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Functional Interactions Between Bottom-Up Synthetic Cells and Living Matter for Biomedical Applications

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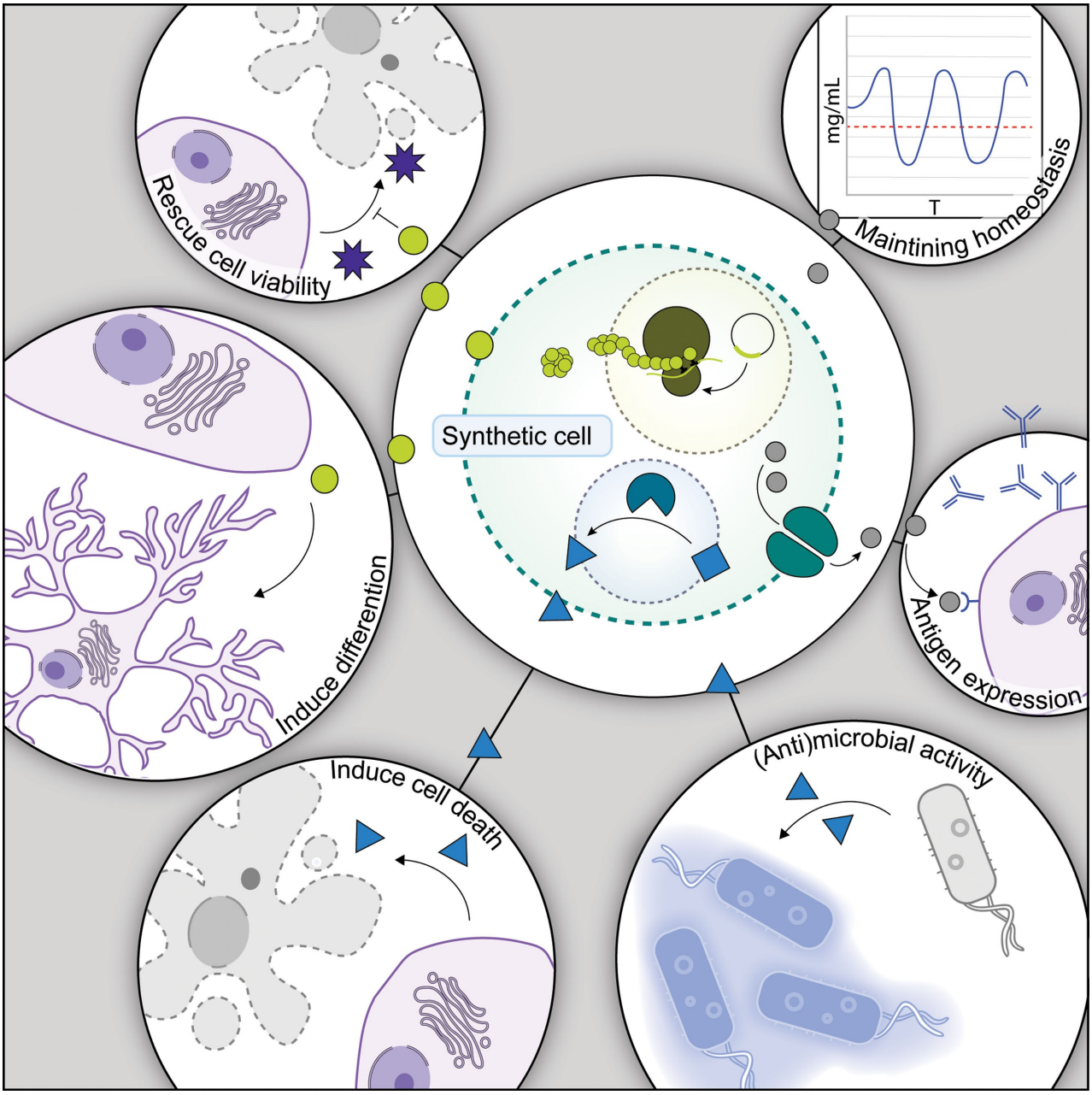

Bottom-up synthetic cells, where diverse non-living materials are combined in creative ways in order to construct increasingly life-like and adaptive systems, are fast approaching a level of function that will enable significant advances in solving specific biomedical challenges. Over the last 10 years, we have seen a wide variety of synthetic cell based approaches to challenges in regulating antimicrobial activity, delivering cargo to mammalian cells, and "growth support". Despite this progress, there has not been a widespread uptake of synthetic cell technologies in biomedical engineering. In this Review, we highlight both the strengths and limitations of these existing synthetic cell applications, as well as give an overview of the state-of-the-art of synthetic cell technology that has yet been applied to cellular contexts. In doing so we aim to identify opportunities for the advancement of this unique intersection of research fields.

1. Introduction

Synthetic biology is a highly interdisciplinary field that focuses on the design and engineering of biological components and systems. Since cells are the main building blocks of life, scientists have been motivated to recreate life in life-like compartments called synthetic cells.[1,2] Over the last years, interest has increased for introducing synthetic cells to their natural counterparts, with the main objective to establish active, bilateral communication pathways, thereby influencing the behavior of both entities. This is believed to have great potential for current biomedical challenges as it could allow for the monitoring of physiological conditions, decision making, and responding accordingly via synthesizing and releasing functional compounds, all by just one type of cell. These synthetic cells could ideally replace specific functions of living cells or perform novel functions in a highly controlled manner, without the disadvantages of using living cells (e.g. high costs, less control).

Synthetic cells can generally be divided into top-down and bottom-up synthetic cells. The first describes the re-designing and simplifying of well-characterized biological elements to study essential life processes. This is achieved by reducing the genome of a living cell to describe the minimal set of genes that are necessary for survival, also referred to as a minimal cell.[3] In contrast to top-down approaches, bottom-up synthetic biology aims to assemble biological systems from scratch using both biological and artificial building blocks. In this review we will focus on this class of synthetic cells.

Bottom-up synthetic cells can been made from a variety of different materials.[4–7] Their development is driven by three central themes;[8] studying the origin of life (i), studying biological processes in a simplified and highly regulated biochemical setting (ii), and finally, applying these soft microparticles in different fields, including the biomedical field (iii), which is the focus of this review. We will first discuss how synthetic cells differ from other synthetic vesicles. Using our definition of a synthetic cell, we will take a closer look at the platforms that have been applied to the biomedical field and consider their advantages and limitations. Finally, we will highlight some recent, cutting edge platforms whose functionality and new behavior could find use in a biomedical context. Synthetic cells have their own unique challenges when introducing them to living contexts, but the effort of overcoming them is worth it because the resulting materials are more adaptive, tunable, and bioinspired than existing static approaches.

2. Synthetic Cells, or Synthetic Microparticles?

Over the last few years, the use of micron-sized particles has been reported in a variety of disciplines including biomedical engineering and synthetic biology. In many cases, however, the nomenclature is not always being used consistently. There are many examples of adaptive, micron-sized particles that are unknown to those involved in synthetic cell research, but could easily be classified as a synthetic cell. For example, in the biomedical field, these particles are often referred to as microparticles, synthetic vesicles or microreactors. On the other hand, in synthetic biology a similar construct might be referred to as a synthetic cell, cell mimic, artificial cell or protocell. To give a complete overview of the progress of synthetic cells in biomedical applications, we will first describe in what ways synthetic cells differ from the simpler synthetic vesicles and provide our definition of a synthetic cell. There is naturally a lot of ambiguity around this definition because there are so many different ways to approach this challenge.

