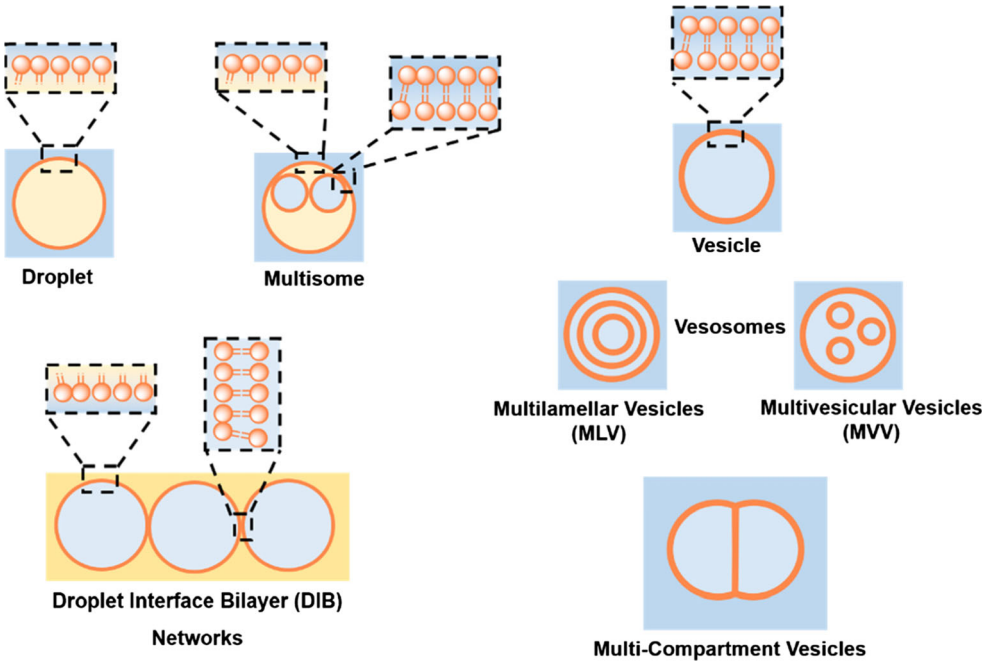

Fig. 8 Different multi-compartment architectures. Monolayers (droplets) or bilayers (vesicles) are generated depend on water/oil environment. The membrane structure is shown in the inset

4.4 Microfluidic device as a cell-like system

A microfluidic chip itself has been exploited to act as a synthetic cell that contains 2D DNA compartments and simple gene network dynamics, and that enabled programmed protein expression, metabolism, and communication (Karzbrun et al. 2014). This model was constructed by grafting a DNA brush on a microfluidic silicon device connected to a channel feeding the device with a cell-free expression mixture through a diffusive capillary. Green florescent protein was continuously produced, and has a long lifetime due to the constant influx of nutrients into the capillary channels and the removal of reaction products. Hence, the fabricated cell-on-a-chip enabled gene expression in DNA compartments, which allowed the investigation of biological networks in a rich, dynamic, and semi-natural system. A nanoliter-scale microfluidic reactor was constructed to study the assembly of steady-state biological networks. In this model, a genetic oscillator was implemented *in vitro*, mimicking the complexity of synthetic gene networks. Transcription and translation were achieved at steady-state for 30 h and implemented diverse regulatory mechanisms on different levels of transcription, translation, and post-translation, such as repression of transcription, activation of translation, and proteolysis (Niederholtmeyer et al. 2013). Another cell-on-a-chip model was developed by Gerber et al., allowing 14,792 on-chip simulation experiments to investigate protein–protein interactions of 43 *Streptococcus pneumoniae* proteins in quadruplicate (Gerber et al. 2009). Combining a highly parallel and sensitive microfluidic affinity assay with a cell-free protein expression system resulted in the revelation of previously unreported physical interactions between proteins of several biochemical pathways. Moreover, a network of 157 interactions showed a denser reaction than expected compared to known networks.

