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Toward Synthetic Life: Biomimetic Synthetic Cell Communication

Abbey O. Robinson[1,*], **Orion M. Venero**[1,*], **Katarzyna P. Adamala**[1,&]

[1]Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN US.

Abstract

Engineering synthetic minimal cells provides a controllable chassis for studying the biochemical principles of natural life: increasing our understanding of complex biological processes. Recently, synthetic cell engineering has enabled communication between both natural live cells and other synthetic cells.

System such as these enable studying interactions between populations of cells, both natural and artificial, and engineering small molecule cell communication protocols for a variety of basic research and practical applications. In this review, we summarize recent progress in engineering communication between synthetic and natural cells, and we speculate about the possible future directions of this work.

Introduction

While often referred to as the “building blocks of life,” living cells are complex, poorly understood entities carrying out millions of chemical reactions every second [1]. The traditional top-down studying of natural cells is complicated by a dense and nebulous network of signaling pathways: membrane-bound and membrane-less organelles, proteins, DNA, RNA, and all other cell constituents and processes that are necessary to sustain life: all contribute to background interference.

On the contrary, building cell mimetic vesicles from the bottom-up, termed synthetic or artificial cells, allows for the isolation of unique variables outside of the complexity of the full molecular and biochemical workings of a natural cell; these synthetic cells can be used to study the biology and biochemistries that allow for life to happen in a less background occluded system.

[&]Correspondence to: kadamala@umn.edu.
[*]These authors contributed equally

Declaration of interests

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.

Biological and biochemical technologies have advanced substantially in recent years; however, the basic concept of artificial cells has remained since it was first proposed by Dr. Thomas Ming Swi Chang in 1957 [2]. Synthetic cells are compartmentalized cell-free systems that mimic the functions of natural cells [3]. The basic chassis of synthetic minimal cells are spherical liposomes, usually around 0.1 to 100 microns in diameter, and often constructed from phospholipids and cholesterol; however, hydrogel droplets and coacervate based systems also exist and provide alternative transduction kinetics and morphologies [4]. Membrane pores in liposome based synthetic cells allow for communication and are created by integrating proteins such as hemolysin into the lipid bilayer. Additionally, transcription and translation machinery including genes, tRNAs, small molecules, other proteins and polymerases necessary for life, such as the ribosome, are encapsulated within a lipid bilayer to create programmable, biomimetic vessels [5].

The engineering of a robust and autopoietic synthetic minimal cell system is the central pursuit of synthetic biology. This burgeoning reductionist methodology can be harnessed to probe the origins of modern life as well as to provide a tool to understand both age-old and emerging biological questions with applications in the biomedical, industrial, and basic science spaces. This review provides commentary on functional aspects of synthetic cells designed from the bottom-up and discusses the development of diverse technologies within recent years that has allowed for advancements in synthetic-synthetic and synthetic-natural communication.

Background

Formation of the compartment

The formation of a phospholipid bilayer is one of the principal methods of bio-compartmentalization and serves as a backbone into which additional chemistries can be combined to functionalize and increase the complexity of synthetic cells. The process of forming nano-scale liposome membranes has been investigated extensively as nano-scale liposomes are of interest in pharmacology; thin film rehydration and subsequent extrusion – which redistributes the upper tail of the size distribution – as well as ethanol injection and microfluidics flow-focusing are amongst the most prominent nano-scale formation methods [6-9].

For the formation of microscale liposomes, thin-film rehydration and extrusion can be used to achieve a size distribution of liposomes which includes micro-scale dimensions; though additionally, a method based on the same principals, where a thin-film is swelled in heated buffer, can be used to form GUVs [10]. Additionally, the same principals which facilitate the formation of liposomes with the reverse emulsion method also have led to the development of microfluidics methods such as droplet-shooting and size-filtration (DSSF), a microcapillary and centrifuge based microfluidics method, as well as octanol-assisted liposome assembly (OLA), an increasingly used PDMS based microfluidics system [11-13]. DLS has shown microfluidics system to be impressively uniform with regard to liposome size distribution which furthers the notion of uniform data associated with synthetic cells.

Membrane characteristic

Computational modeling and molecular dynamics has just begun to compile and elucidate the phospholipid bilayer of living cells and its proteins and cofactors [14]. Similarly, membrane pores like hemolysin are being used to tailor and elicit membrane fluidity and logic-controlled cross-membrane communication in synthetic cells [15,16]. Furthermore, there has been considerable success with regard to reconstituting functional membrane proteins such as ATP-synthase in synthetic cell systems [17,18].