A common starting point is the formation of a biomimetic, membranous compartment. In cell biology, all membranes are comprised of a lipid bilayer, and as such, the majority of synthetic cells researchers utilize liposomes. Besides phospholipids, other fundamental building blocks, including polymers and proteins or a combination (capsosomes) have been used to recreate this biological boundary (Figure 1).[4,6,7,9] However, in order to qualify as a synthetic cell, the compartmentalized systems must display a degree of adaptive functionality in order to mimic the behavior of their biological counterpart. This can be accomplished via the incorporation of membrane-bound elements,[10] subcompartmentalization,[11] encapsulated catalytic elements,[12] DNA based switches,[13] and protein synthesis

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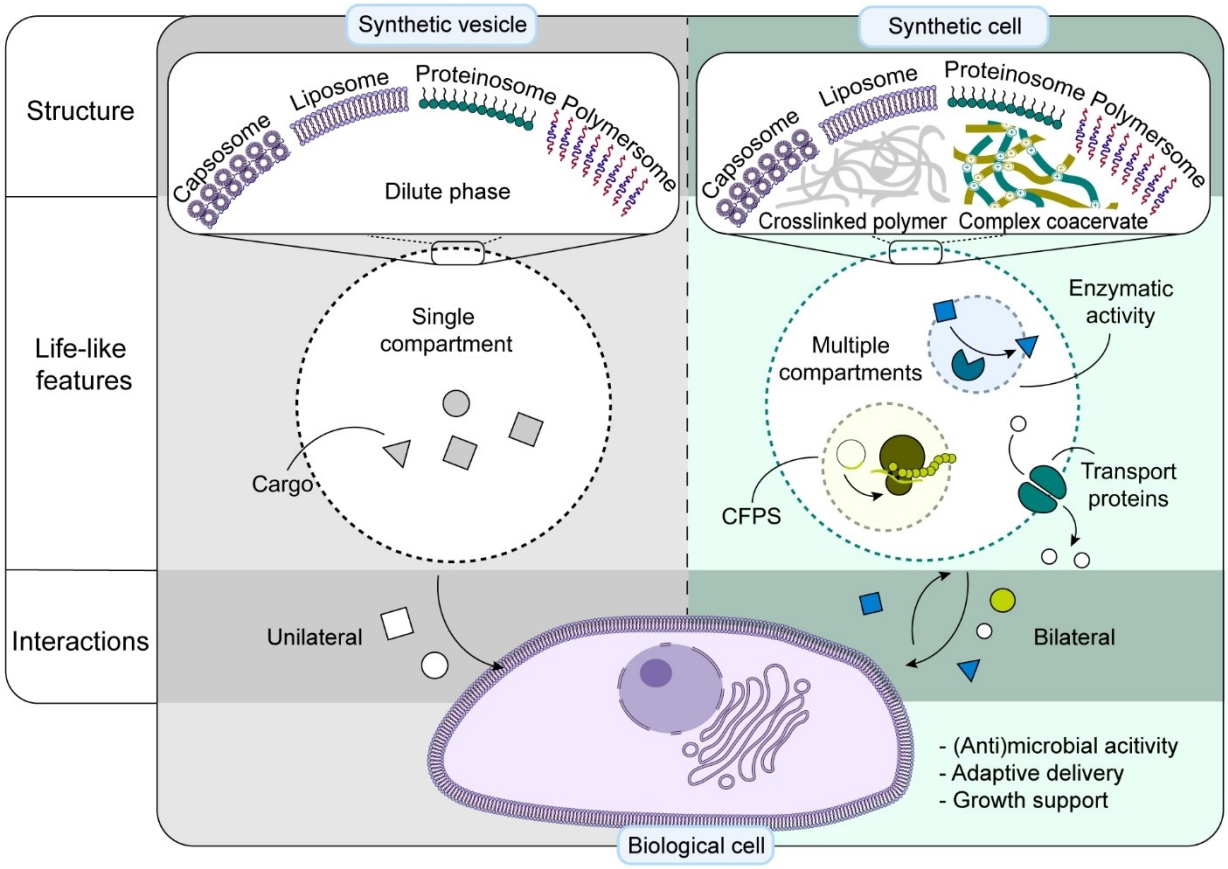

Figure 1. Scheme showing the differences and similarities between synthetic vesicles and synthetic cells. Both share structural elements, but only synthetic cells possess life-like features, or functional elements that allow for bilateral/adaptive interactions with biological cells.

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Alexander F. Mason received his PhD in Chemistry from the University of New South Wales in 2017. His thesis was focused on the development of block copolymer vesicles as synthetic cell scaffolds. During his first post-doctoral position in the van Hest lab, he turned to coacervate-based synthetic cells and their application to cell-sized, hierarchically self-assembled systems.

Jan van Hest obtained his PhD from Eindhoven University of Technology (TU/e) in 1996 with prof E. W. Meijer. In 2000 he was appointed full professor at Radboud University Nijmegen. As of September 2016, he holds the chair of Bio-organic Chemistry at TU/e. Since May 2017 he is the scientific director of the Institute for Complex Molecular Systems (ICMS). The group's focus is to develop well-defined compartments for nanomedicine and artificial cell research, using a combination of techniques from polymer science to protein engineering.

machinery.[14,15] Without these functionalities, providing the potential to operate as a feedback mechanism, the interactions between these synthetic compartments and living cells remain inherently one-sided and non-adaptive. In other words, they are unable to dynamically adjust their function as a response to changing environmental and cellular cues. Synthetic nano- and microparticles are finding increased academic and clinical use for drug delivery,[16] vaccine development,[17] and tissue culturing,[18] however their inherently passive nature precludes such platforms from being classified as synthetic cells. The synthetic cells described herein are thus defined as membranous compartments that comprise some type of feedback mechanism that facilitates participation in adaptive interactions.

Despite the incorporation of all the right components for feedback mechanisms, establishing truly adaptive behavior is still elusive. As the degree of complexity and the number of different functional components increases, it becomes much more challenging to characterize and control these processes at the molecular level. Nevertheless, the first steps towards applying the functional elements in synthetic cells to living cell communities have already been made and are outlined herein.

3. Synthetic Cells in Biomedical Applications

The exploration of synthetic cells in the biomedical field has only commenced in the last two decades and the reports of interactions between synthetic cells and living cells or tissues are typically of fundamental nature. Moreover, in many studies these interactions are often still passive, despite the incorporation of relevant feedback mechanisms. Herein we will give an overview of representative works where synthetic cells have been used for the regulation of (anti)microbial activity, adaptive delivery of functional compounds to mammalian cells, and growth support.

3.1. Regulating (Anti)microbial Activity

Most interactions between complex synthetic cell systems and biological cells have been established using prokaryotes. The goal of these studies is to be able to determine and/or define whether or not a synthetic cell is indeed 'alive', by demonstrating the ability to influence the behavior of natural cells. These interactions are mostly based on the well-studied quorum sensing abilities of prokaryotes. Quorum sensing is used by prokaryotes to access their population density and subsequently express various genes to direct certain behaviors or functionalities beneficial for the survival of the population.[19] Various quorum sensing signals, called autoinducers are produced at different stages of population growth and control, (e.g. luminescence, pathogenicity and biofilm formation)[20] These synthetic cell-prokaryote interactions could in the future have clinical potential, for example by regulating (anti)microbial activity.