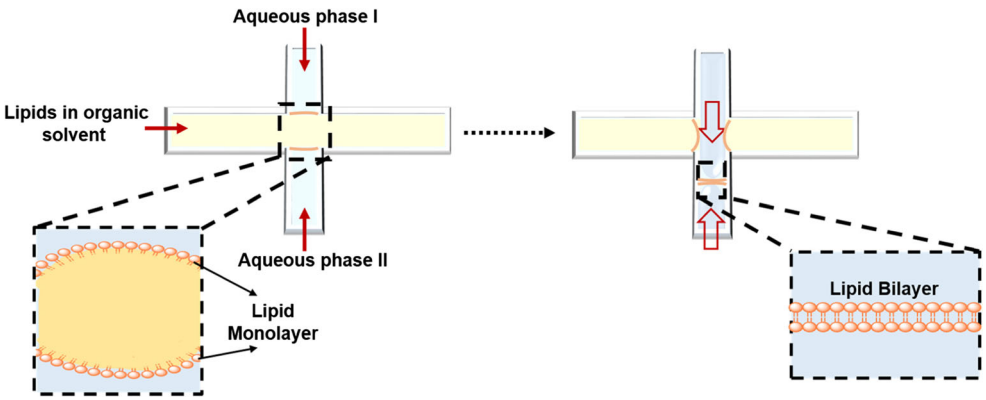

Fig. 9 Schematic of bilayer formation process in microfluidic channels. Two aqueous solutions inject into one channel in opposite directions while lipid solution injects into the crossing channel which leads to organization of lipids molecules on the hydrophobic-hydrophilic interface. By infusing the aqueous solutions, the two lipid interfacial monolayers approach each other resulting in generation of bilayer membrane

Table 1 Summary of some advantages and disadvantages of the different biomimetic models utilizing microfluidics. Several points are common among the different models (Trantidou et al. 2018; Malmstadt et al. 2006; Dressler et al. 2014; Holtze et al. 2017; Sato and Takinoue 2019; Schneider et al. 2013)

Advantages	Disadvantages
Droplet-Based Microfluidics	
- suitable for high-throughput screening applications	- requires using of very sensitive techniques due to small quantities of analytes
- reduces the consumed samples/reagents volumes	- droplet volume limitations
- enables biological or chemical process or compositions in drops rather than in a single phase microfluidic flow.	- the surface to volume ratio in drops is large compared to microtiter wells which may affect interfacial adsorption inside drops
- small scale offers a good impact on mass and heat transfer due to fast mixing which enhances reaction kinetics.	- Drops destabilization and components leaching can disrupt the experimental investigations.
- less surface fouling due to prevention of the direct contact between the droplets components and the device wall	
- minimizes cross contamination and thus improve the sensitivity.	
Multi-Compartment Synthetic Cells	
- compartmentalization of cells and cell components	- similar to the disadvantages of droplet-based microfluidic models
- the spatial arrangement of chemical species in space allows isolation of interfering processes and maintain the non-equilibrium states	- requires experts with good skills in chemistry and biology
- the possibility of optimizing the spatially distinct microenvironments chemically for particular investigations	
- enables generation of synthetic membrane-bound microsystems	
- allows communications between molecular components and communications between non-living systems and living systems	
Constructing Synthetic Lipid Bilayer Membranes in Microfluidics	
- enables creation of asymmetric lipid membranes in the absence of an organic solvent	- requires experts with good skills to carefully manipulate the device during the lipid membrane generation
- offers a defined space allows mimicking of biochemical reactions	- the solvent in the membrane precursor solution surrounds the membrane which limits the degree of miniaturization of membrane
Microfluidic Device as a Cell-Like System	
- offers good stability during the fabrication of the biochemical processes	- requires experts with good skills to carefully manipulate the device
- allows mimicking of biochemical reactions such as metabolism, protein synthesis, and communications on a single chip	- some polymers that used to construct microfluidic devices can absorbed small hydrophilic biomolecules
- allows controlling of cellular functions based on individual interactions	
Tissue-Like Structures	
- DIBs mimic the phase behavior as oil-free membrane structures	- DIBs structure is not thermodynamically stable and droplets coalescence is a common problem
- DIBs enable hosting of transmembrane proteins	- amphiphilic biomolecules may denature when accumulate on water-oil surface
	- the biophysical properties of the membrane and the membrane thickness can be affected by the degree of incorporation of oils at the droplet interface
	– transferring of DIB into a physiological conditions is limited due to membrane assembly in a bulk oil environment