Membrane fluidity and permeability can also be augmented through the presence of divalent ions such as Mg^{+2} as well as the presence of fatty acids or other amphiphiles within a heterologous phospholipid membrane [19,20]. Membrane permeability can also be affected by the chirality of the lipids, with homochiral membranes exhibiting enantioselective permeability. [21]

Live cells rarely conform to a circular geometry and therefore various efforts have investigated the deformation and shaping of synthetic cell membranes [22]. The confinement of synthetic cells in PDMS based microfluids traps has been used to study internal fluid kinematics of synthetic cells; likewise, cholesterol and the internal polymerization of proteins such as MreB have been used to form tetrahedral and rod-shaped vesicles respectively [23-25].

Growth and division

Living systems must grow and divide to perpetuate. The same is true for artificial cells, thus there is much interest in the development of an autopoietic cell-free system. Without self-production of membrane components, descendants of the initial artificial cell will undergo significant shrinkage [26].

When a cell grows, the lipid bilayer increases in size to incorporate new lipid molecules into the membrane (Figure 1). Various bottom-up efforts have focused on synthesizing lipids within liposomes for incorporation into the membrane to stimulate growth [27-29]. Another approach to liposomal growth describes the fusion of smaller vesicles to create a larger vesicle as mediated by osmotic pressure [30]. It has been shown that the presence of fatty acids or other small molecules can catalyze membrane growth, division, or regulate membrane characteristics such as permeability [31-35] Recently, it was observed that when phospholipid vesicles are incubated in a bath of fatty acids, changes in the concentration of those fatty acids, vesicle size, and osmotic pressure differences across the membrane can lead to insertion of fatty acids into the membrane of vesicles that contain high levels of osmolytes, resulting in vesicle growth [33]. Also due to osmotic pressure, these vesicles became unstable and divided into daughter vesicles. This approach describes liposomal growth and division in one environment, a phenomenon crucial for the development of lifelike synthetic cells.

Additional methods for catalyzing the division of synthetic cells or vesicles have been investigated, including chemical stimuli, temperature driven self-replication [36], microfluidic approaches [37], the internal organization of polymeric structures [38], osmolarity induced osmotic stress [39-41], and internal lipid or amphiphile synthesis

[42,43]. However, a truly *de novo* system has yet to be described, as the findings reported here are limited by starting reagents or require intervention to induce non-persistent catalysis or reagent reuptake.

Quantitative analysis

The development of characterization methodologies applicable to synthetic cell systems has been of interest in the synthetic cell community for some time. Chiefly, FRET and various electron and light microscopy modalities are used in order to observe membrane growth and stability as well as to validate formation and examine morphology[44,45]. The imaging of liposomes and synthetic cells is confounded due to the fact that liposomes readily wet glass and are therefore effectively annihilated on contact with glass slides or cover slips; however the immobilization of the vesicle with PEG linkers, the treatment of glass with blocking agents such as BSA, or the formation of planar bilayers on cover slips or glass slides has shown to be effective at increasing membrane stability [46]. Similarly, sugars have been shown to increase membrane stability likely due to hydrogen bonding [47]. Parallel work in the field of NMR with regard to 31P spectroscopy and DOSY represent a promising area of study regarding possible synthetic cell related assays which might provide useful insights into vesicle morphology and shape, size, and laminarity as well as the relative amount of encapsulated material within the lumen as opposed to exogenously within solution [48-52].

Genetic robustness

Self-replication of DNA—Perhaps the most critical aspect of creating a functional and prolific synthetic cell is the ability to transcribe and translate DNA into proteins. As discussed, synthetic cells need to grow and divide without losing material to perpetuate; thus, creation of a *de novo* DNA self-replicating system with transcription and translation capabilities is a significant objective for synthetic biologists. Protein synthesis Using Recombinant Elements (PURE) uses RNA polymerases and machinery from *E. coli* to synthesize proteins [53,54].

A modified PURE system in conjunction with Phi29-transcription-translation-coupled DNA replication (TTcDR) has been used to self-replicate a 116 kB genome [55]. While this system provides a framework for gene replication, protein expression was at a minimal level. Similarly, this system requires multiple proteins for replication, and a simpler method would be preferred. A subsequent system utilizes rolling-circle replication of circular DNA by a self-synthesized phi29 DNA polymerase and Cre recombinase [56].