Interactions between prokaryotes and synthetic cells are mostly based on enzymatic cascades[21,22,23] or cell free protein synthesis (CFPS).[10,24] Complex genetic circuits used in CFPS have been used to demonstrate active interactions, rather than more passive interactions reported via encapsulation of a catalytic reaction. Moreover, via the incorporation of CFPS, it is possible to produce diverse correctly-folded and active proteins inside synthetic cells.[25] This protein can for example be an enzyme,[11,24,25,26,27,28,29] a receptor[24] or a pore forming complex.[10] The advances in these sorts of interactions have also been reviewed elsewhere.[30,31]

In an early study, Gardner et al. used an autocatalytic formose reaction encapsulated inside a liposome to establish communication with prokaryotes (Figure 2A).[21] The autocata-

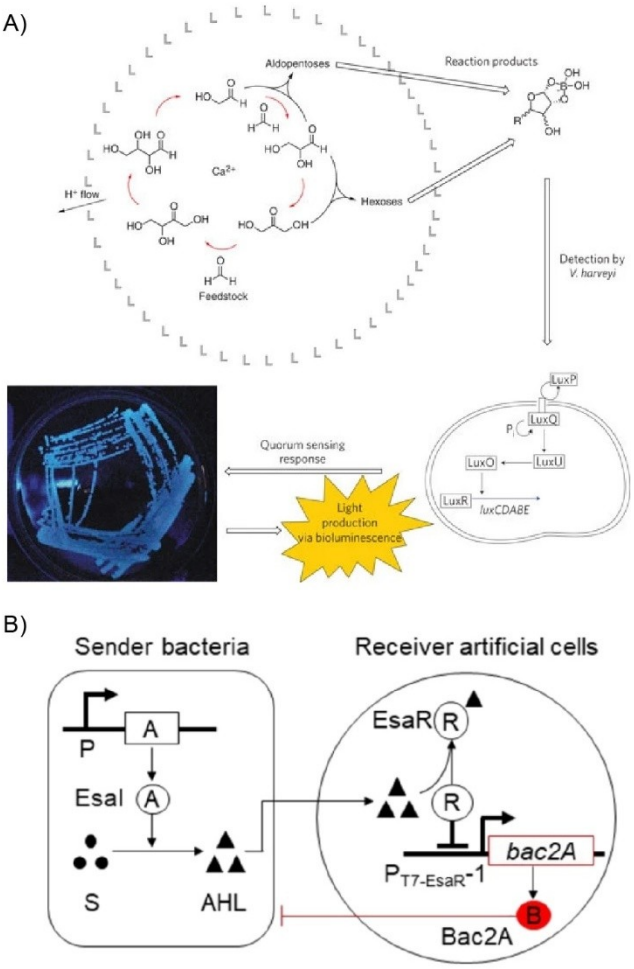

Figure 2. Regulating (anti)microbial activity. A) Autocatalytic formose reaction encapsulated inside a lipid-based synthetic cell. The synthetic cells produce carbohydrate-borate complexes which, upon diffusion outside the liposomes activate the quorum sensing LuxP/LuxQ signal transduction pathway and induce bioluminescence in *Vibrio harveyi*. B) A negative feedback loop between synthetic cells and bacteria. Quorum sensing N-acyl-homoserine lactones (AHLs) produced by AHL synthase (EsaI) in sender bacteria are detected by the receiver synthetic cells. Inside the receiver synthetic cells, AHL binds to transcriptional repressor (EsaR), thereby triggering its release from the $P_{T7\text{-}EsaR}$ promotor and activating the expression of the antibacterial peptide Bactenecin 2 A (Bac2 A). Upon release from the receiver synthetic cells, Bac2 A kills the sender bacteria. A represents EsaI, S represents substrates of EsaI, and R represents EsaR. Adapted with permission from Refs. [21, 33]. Copyright 2009 Springer Nature and 2018 American Chemical Society.

lytic formose reaction is thought to have played a role in the origin of life as it yields complex sugar complexes from small molecule precursors. Within the synthetic cells, carbohydrate-borate complexes were produced from formaldehyde, which subsequently diffused out. These complexes activated the quorum sensing LuxP/LuxQ signal transduction pathway, resulting in bioluminescence in *Vibrio harveyi*. This was the first time that such autocatalytic metabolism was incorporated inside synthetic cells for the communication with prokaryotes. Although selectivity was lacking (many non-functional by-products were formed) and harsh reactions conditions were required in the presence of living cells, this study represented an important step forward in the field.

Later, an enzymatic cascade that produces autoinducer-2 (AI-2), was used to setup communication between synthetic cells and prokaryotes.[22,24] This enzymatic cascade, as part of a nano factory, was introduced by Fernandes *et al.* and converts substrate S-(5′-deoxyadenosine-5′)-L-homocysteine (SAH) to AI-2.[32] The other part of the nano factory consisted of a targeting antibody, enabling the nano factory to bind prokaryotic cells. In one study, the nano factory was encapsulated in alginate/chitosan capsules, consisting of an alginate core and shell of chitosan, crosslinked by tripolyphosphate ions.[22] Upon addition of SAH, the capsules generated AI-2, and *E. coli* responded by producing GFP. In a follow-up study, these alginate/chitosan capsules were decorated with the AI-2 kinase LsrK.[23] Upon addition of the synthetic cell capsules, AI-2 mediated quorum sensing was quenched in *E. coli* through the action of LsrK. These studies nicely demonstrate the possibility to influence the behavior of bacterial communities by their directing quorum behavior.

Moving away from these catalytic functionalities, Lentini *et al.* were the first to report CFPS of a pore forming complex into lipid-based synthetic cells.[10] The production of this pore forming complex expanded the senses of *E. coli*. These bacteria normally do not sense theophylline, a drug used in respiratory disease treatment. A vesicle was constructed that contains a riboswitch which is activated in the presence of theophylline and encodes for the pore forming complex α-hemolysin. Subsequently, co-encapsulated isopropyl β-D-1-thiogalactopyranoside (IPTG) was released through α-hemolysin and bound to an IPTG-responsive lac operator in *E. coli*, resulting in the production of GFP. Recently, the same group used CFPS of *N*-acyl-homoserine lactones (AHL) synthases for the synthesis of autoinducers to induce bilateral communication between synthetic cells and *Vibrio fisheri*.[24]

Although these studies demonstrate the possibilities to establish communication between synthetic cells and prokaryotes, many only demonstrate short-term coexistence in the same spatial location, long-term co-culturing (>24 h) can perhaps still pose a significant challenge. Moreover, these proof-of-principle studies illustrate the ability to influence prokaryotic behavior using a luminescent and fluorescent readout. However, these models could be expanded to direct important processes in prokaryotes, for example for overcoming resistance or by sensing and directing the health of the human microbiome.[34] In this context, pro- and anti-quorum sensing therapies could be considered. For example, synthetic cells could be used to produce autoinducers that repress biofilm formation or induce the expression of virulence factors, (e.g. by activating luminescence).[35] Alternatively, synthetic cells could express enzymes that cleave autoinducers important for biofilm formation, or express antimicrobial peptides.[23] Recently, Ding *et al.* created an artificial negative feedback loop between lipid-based synthetic cells and prokaryotes that co-existed in the same spatial location (Figure 2B).[33] Autoinducers that were produced by prokaryotes, were detected by synthetic cells, which in return produced the antimicrobial peptide Bactenecin 2 A (Bac2 A) that killed the prokaryotes. This feedback loop was shown to work in three different chemical conditions (H_2O, phosphate buffer and nutrient-rich medium), demonstrating the robustness of these synthetic cells.