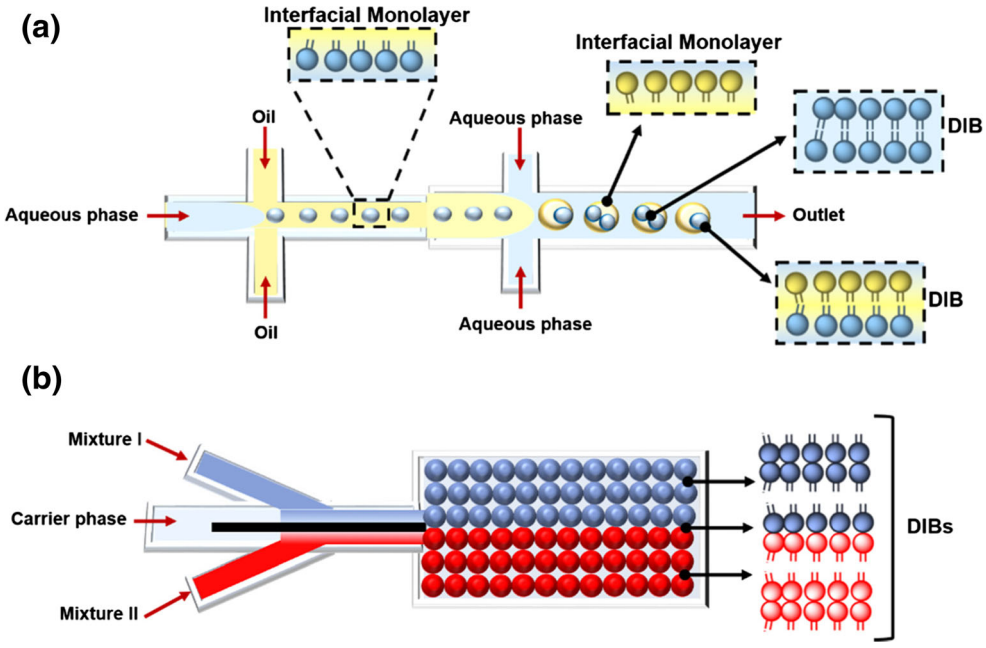

Fig. 10 Strategies for generating droplet interface bilayer (DIB) networks (multisomes) in microfluidics which can be exploited further as cell-like or tissue-like reactors. **a** Schematic of formation of multisomes by encapsulating water-in-oil (W/O) droplets in oil droplets and thus two lipid mono-layers are brought in contact to generate a bilayer. **b** Generation of a dense arrangement of droplets utilizing microfluidic employs two droplets generators. Generated droplets are monolayers and present bilayers with neighbor droplets in the growing network

4.5 Tissue-like structures

Synthetic multi-cellular structures can be constructed by the assembly of multi-units at large scale, acting as a tissue engineering substrate or as a mimicry model of living tissues. Connecting individual cells together mimics biological tissues with higher-order properties compared to single-unit synthetic cells. A promising example is droplet interface bilayers (DIBs) (Fig. 10). DIBs are synthetic membrane models formed by a self-assembled W/O monolayer coated with another O/W monolayer to form a bilayer membrane. A network of DIBs is constructed by bringing three or more droplets together, and can be extended by hundreds or thousands of droplets. DIB networks can act as soft biodevices when functionalized with transmembrane proteins for better understanding of biological reactions. Several properties associated with DIBs make them pioneering synthetic models involving high kinetic stability, long life-time (i.e., days to weeks), and make it possible to generate asymmetric bilayer membranes (Elani 2016; Funakoshi et al. 2006; Bayley et al. 2008). Microfluidic devices enable the construction of 2D and 3D DIB networks composed of thousands of droplets. A 3D DIB network resembling tissues was developed by Villar et al., using the 3D printing of thousands of individual pico-liter droplets. The constructed networks can be modified with membrane proteins to perform specific cooperative behaviors (e.g., enabling electrical communication in a specific path), and can be programmed by osmotic differences to make geometry changes over time (Villar et al. 2013).

5 Conclusions

While necessity is the mother of invention, inspiration from nature allows for the continuous improvement of technologies that have an impact on the quality of research and subsequently on quality of life. Biomimetic models can be constructed by a direct coping from nature or indirect by getting inspiration from nature. The development of human-made bioarcheticture systems is attributed to multi-disciplinary collaborations between the fields of bioengineering, gene technology, and biophysics, because the integration of different approaches is needed to construct a relatively complex mimicry system. A major obstacle in a biomimetic manipulation is the limitation to fully mimic the cellular life. Therefore, merging synthetic biology with microfluidic technology may offer new opportunities and improve the engineering of synthetic cells for biotechnological applications (Table 1). Microfluidic devices open up new areas of research with simplicity that enables plug-and-play operability to construct uniform-sized synthetic cells with cellular dimensions and defined internal and membrane components. The construction of cell-like structures exploiting microfluidic devices as a synthetic cell chassis or the construction of tissue-like structures can be adapted for high-throughput drug screening and toxicology, as well as membrane protein investigations. Although microfluidic system is a relatively new field in biomimetic applicability, the future impact of this technology may accelerate applications development which aids the investigation of biophysical processes of living biological systems and achieve a significant progress in the field of synthetic cells construction.

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Compliance with ethical standards

Conflict of interest The author declares no conflict of interest.

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