DNA logic gates/cascades

Parallel to engineering systems for replication of genomic information, controlling the expression of genes beyond replication machinery is a key objective. Encapsulation of TXTL constituents within a liposome enables a compartment to function as a synthetic minimal cell [57]. This general scheme has enabled the engineering of genetic circuits and cascades within a synthetic cell system, allowing for the control of communication by external signals, the combination of otherwise incompatible reactions, fusion of liposomes, and catalysis of enzymatic reactions [15,58,59].

Boolean logic gates provide a framework for controlling genetic circuits, but the non-binary nor discrete nature of biological signals means that quantum and multi-valued logics may be used in the design of biological circuits. NAND/NOR logic gates have been used to create a modular regulatory network by controlling binary inputs to effect binary outputs [60]. A recent study documents a membrane AND logic gate used to release membrane bound cargo in response to a stimulus, which could be harnessed to work with other genetic circuits to add another level of control within artificial cells [61]. However, differing sizes of liposomes, the effect of crosstalk between circuits, and undesirable interactions between chemical components leave room for further investigation and demonstrates the quasi-evolutionary necessity of further sub-compartmentalization: synthetic organelles.

Communication – between synthetic cells and with natural cells

With other microorganisms—Synthetic cells are an ideal vessel for studying communication from the bottom-up as they can be programmed to carry out select functions without the busy environment of a living cell. There has been much work to create mechanisms of communication among synthetic populations in consortium with natural cells (Figure 3). Harnessing the power of quorum sensing in synthetic cells in conjunction with natural cells has proven to be a useful approach to this challenge. A quorum sensing-based sender-receiver model using IPTG and AHL as inducers has been described [62]. Receiver cells composed of encapsulated bacteria express GFP under the inducible pLuxR promoter in response to AHL produced by sender cells. GFP fluorescence was visualized diffusing into both adjacent and distal receiver cells. Similarly, diffusion was observed from AHL synthesizing bacteria into neighboring receiver droplets. A similar study used bacterial quorum sensing molecules expressed in artificial cells to create a biological-Turing test applied to natural cells to determine the level of bio-mimesis achieved with these artificial cells [63]. Using fluorescence, luminescence, and RNA sequencing to compare the expression of sequences normally produced when *V. fischeri* bind a quorum molecule, it was found that while some overlap in expression was present, a natural cell's response to an artificial cell is not identical, thus failing the Turing test. Studying artificial cells in the presence of natural cells provides a substantial opportunity to explore and expand the capabilities of synthetic cells using a well-characterized system.

With other synthetic cells—Less is known about communication among entirely synthetic populations. A population of coacervate-based droplets containing proteases were able to electrostatically attach to proteinosomes, a type of protocell with a flexible, semi-permeable membrane constituted of amphiphilic enzymes and other protein-polymer building blocks [64]. After attaching, the coacervate-proteases degraded the proteinosome membrane and seized its internal contents. This work is an exciting display of an exclusively "protocell ecosystem". While one of the populations was droplet-based, this displays the diversity of possible synthetic ecosystems. Moreover, this work highlights the predatory capabilities of synthetic populations. However, communication between populations of synthetic cells composed of a biomimetic lipid bilayer has yet to be described.

Organelles

The development of discrete intracellular compartments was a major evolutionary breakthrough in terms of eukaryogenesis and facilitated an increase in the complexity of intracellular biochemistry; moreover, it is widely accepted that mitochondria and plastids are the result of precursory endosymbiosis events [65-67]. Because of their ability to biorthogonally increase functionality and complexity, synthetic organelles could be used to build-up or increase the complexity of synthetic cell systems (Figure 3). Various efforts have investigated the engineering of synthetic internal compartmentalization structures based off of natural endosymbionts [68-71], oligolamellar vesicles [17,18], lipid sponge droplets [72], proteinaceous microcompartments [73-75], and coacervate-like structures [76-78]. The inherent complexity of organelle systems from the standpoint of biochemical communication should also be noted. For example, recent work to encapsulate living cells within a liposome epitomizes intramembrane communication between a synthetic cell chassis and a natural cell endosymbiont [68]. Similarly, these intramembrane communication logics associated with organelles can be reconstructed entirely from synthetic components and demonstrate Szostakian compartmentalization.