The use of synthetic cells for antimicrobial purposes has some advantages over simpler synthetic vesicles as they could be programmed to monitor the environment for an extended period of time and tailor the conversion or synthesis of signaling molecules to specific environmental cues. At the moment, such applications are still elusive as it not only required long-term co-culturing of synthetic cells with prokaryotes, but also continuous protein synthesis for prolonged time periods, which is often limited by the amount of resources that can be incorporated inside the synthetic cell.

3.2. Triggered Delivery to Mammalian Cells

Active and predictable interactions via comprehensive synthetic gene circuits, found between prokaryotes and synthetic cells, have not yet been reported for mammalian cells. A possible explanation is perhaps the fact that eukaryotic cells do not possess a relatively straightforward mechanism analogous to quorum sensing. Over the last decade, one-way interactions between synthetic cells and mammalian cells have however been reported using different synthetic cell platforms. These studies mainly describe the delivery of functional molecules in an adaptive fashion using enzymatic cascades, CFPS and catalytic activity. The earliest synthetic cell designs for the adaptive delivery of functional compounds to mammalian cells was reported by Amidi *et al.*[36] In this study, components for CFPS of the *E. Coli* β-galactosidase were incorporated inside lipid-based synthetic cells, in order to make a genetically programmable vaccine. Possible advantages of such antigen-expressing immunostimulatory liposomes (AnExILs) over standard vaccines include increased safety, as no bacterial or viral particles are included, and high modularity. For example, the target for the vaccine can be easily altered by exchanging DNA templates. This way the vaccine formulation doesn't require any alterations. AnExILs were reported to induce high serum antibody responses after intra-muscular immunization, which were superior to the responses of conventional liposomal protein or DNA vaccines. In a follow-up study, the authors demonstrated that these particles were not only able to induce a humoral (secretion of antibodies) but also a T-cell response, which is of interest for the development of cancer immuno-

therapies. In this study, an epitope for special killer T lymphocytes called cytotoxic T-cells, was genetically fused to the C-terminus of reporter enzymes including β-galactosidase. The observed T-cell responses were found to be the effect of cross-presentation of AnExIL-produced antigens by antigen presenting cells, but also endogenous antigen production. The specificity and modularity of AnExILs, together with the reported ease of preparation demonstrates the great potential of these group of synthetic cells as candidates for therapeutic vaccines against both infections and cancer.

Krinsky *et al.* also used liposomes as a synthetic cell model for the delivery of a therapeutic compound via CFPS.[14] More specifically, these liposomes were equipped with CFPS tools for the synthesis of Psodomas exotoxin A (PE), a cytotoxin that, ironically, blocks protein synthesis. The particles were constructed from 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), which formed a soft liquid-phase membrane at physiological temperature that is permeable to small molecules like amino acids and nucleotides (Figure 3A). In the presence of 4T1 breast cancer cells, PE was synthesized inside the liposomes. Finally, these particles were able to induce cytotoxicity *in vitro*, and *in vivo* (Figure 3B). Whereas the production of this toxin was not a result of specific interactions between the tumor cells and synthetic cells, this platform has the possibility to be expanded to enable bilateral interactions in the future by linking the expression of PE to specific signal molecules excreted by cancer cells.

One of the most elegant synthetic cell designs for the delivery of therapeutic agents to mammalian cells was reported by Chen *et al* using a combination of an enzymatic cascade and membrane fusion.[37] In this study, the authors prepared compartmentalized liposomes that mimic the glucose-responsive insulin secretion of pancreatic beta cells (Figure 4A). The secretion of insulin was facilitated by vesicle fusion of enclosed insulin harboring small liposomes, with a giant liposome. In this equation, the small and giant liposomes represent the secretory granules and beta cells respectively. The glucose responsive behavior was achieved via the incorporation of a transporter protein and enzymatic cascade. More specifically, at high concentrations, glucose was taken up by the glucose transporter GLUT2 which was anchored to the giant liposome membrane. Glucose was subsequently oxidized by glucose oxidase (GOX), which decreased the pH inside the system. The net pH variation was balanced by a proton efflux through the proton channel gramicidin A, which was inserted in the same membrane and allowed for greater variation in the internal pH levels under hyperglycemic conditions. The low pH triggered deshielding of peptide K, and the formation of coiled coils with peptide E on the inside of the giant liposome membrane. This process initiated fusion and release of insulin. Upon the decline of glucose concentration to a normal glycemic range, glucose uptake stagnated, and the inner pH level increased. This allowed for the re-shielding of peptide K and inhibition of the fusion events. The authors stated that the synthetic cells could cycle between the high and low glucose state, and thereby temporally control the insulin secretion. Finally, they demonstrated that the synthetic cells could return the dynamic

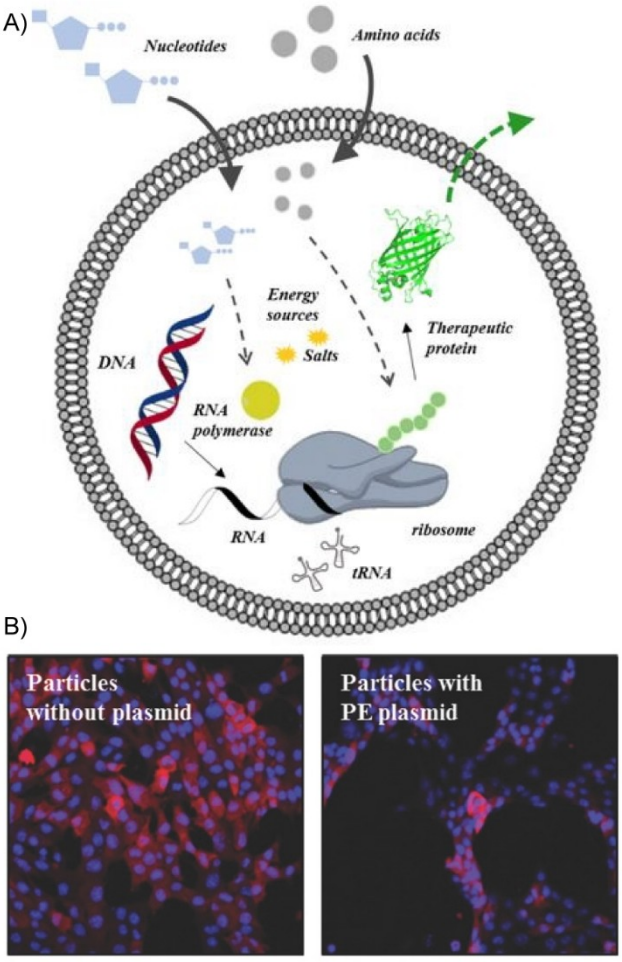

Figure 3. Synthesis of a therapeutic protein inside a lipid-based synthetic cell. A) Schematic illustrating the cell free protein synthesis of a therapeutic protein inside the synthetic cells. B) Confocal micrographs showing the therapeutic effect of the synthetic cells in a 4T1 breast cancer cell culture. Left; synthetic cells without plasmid for the synthesis of the therapeutic protein Pseudomas exotoxin A (PE). Right; Synthetic cells with PE plasmid. The cytoplasm of the 4T1 cells is labeled red, and the nucleus is labeled blue. Adapted with permission from Ref. [14] Copyright 2018 Wiley.

