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Synthetic Immunology—Building Immunity from the Bottom-Up with Synthetic Cells

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Synthetic cells can advance immunotherapy, offering innovative approaches to understanding and enhancing immune responses. This review article delves into the advancements and potential of synthetic cell technologies in immunology, emphasizing their role in understanding and manipulating immune functions. Recent progress in understanding vertebrate immune systems and the challenges posed by diseases highlight the need for innovative research methods, complementing the analysis of multidimensional datasets and genetic engineering. Synthetic immune cell engineering aims to simplify the complexity of immunological systems by reconstructing them in a controlled setting. This approach, alongside high-throughput strategies, facilitates systematic investigations into immunity and the development of novel treatments. The article reviews synthetic cell technologies, focusing on their alignment with the three laws of immunity: universality, tolerance, and appropriateness. It explores the integration of synthetic cell modules to mimic processes such as controlled T-cell activation, bacteria engulfment and elimination, or cellular maturation into desirable phenotypes. Together, such advancements expand the toolbox for understanding and manipulating immune functions. Synthetic cell technologies stand at the innovation crossroads in immunology, promising to illuminate fundamental immune system principles and open new avenues for research and therapy.

1. Introduction

Immunity is critical for all forms of life. Whenever a living system concentrates significant amounts of energy in a small confinement (e.g., a cell), it inevitably attracts other organisms, typically smaller ones, seeking to steal this energy. Thus, an immune system can be described as any molecular or cellular mechanism employed by life forms to protect themselves from the exploitation of their resources by other beings. Not all of these systems are as sophisticated and complex as those found in vertebrates. Even basic molecular strategies, like the CRISPR/Cas system in bacteria, serve a similar protective function. As dynamic chemical systems, life forms gather, store, and process vast amounts of energy to sustain their nonequilibrium state. Engaged in close interactions with their environment and in competition with other organisms, all life forms eventually develop some form of immunity. Therefore, immunity can be considered as a fundamental aspect of life, reflecting the complexity and environmental challenges faced by organisms.

However, understanding and mastering immunity is not only crucial for mechanistic insights into the core principles of life but also stands at the forefront of medical advancements, playing a key role in the sociocultural and economic development of human societies. The past century has witnessed significant progress in our comprehension of vertebrate immune systems, spanning molecular details of cellular defense mechanisms,[1] and their implications for public health.[2] The recent COVID-19 pandemic has underscored the urgency of gaining control over immune responses, a need further highlighted by the rising challenges of cancer, autoimmune disorders, and antibiotic-resistant bacteria. Consequently, there's a growing need for innovative methods to investigate immunity at its most basic levels and for the development of new technologies that promise more effective treatments. Therefore, contemporary research in immunology is currently mostly moving toward the analysis of multidimensional dataset, based on sequencing and other -omics approaches.[3] Although these datasets have a high informational content, they are often not straightforward to interpret and require advanced analytical pipelines for dimensionality reduction and experimental designs that are meticulously tailored to the hypotheses being tested. This approach aims to capture and integrate the full molecular,

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cellular, and functional complexity of the immune network to gain mechanistic insights.

A promising, albeit opposing direction to complement these high-throughput strategies, involves simplifying the complexity of the immunological systems being studied. Such a minimalist approach aims to distill the fundamental aspects of immunological processes and reconstruct them in a controlled, isolated, and well-defined setting (**Figure 1**). This would facilitate more systematic and comprehensive investigations of immunity. In pursuing such reductionistic experimental strategies, novel treatment possibilities may also emerge. Within this framework, the developing field of synthetic cell engineering (also referred to as bottom-up synthetic biology) presents an interesting approach.[4] The construction of synthetic cells through a bottom-up approach, assembling them from individual molecular components step by step, is primarily motivated by the desire to understand the processes at the origin of life in more detail and to grasp the physical fundamentals of life by producing living cells from scratch.[5–8] Achieving the milestone of the first living synthetic cell would mark the introduction of the first life form created by humans from scratch, distinct from all naturally evolved life.