Recently, bottom-up synthetic oligolamellar organelles capable of anabolic catalysis mimicking photosynthesis were successfully engineered, though such systems remain non-autopoietic [17,18]. A clay-based DNA hydrogel was engineered to serve as a means of genetic compartmentalization in synthetic cells, and in addition, a proto-nucleus was shown to sequester transcriptional factors where transduction of such factors intracellularly represents an internal communication or passive logic [78]. Similarly, protein based microcompartments have served as the basis for organelles designed to sequester iron atoms [73], replicate and optimize the function of carboxisomes [75], and serve as a site of cell-free TXTL [74]. Moreover, sponge phase lipid droplets have been used to mimic the interconnected structure of the endoplasmic reticulum and Golgi apparatus from a biophysical or morphological standpoint [72]. Droplet systems were also used to facilitate synthetic cell – like bioreactors for evolution of active enzymes. From a top-down perspective, both prokaryotic, and mammalian cells have been used for the basis of synthetic endosymbionts [68-71]. Encapsulated *E. coli* can function as a sensing component within the top-down synthetic cell system and exhibit a communication logic whereby environmental lactate results in the fluorescence of the *E. coli* synthetic organelle [69].

In terms of fully synthetic organelle systems, communication has been observed within an artificial cell between two proto-organelles [79]. In this system, a glucose oxidase (GOx) containing proto-organelle was encapsulated within a GUV, followed by inward budding of the GUV to create a secondary proto-organelles encapsulating horseradish peroxidase and Amplex red. Glucose diffused into the GOx vesicle creating hydrogen peroxide, which then transduced the membrane of the HRP vesicle, oxidizing Amplex red to produce a fluorescent readout. This model describes successful uni-directional communication between fully synthetic organelles, although a reciprocal communication system has yet to be established.

Biosensing

There is a need for the development of synthetic biosensors able to detect small molecules for agricultural, biomedical, and environmental applications. For example, a paper-based, cell-free biosensor that expresses sfGFP in the presence of heavy metals as well as certain date rape drugs has been described [80]. However, artificial cells may allow for expanded capabilities beyond whole-cell and *in vitro* sensing.

Synthetic cells consisting of encapsulated bacteria have been useful for biosensing efforts. *E. coli* encapsulated within a liposome has been used to measure lactate levels in the external environment [69]. By using engineered bacteria employing the lldPRD operon that expresses GFP in the presence of lactate, fluorescence can be used to assess the amount of lactate present in a sample. Liposome-based approaches are particularly useful for pathogens that secrete pore-forming toxins, such as *Listeriolysin O (*LLO) secreted by *Listeria monocytogenes (L. monocytogenes)*. A recent paper describes an assay for detecting the presence of LLO [81]. LLO forms pores on liposomes containing encapsulated cysteine, resulting in the release of the cysteine and the subsequent aggregation of gold nanoparticles. This aggregation results in a colorimetric change from red to purple/blue, denoting the presence of the bacterial toxin.

Encapsulating sensing pathways within the lipid membrane of an artificial cell allows for the compartmentalization of reactions from external environments where they may have been otherwise incompatible. This would represent the creation of simple and programmable bioreactors capable of sensory logics and may be better suited for instances where introducing bacteria or similar microorganisms is undesirable.

Conclusion

Within recent years, there has been sufficient progress in the engineering of biomimetic synthetic minimal cells. Emerging technologies in microfluidics, microscopy, NMR, biophysics, biochemistry, etc. have contributed to advances in synthetic cell formation, growth and division, organelle development, communication, biosensing, protein evolution [81] and the development of robust and self-sustaining genomic constructs. (Figure 3) Looking towards the future, one should expect rigorous research in the entirety of the field of synthetic cell engineering due to its burgeoning exposure. More specifically, it is likely that research will focus on the construction of autopoietic systems capable of more diverse communication with other synthetic cells and natural cells alike. In this way, self-sustaining synthetic cells capable of passing the equivalent of a biological Turing test – that is, the predation on and by natural cells – will be one of the next major evolutions of the field. As a result, the field of synthetic cell research will have to develop a more taxonomically useful characterization methodology for synthetic cell lines, possible arising out of the molecular and biological provenance of constituent parts.

Additionally, it is likely that the design and engineering of synthetic tissues, predicated on the development of synthetic extracellular matrixes, will develop into a robust field of its own withing the next few years.