regulation of blood glucose levels in an *in vivo* type 1 diabetic mouse model to normal (Figure 4B). Besides liposomes, capsosome-based synthetic cells have been used for the conversion of toxins in the presence of biological cells.[38] Leticia *et al.* used capsosomes and silica core shell particles for the conversion of phenylalanine to trans-cinnamic acid to treat phenylketonuria (PKU), an inborn error of metabolism. The particles were prepared by the deposition of poly(l-lysine) (PLL)/poly (methacrylic acid)-co-(cholesteryl methacrylate) (PMAc) on silica particles, followed by a layer of phenylaniline ammonia lyase loaded liposomes, another layer of PMAc and a shell of poly (dopamine) (PDA). To obtain capsosomes, the silica core was dissolved at low pH. The conversion of phenylalanine is dependent on its permeation through the liposome membrane, which only occurs at temperatures that are equal or above the phase transition temperature of the lipid mixture. The authors demonstrated that the enzymatic reaction could proceed in the

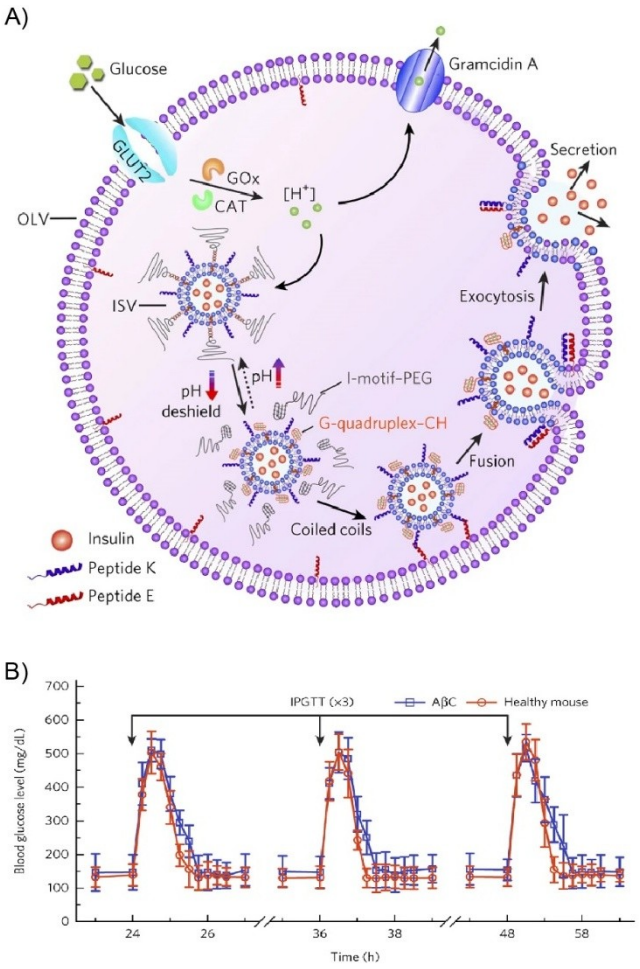

Figure 4. Synthetic beta cells (AβCs) that dynamically secrete insulin. A) Schematic illustrating the biochemical processes inside the compartmentalized synthetic cells leading up to insulin secretion. OLV, Outer large vesicle; ISV, inner small vesicle; GOx, glucose oxidase; CAT, catalase. B) Blood glucose regulation over time in diabetic and healthy mice after challenging with an in vitro intraperitoneal glucose tolerance test (IPGTT). AβCs were transplanted subcutaneously in diabetic mice. Adapted with permission from Ref. [37] Copyright 2018 Springer Nature.

presence of HT-29 human colorectal adenocarcinoma cells and foresee that in the future these particles could traffic through the stomach to reach the intestine, where they could decrease toxic phenylalanine levels.

Synthetic cells that induce cell death, instead of rescuing cell viability, were reported in a study of Zhang *et al.* In this study the authors designed interacting synthetic cell communities exhibiting a simple form of invasion-defense mutual interactions, in which a population of enzyme-active coacervate protocells ("invaders") infect a coexisting population of living cells ("resisters").[39] The synthetic cells were prepared from single stranded DNA (ssDNA) and diethylaminomethyl (DEAE) dextran, and sequestered glucose oxidase (GOX). After uptake of the synthetic cells inside HepG2 cells, GOX mediated reactive oxygen species (ROS) production was shown to reduce cell viability. Pre-incubation of HepG2 cells with catalase, not synthetic cells, in turn rescued cell viability. The sequestration ability of enzymes inside complex coacervate-based synthetic cells was also used for the production of nitric oxide *in vivo*.[12] Synthetic cells were again prepared via the coacervation of DEAE-dextran and low molecular weight dsDNA, followed by sequestration of GOX and the spontaneous interfacial assembly of negatively charged erythrocyte membrane fragments. Using this procedure, hemoglobin, originating from the erythrocyte fragments, and GOX were spatially positioned on the periphery and in the core of the synthetic cells, respectively. Using this synthetic cell platform, the authors were able to enhance the blood circulation time of the particles, compared to the uncoated particles, and produce NO inside the veins in vivo. The production of NO finally resulted in the vasodilation of blood vessels in the presence of endogenous glucose and supplemented hydroxyurea.

These studies mostly addressed synthetic cells sending signals to biological cells. However, in another study, Elani *et al.* reported a hybrid cellular system in which synthetic cells encapsulated colon carcinoma cells and were able to respond to their cues using the enzymatic GOX/Horse Radish Peroxidase (HRP) cascade. In order for this interaction to be established, the colon carcinoma cells were transfected with a construct for the expression of β-galactosidase. β-galactosidase converts lactose into glucose, which is subsequently oxidized by GOX inside the liposomes. H_2O_2, is produced during this reaction, and triggers the conversion of amplex red into the fluorescent resorufin. The authors state that the encapsulation shielded the colon carcinoma cells from toxic surroundings, enabling them to act as a bioreactor module inside a synthetic cell. This study also exemplifies one of the major challenges of establishing meaningful bilateral interactions between eukaryotic cells and synthetic cells, as a clear signal from the eukaryotic cells could only be established by transfecting and thus engineering the colon carcinoma cells. Nonetheless, these studies represent the first steps towards engineering active interactions with biological cells, for the adaptive delivery of therapeutic compounds or conversion of toxic compounds. For this purpose, both CFPS and enzymatic cascades represent promising tools.