Despite ongoing debates around the defining characteristics and essential features of a living synthetic cell,[9] significant advancements have been made in engineering increasingly complex molecular systems. Various forms of synthetic cells have been developed, including those based on giant unilamellar vesicles (GUVs),[10] coacervates,[11] inorganic protocells,[12] and polymerosomes,[13] which are capable of performing increasingly sophisticated functions such as division,[14] migration,[15] and energy harvesting[16] (**Figure 2**). Advancements in molecular systems engineering as well as new approaches in microfluidic technologies and 3D printing have catalyzed the creation of more and more complex synthetic cells models.[17,18] Some approaches, such as droplet-based microfluidics, are designed to produce single synthetic cell chassis with high control and little variability but with reduced throughput.[19] Bulk emulsification approaches produce synthetic cells with higher variability in the population but at higher throughputs (mg to kg range), suitable for in vivo application.[20,21] Current approaches in synthetic cell engineering have thoroughly been reviewed elsewhere.[22] Notably, significant advancements have also been made in leveraging synthetic cells for new biomedical applications.[23–25] These advancements include the development of synthetic or cell-mimicking systems designed to restore functions disrupted by diseases, such as synthetic cells engineered to produce insulin or vascular growth factors,[26,27] demonstrating a potential new frontier in medical treatment possibilities with cellular bionics.

The specific advantage of applying synthetic cell systems over traditional cellular therapies lies in their fully artificial and reductionist nature. Synthetic cells are often precisely defined at a

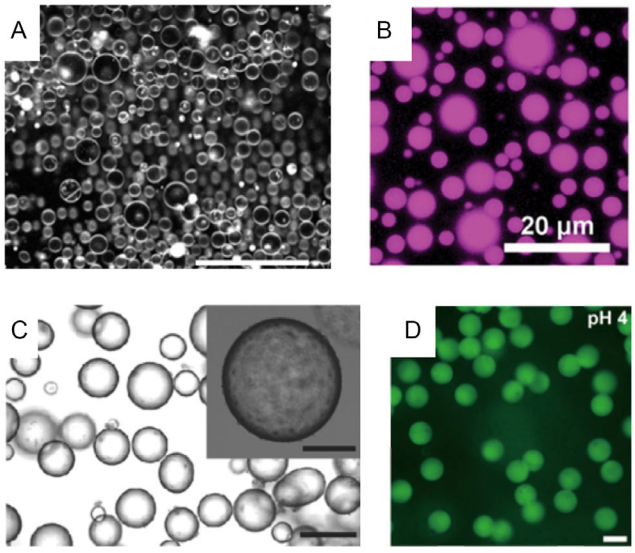

Figure 2. Synthetic cell model compartments. A) Exemplary microscopy images from frequently applied synthetic cell compartments including GUVs, B) coacervates, C) colloidosomes, and D) polymerosomes. Adapted with permission.[10,11,13,82]