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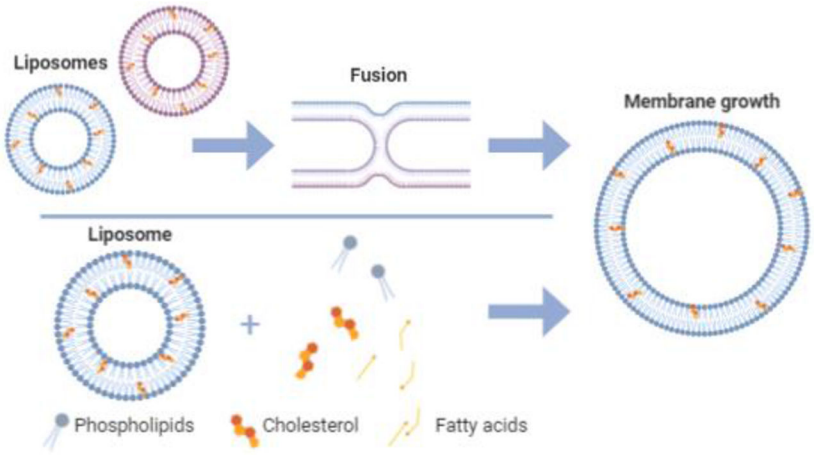

Figure 1:
Membrane growth of liposomes driven by liposomal fusion and fatty acid/lipid insertion.

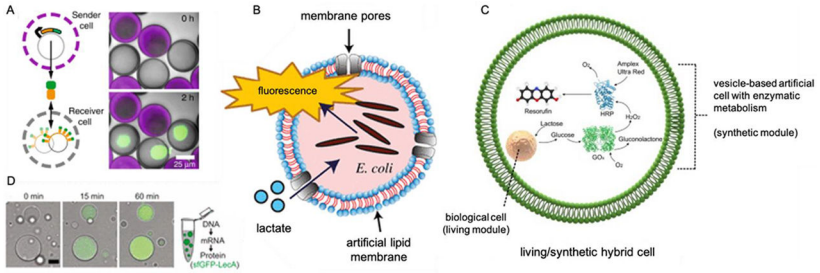

Figure 2: Synthetic organelles and communication.

A) A porous synthetic cell with a nucleus-like DNA-hydrogel that expresses display proteins and communicates with neighboring synthetic cell through protein diffusion [78]. **B)** encapsulated E. coli functions as lactate sensor in synthetic cell [69]. **C)** Internal lipid droplet with sponge morphology enables expression regulation and mimics interconnected membrane morphologies of Golgi apparatus and endoplasmic reticulum [72]. **D)** internal synthetic cell chemistry communicating with encapsulated bacterial and eukaryotic cells to display logic gate encodable mutualism [68].

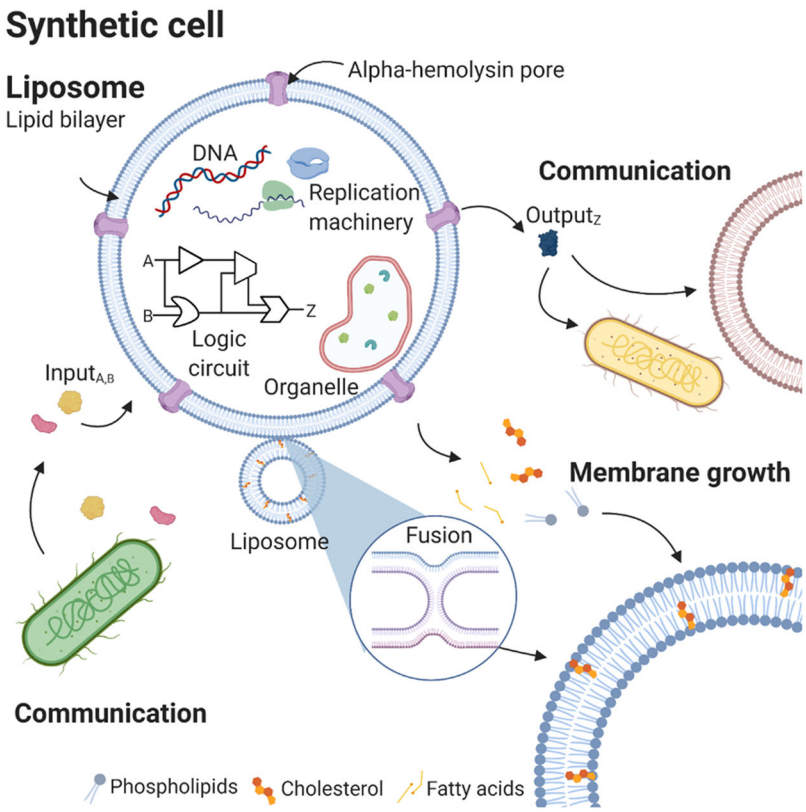

Figure 3: Schematic of synthetic cell.

Synthetic cell encapsulating various DNAs and proteins. Synthetic cells can receive chemical signals from natural cells as well as secrete signals through hemolysin pores to drive communication. Membrane growth can result from fusion with another vesicle or insertion of fatty acids into the existing membrane.