3.3. Growth Support

Another promising application of synthetic cells in the biomedical field is providing growth support for tissues, for example by mimicking a new or lost specific cellular function that allows for better tissue growth. Recently, Itel *et al.* reported alginate-based cell mimics that induce bone mineralization in a similar fashion to osteoblasts (Figure 5).[40] The authors prepared the synthetic cells from alginate crosslinked with calcium, and a PLL coating, to allow for cellular adhesion. Additionally, these particles were decorated with either biological, or artificial matrix vesicles (MV). Biological MVs accumulate calcium and inorganic phosphate (Pi), which upon secretion from osteoblasts into the extracellular matrix, eventually results in precipitation, thereby inducing bone mineralization. For this purpose, MVs contain Ca^{2+} ions, Pi, tissue non-specific alkaline phosphatase (TNAP), and different Ca^{2+}- and Pi-channel

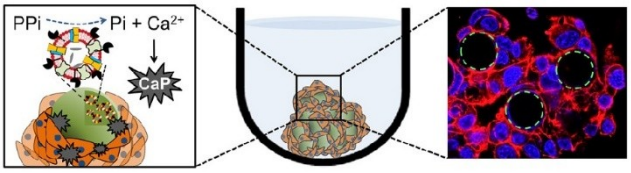

Figure 5. Alginate-based synthetic osteoblasts convert inorganic pyrophosphate into inorganic phosphate and calcium inside a 3D osteoblast-like SaOS-2 spheroids resulting in calcium precipitation. In the confocal micrograph synthetic osteoblasts are indicated with a green dashed line, the nucleus is labeled blue, actin filaments are labeled red. Adapted with permission from Ref. [40] Copyright 2018 American Chemical Society.

proteins. Artificial MVs were prepared using liposomes equipped with TNAP, but proved to be much less active compared with biological MVs. When co-assembled in an osteoblast-like SaOS-2 spheroid, microreactors prepared with biological MVs significantly increased the Ca^{2+} content and spheroid volume compared to empty synthetic cells. The authors speculate that these synthetic cells provide the basis for 3D- bioprinted bone tissue as the next-generation materials. For this purpose, the synthetic cells should display a higher degree of adaptivity. This could be achieved via incorporation of additional functional elements (e.g. tools for the CFPS of TNAP for example in response to high calcium concentrations).

In addition to induction of bone mineralization, many studies have also used increased cell viability as a readout for growth support. The reason for this is likely the relative straightforwardness of measuring cell viability, compared to other cellular readouts. In these studies, biological cells were first purposely challenged by a toxic compound and subsequently rescued by their synthetic counterparts. The first report of this approach was made by Zhang *et al.*[41] Synthetic cells were prepared from silica particles, coated with PLL, followed by the deposition of catalase loaded liposomes, and a final polymer and polydopamine layer. These core-shell cell mimics increased cell viability after exposure of the cells to H_2O_2. However, these particles possessed some disadvantages, as the layer-by-layer based assembly is labor intensive and the loading capacity with liposomes was limited. Therefore, in a follow-up study the synthetic cell design was changed from silica core-shell particles to alginate (Alg) particles coated with PLL and cholesterol-modified polymethacrylic acid (PMAc) in a microfluidic setup (Figure 6A).[42] Two types of microreactors were assembled: Alg carrier particles with entrapped catalase-loaded liposomal microcompartments ($AlgL_{cat}$) and Alg carrier particles with encapsulated catalase (Algcat). Both synthetic cells were able to preserve the viability of HepG2 cells upon H_2O_2 exposure (Figure 6B). Algcat proved to be better in rescuing cell viability than $AlgL_{cat}$, possibly because of the reduced access of the enzymes for H_2O_2 inside the liposomes. On the other hand, $AlgL_{cat}$ demonstrated enhanced preservation of enzymatic activity over time compared to AlgCat. Whereas biomedical applications for these types of synthetic cells (e.g. adaptive 3D printed tissue engineering scaffolds or next-generation extracorporeal temporary (liver) support devices) are still far in the

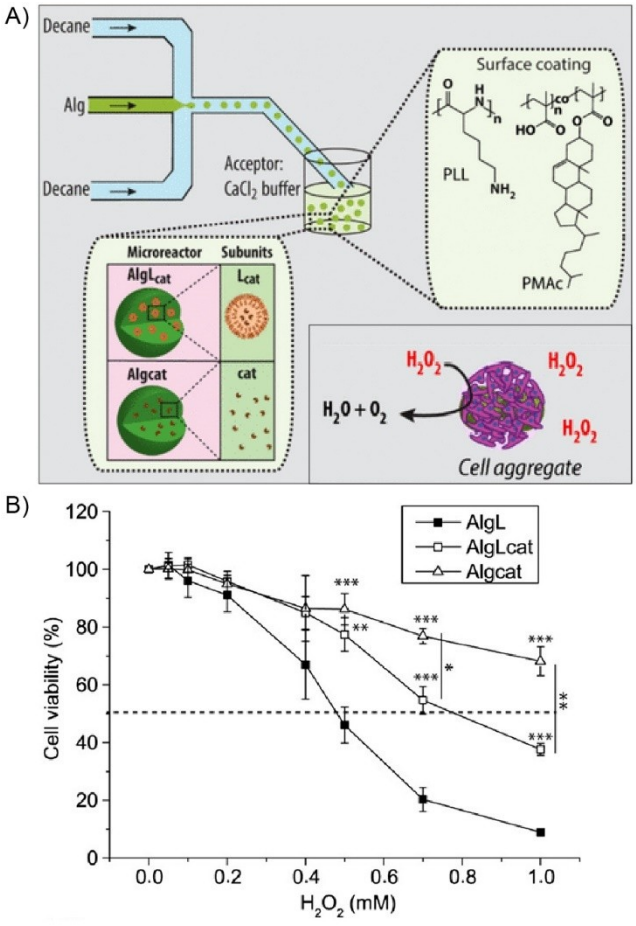

Figure 6. A) Schematic illustrating the assembly of alginate (Alg)-based synthetic cells using microfluidics. Two types of synthetic cells were assembled: $AlgL_{cat}$, alginate particles with encapsulated catalase-loaded liposomal microcompartments; Algcat, alginate particles with encapsulated catalase. $AlgL_{cat}$ and AlgCat were co-cultured with 3D HepG2 spheroids and converted toxic H_2O_2 into H_2O and O_2. B) Dose–response curves of HepgG2 cells in a 3D coculture with Algcat and Alg_{Lcat} when exposed to different concentrations of H_2O_2 for 24 h. AlgL, Alg particles with empty liposomes were used as negative control. Adapted with permission from Ref. [42] (DOI: 10.1021/acsomega.7b01234) 2017 American Chemical Society. Further permission related to the material excerpted should be directed to the ACS.

future, these studies contribute to the fundamental understanding of integrating synthetic cells in biological cell communities.

In a different study, polydopamine-based synthetic cells were reported as astrocyte mimics.[43] Astrocytes are non-neural cells in the central nervous system that perform many supporting functions including the conversion of glutamate (L-Glu) and ammonium to glutamine using glutamine synthetase.[44] Pathologically high glutamate levels can result in excitotoxicity, a form of neuronal damage characterized by the production of ROS and ammonia (NH_4^+). The protective function of astrocytes was mimicked by incorporating citrate-capped platinum-nanoparticles (Pt-NP) inside polydopamine-based synthetic cells (M^P) (Figure 7A). Pt-NPs act as ROS scavengers due to their activity similar to superoxide dismutase and catalase. The synthetic cells were assembled by coating