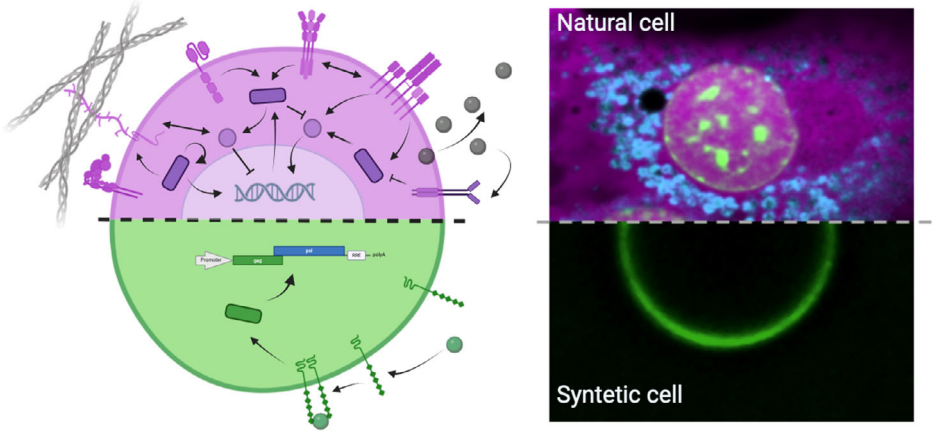

Figure 1. Bottom-up synthetic immunology, a reductionistic approach to immunity. Schematic illustration (left) of the complexity of cues and signaling processing within natural cells (magenta) and a reconstituted, lower complexity approach within a synthetic cell (green). On the right, confocal microscopy images of a natural cell (top) with labeled cytoplasm (magenta), endosomes (cyan), and nucleus (green). Bottom part shows the membrane of a giant unilamellar vesicle as reductionistic synthetic cell model.

molecular level, making them, in many respects, safer than treatments relying on genetically modified cells. Their simplified complexity allows for a concentrated focus on singular functional targets, streamlining processes that are critical to therapeutic outcomes. As the field of synthetic cell engineering advances, producing synthetic cells capable of interfacing with and responding dynamically to the environments with natural cellular,[20,28] their application in immunological research becomes increasingly viable. This development represents a continuation of the longstanding integration of biomaterial sciences within immunology, such as using supported lipid bilayers to study immunological synapses[29] or specially designed surface coatings to examine cell migration and homing.[30] The distinction between these traditional biomaterial approaches and the emerging role of synthetic cells lies in the latter's enhanced biomimicry and active, potentially nonequilibrium nature. This allows for a more responsive interaction with natural immune components, potentially initiating a new era of applications in immunological research and therapy.

This review summarizes and discusses synthetic cell technologies specifically designed for use in synthetic immunology, as well as those synthetic cell modules that may be utilized in the field in the future. The technologies relevant to bottom-up engineering of synthetic immunity are organized and categorized around the three laws of immunity: universality, tolerance, and appropriateness.[31] Of note, the focus of this review is on the concepts and overviews of technologies for assembling synthetic cells from the bottom up within the realm of synthetic immunology. This is distinct from genetic engineering and top-down synthetic biology approaches, which, while more prevalent in clinical settings and often seen as complementary, diverge from the bottom-up strategies emphasized here.

The immune system, perhaps more than any other system within the vertebrate body, relies heavily on surface interactions, membrane signaling, and the binding of membrane receptors to ligands which initiate crucial signal transduction pathways. The membrane plays a pivotal role in these processes, acting as a central structure essential for sustaining signal transmission and downstream signaling events.[32] Furthermore, the immune system's compartmentalized and somewhat decentralized architecture, which operates on a divide-and-conquer principle by distributing effector and regulatory functions among various cell types, underscores the significance of compartmentalization. Bottom-up synthetic biology, with its vast array of tools, is particularly suited to addressing these two aspects of immune system functionality. This approach has been the focus of significant research efforts in synthetic cell engineering, aiming to replicate and harness the compartmentalized nature of immune responses (Figure 2). Therefore, the potential for advancements in bottom-up synthetic immunology is considerable, as evidenced by the systems and technologies reviewed in this article, which primarily concentrate on compartmentalization principles.