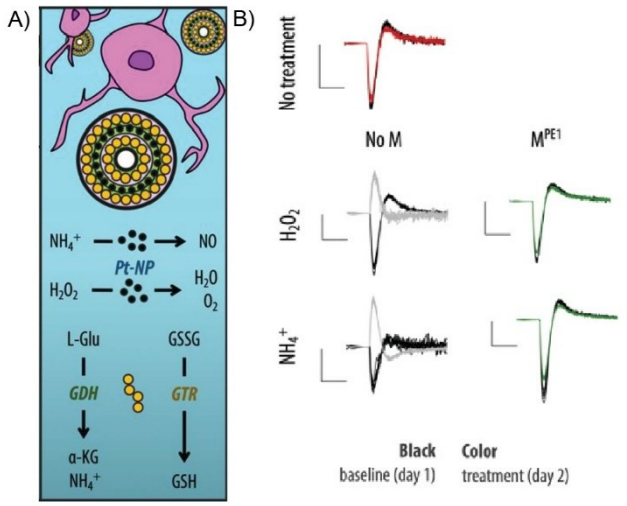

Figure 7. Synthetic astrocytes for relieving excitotoxicity in rat primary cortical neurons caused by the build-up of toxic compounds. A) Schematic illustrating the multi-compartmentalized nature of the synthetic astrocytes, which are built up from polydopamine particles, platinum nanoparticles (Pt-NPs) and liposomes containing glutamate dehydrogenase (GDH) and glutathione reductase (GTR). Pt-NPs convert toxic ammonia (NH_4^+) into nitric oxide. GDH and GTR convert L-Glu to alpha-ketoglutarate (α-KG) and oxidized glutathione (GSSH) to reduced glutathione (GSH) respectively. B) Extracellular electrophysiological recordings showing neuronal activity in cells upon incubation with (M^{PE1}) or without (no M) the astrocyte mimics when treated with H_2O_2 and NH_4^+. Adapted with permission from Ref. [45] Copyright 2020 Wiley.

polystyrene particles with polydopamine and PLL prior to the deposition of the catalytically active Pt-nanoparticles and different terminating polymer layers that increased the interaction with the neuroblastoma cells. After incubation with these neuroblastoma cells, the synthetic cells were found to partly rescue cell viability after challenging the cells with H_2O_2 and NH_4^+. In a recent follow-up study, the biocompatibility and interaction of the astrocyte mimics was further investigated using rat primary cortical neurons.[45] To expand the function of the synthetic cells, liposomes containing glutamate dehydrogenase (GDH) and glutathione reductase (GTR) were incorporated in the synthetic cells (M^{PE}) (figure 7A). These enzymes were able to convert toxic L-Glu to alpha-ketoglutarate (α-KG) and NH_4^+, and oxidized glutathione (GSSH) to reduced glutathione (GSH) respectively, thereby increasing cell viability. The activity of the synthetic cells was measured via extracellular electrophysiological recordings that showed baseline neuronal activity in cells upon incubation with the astrocyte mimics when challenged with H_2O_2 and NH_4^+ (Figure 7B). These studies nicely demonstrate the capabilities of synthetic cells to counteract toxicity induced by ROS. Incorporating the synthetic cells inside the body (e.g. the brain) remains a major challenge. Although not yet fully adaptive, these studies represent steps towards incorporating synthetic cells in tissues *in vitro* for providing growth support by shaping the extracellular matrix or by improving cell viability. Toperlak *et al.* recently took this supporting role a step further, by influencing the differentiation behavior of mammalian cells. More specifically, lipid-based synthetic cells were used for the differentiation of mouse embryonic stem cell-derived neural stem cells (mNS).[46] Interestingly, the authors used a quorum signaling pathway to establish communication between the synthetic cells and mammalian cells (Figure 8A).[24,10] The liposomes were equipped with a genetic AND-gate for the expression of brain derived neurotrophic factor (BDNF). This factor drives the differentiation and survival of neuronal cells via activation of tropomyosinreceptor kinase B (TrkB) and its downstream signaling pathway, resulting in neurite outgrowth, branching, synapse formation and stabilization. The genetic AND-gate consisted of CFPS machinery and DNA templates for the production of BDNF, LuxR, and the pore forming protein perfringolysin O (PFO). In this design, BDNF was always synthesized, but could only be excreted from the synthetic cells when the autoinducer 3OC6 HSL was added and induced the transcription of PFO. The authors demonstrated that the synthetic cells activated downstream TrkB signaling in mNS cells and increased axon outgrowth velocity in *Xenopus laevis ex vivo* eye organocultures (Figure 8B). Moreover, these synthetic cells were stable in physiological conditions and did not induce substantial toxicity. As these synthetic cells are

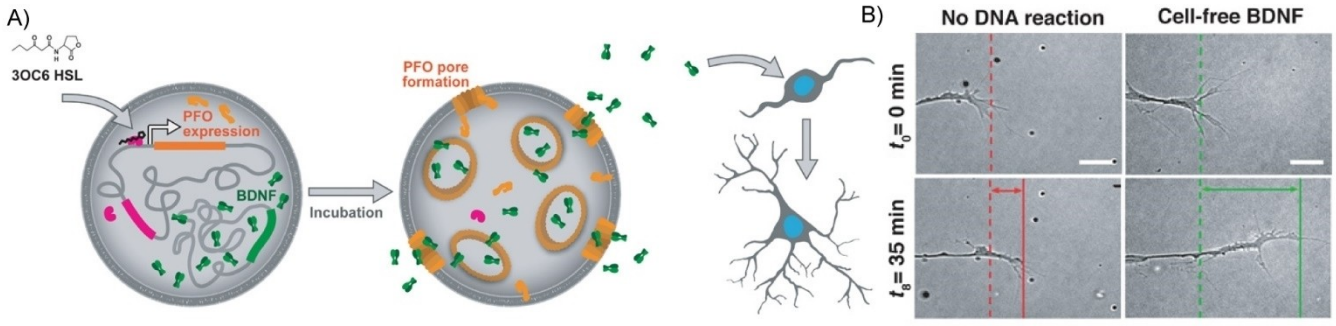

Figure 8. Synthetic cells drive differentiation of neural stem cells into neuronal cells. A) Schematic showing a genetic AND-gate that allows for the communication between synthetic and neural stem cells via the release of brain derived neurotrophic factor (BDNF). BDNF is continuously synthesized inside the synthetic cells, but is only released when the pore forming protein perfringolysin O (PFO) is produced. Synthesis of PFO is regulated by the addition of quorum sensing molecule *N*-3-oxohexanoyl homoserine lactone (3OC6 HSL). B) Outgrowth of retinal ganglion cell axons in the absence or presence of a DNA reaction resulting in BDNF release. Scale bar represents 10 μm. Adapted under terms of the CC-BY license from Ref. [46] Copyright 2020, The Authors (published by the American Association for the Advancement of Science).

responding to quorum sensing, which naturally occurs in the gut, the authors envision that they could be used for example within the gut-brain axis, regularly monitoring the environment and, in response, synthesize and release different compounds. This study exemplifies one of the most established interactions between synthetic and mammalian cell communities. The use of autoinducer responsive CFPS elements represents a major step forward in the establishment of meaningful feedback mechanisms. These mechanisms could in the future be interesting for the spatiotemporal differentiation of cells inside self-organized three-dimensional tissues, allowing these tissues to better mimic the complexity of organs.

4. Unrealized Avenues for the Application of Synthetic Cells to Biomedical Challenges

In this last section, we will highlight a limited selection of recent developments in the synthetic cell literature that have not yet been applied to biomedical contexts, but have the potential to do so. Building a synthetic cell from the bottom up has been approached from many different angles, and is continuing to expand rapidly. Covering the potential impact of every single different synthetic cell platform is beyond the scope of this review, and as such we will focus on three areas that we believe will have a significant impact in the years ahead: coacervate-based synthetic cells, synthetic tissues, and synthetic cells capable of autonomous propulsion.

4.1. Coacervate-Based Synthetic Cells

While there are an impressive variety of approaches that can be used to create compartmentalized structures, the inner phase of these synthetic vesicles is often the same, typically consisting of a dilute aqueous solution of buffer salts and (relatively, compared to the cell cytosol) low concentrations of functional biomacromolecules.[2] However, synthetic cells that are constructed upon condensed phases of matter, such as aqueous two phase systems[47] (ATPS) and complex coacervates, provide platforms that have a greater physicochemical resemblance to the highly molecularly crowded cytoplasm. For biomedical applications perhaps the biggest advantage that these condensed phases have is the ability to sequester a great variety of molecules.[48,5] This ability has already been adopted to applications, including the development of sensors, biomimetic adhesives, and delivery platforms.[49] Encapsulation can be achieved by using the cargo as part of the coacervate matrix,[50] as a result of specific interactions,[51] or by preferential partitioning.[39,52,53,54,55,56] These synthetic cells could be used to build better *in vitro* models of biological cascades, as the microenvironment of encapsulated protein-protein or protein-small molecule interactions is more similar to their eventual, highly concentrated, cellular environment.

The development of responsive coacervate-based synthetic cells to act as cell-sized reservoirs that can autonomously dose biomedically relevant cargoes, such as small molecule drugs, proteins, or DNA/RNA could have a significant impact on drug release or aiding cell culture. For example, we have recently shown that programmed cargo loading and release can already be achieved by carefully balancing attractive and repulsive forces between functional cargo and the coacervate core.[51] Another approach is to use the coacervate to deprive the cells of vital components, as described in a recent paper from Ikeuchi *et al.*[57] Here they use UV-responsive coacervates based on ureido polymers, which can spatiotemporally recruit proteins and influence cell morphology. HeLa cells that grew in the presence of coacervates showed a more rounded morphology as the coacervates inhibit interactions with the surface of the dish. When the coacervates 'dissolved' after irradiation of a small area in the dish, the Hela cells showed a more elongated morphology. This sort of responsive, high-concentration loading of functional material is currently very difficult to achieve via conventional compartmentalized structures and is a unique strength of coacervate-based synthetic cells.[58] By incorporating multiple parallel, compatible yet orthogonal release mechanisms, it is possible to envisage a synthetic cell that can autonomously respond to a wide range of cellular cues, which would be of interest to a number of biomedical challenges, such as the controlled differentiation of cells to form organoids with a stronger resemblance to naturally occurring tissues.

4.2. Synthetic Tissues

To date, the majority of synthetic cell research has been focused on making increasingly lifelike populations of single cells. However, in a biomedical context, where researchers are dealing with tissues, organs, and entire organisms, this "single cell" environment is rare, with cells more often than not packed in tight 3D configurations with other cells like themselves or working together with different cell types to provide a higher-order functionality. To start with, there have been a multitude of systems set up to study the diffusion of small molecules in tightly packed 2D arrangements of synthetic cells, using droplet interface bilayers.[59,60,61] However, a growing number of groups are attempting to construct three-dimensional patterns of synthetic cells, towards synthetic tissues.[62,63] These efforts have been pioneered by the Bayley group, who have developed a 3D printing technique that enables the positioning of droplet-interface bilayers.[64] Such synthetic tissues can be used to study diffusion-based communication,[65] which is vital for understanding signaling in multicellular environments.

These are the first exciting steps in a rapidly developing field. There are many opportunities for hybrid systems, where synthetic tissues interface with living tissues to provide a dynamic scaffold during wound healing or to act as a soft material interface between electrical components and tissues.

4.3. Motile Synthetic Cells

Motion is another important feature that researchers have tried to mimic in order to make synthetic cells more life-like.[66] Motile synthetic cells, or so-called micromotors or micro swimmers, use currently different sources to induce motion, including ultrasound,[67] light,[68] bubble generation[69] and molecular gradients.[70] While all of the examples mentioned in section 3 had promising responsive behaviors, they were still static in terms of their position over time. Motile synthetic cells could have a range of biomedical applications, such as sensing, imaging, and drug delivery. However, the holy grail would be the ability for a synthetic cell to move chemotactically, as it could allow synthetic cells to navigate autonomously within tissues and organs towards a specific target, improving drug delivery via higher local concentrations of a therapeutic and increased specificity.[71] There have been some recent advances of chemotaxis with liposomes, utilizing membrane bound urease and catalase,[72] but in order to sense more biologically relevant chemical gradients, these motility-inducing components will need to be coupled to a secondary sensing system, which leads to a signal amplification and an enhanced response.

While these aforementioned capabilities would certainly be of interest in a biomedical context, there will inevitably be stumbling points. Firstly, as bottom-up synthetic cells grow in the number of different functional components, with stimuli-responsive behaviors, their impact on biological systems could become more difficult to predict. If non-desirable off-target or generally cytotoxic effects are observed, it becomes more difficult to pinpoint the exact molecular basis of this behavior. However, an inherent strength of all synthetic cells is that we know exactly what is contained within them, thanks to their bottom-up design, and can undertake a component-based approach to investigate the cytotoxicity of individual components. We have recently reported such a systematic approach, which clarified exactly which component caused cytotoxic effects, and enabled us to modulate the formulation of the synthetic cells to make them biocompatible.[73] Secondly, many of these synthetic cell systems utilize the same biological machinery, such as the aforementioned quorum sensing pathways and GOX to produce hydrogen peroxide in situ. We should not limit ourselves to these admittedly robust and easy to implement components, and instead focus on designing synthetic cells that are more amenable to the incorporation of a diverse, perhaps more fragile, toolbox of biological functionalities. The engineering of materials in synthetic cells is also vitally important to be able to effectively control their lifetime in biomedical contexts. A careful balance needs to be found wherein synthetic cells are not degraded/cleared before they have performed their designed function; but do not accumulate for extended periods of time that may lead to chronic, undesired side effects. These challenges are not trivial, however, we expect that as our understanding of bottom-up self-assembly continues to improve, these problems will not be insurmountable.

5. Summary and Outlook

Bottom-up synthetic cell research provides an avenue towards the construction of micron-sized, adaptive, and inherently engineerable systems. When these systems are designed with biocompatibility in mind, their application to biomedically relevant problems is a logical and useful research direction to pursue. Indeed, there are already many examples of such synthetic systems having an impact on biomedical research via regulation of (anti)-microbial activity, adaptive delivery of compounds and supporting cellular growth, but sometimes nomenclature can have a detrimental effect. A logical step forward is to ensure that the synthetic cells are compatible with the environment in which living cells operate. This means that on the one hand, the compartmentalized structure should provide protection against protease and nuclease activity, or undesired interactions with biomolecules present in cell medium. On the other hand, the synthetic cells should not induce toxicity to the cells. Taking these aspects into consideration at the design stage will enhance the opportunities to apply them in cellular communication.

Synthetic cell researchers have already developed an impressive array of molecular building blocks to build increasingly lifelike systems. There is a unique opportunity for these systems to be adapted to enable cellular compatibility and communication, and regardless of what they are called, we believe these cell-sized, responsive, biomimetic compartments will play an increasingly important role in biomedical technology in the years to come.

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Conflict of Interest

The authors declare no conflict of interest.

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