2. The Central Engineering Problem

Over the last century, immunological research has made significant strides, delving deeper into the molecular mechanisms, cellular interactions, and progression of diseases within the immune system. This expanding knowledge base has led to some of humanity's most significant achievements, such as the development of the smallpox vaccine, which effectively eradicated the virus globally.[33] Insights into antigen processing and immune memory have enabled the harnessing of immunity's precise and potent force, capable of providing lifelong protection with just a single administration of a few micrograms of antigen. However, immunological research has also uncovered the double-edged nature of this power. When dysregulated, it can lead to severe pathologies, particularly in autoimmune diseases where the immune system mistakenly attacks the body's own cells. This intricate balance and fine-tuning are at the core of the immune system's function, illustrating why mastering and studying immunology remains an exceptionally complex challenge. The immune system manages this delicate equilibrium and processes information through the integration of hundreds of different cell types and an even greater number of signaling molecules, a complexity that surpasses intuitive understanding.[34,35]

In the pursuit of engineering synthetic immunological components, it is crucial to adopt a systematic approach that facilitates the integration of various synthetic cell modules to mirror the regulatory sophistication seen in natural immunity. This effort must also incorporate strategies for controlled interactions and possible integration with natural immunological elements once synthetic cells interact with the body's immune system (**Figure 3**). Mastering such precise regulation, adaptability, and versatility presents a significant engineering challenge, necessitating ongoing cycles of re-evaluation and adjustment. It is therefore tempting to lay out and categorize such synthetic immunology efforts according to the fundamental principles by which the natural immune system functions. Drawing inspiration from the central laws of physics as foundational benchmarks for synthetic cell research, synthetic immunology could draw inspiration from the three laws of immunity, as developed by William E. Paul: universality, tolerance, and appropriateness.[31] These principles offer a framework to streamline the development of bottom-up approaches in synthetic immunology.

3. The Laws of Immunity

The three laws of immunity can be briefly summarized as follows:

3.1. Universality

This principle states that the immune system is capable of recognizing and responding to a vast array of foreign antigens, ensuring comprehensive protection against diverse pathogens or transformed cells. The immune system achieves universality through a complex repertoire of receptors, enabling it to identify and combat almost any foreign molecule encountered.

3.2. Tolerance

Tolerance is the mechanism by which the immune system avoids attacking the host's own cells and tissues, distinguishing between self and nonself. Central and peripheral tolerance are

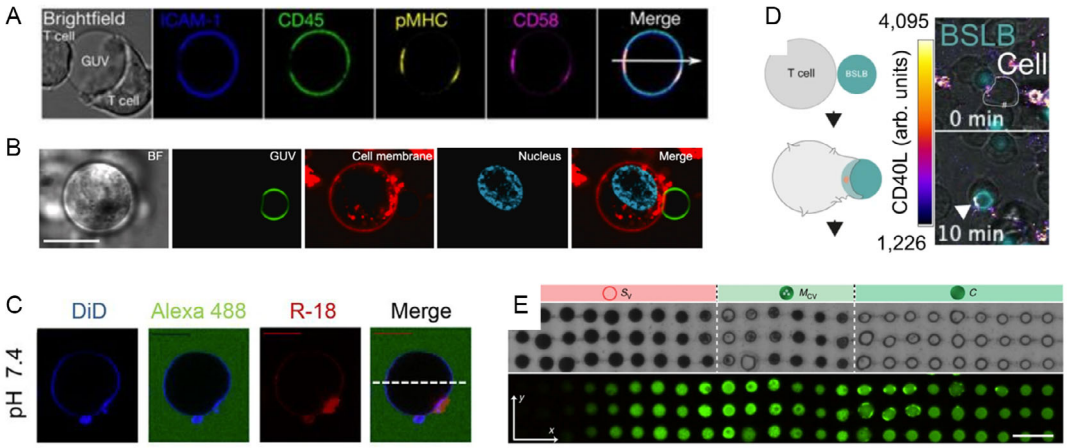

Figure 3. Synthetic cells in the service of immunology. A) GUV-based synthetic antigen-presenting cells incubated with human T cells for activation. B) GUV-based synthetic T effector cells incubated with human leukemia cells for targeted killing. C) GUV-based synthetic target cells for control analysis of influenza fusion (R-18) to host cell membranes. D) Bead-based synthetic cell presenting costimulatory ligands to human primary T cells. E) Coacervate-based synthetic cells under phenotypic differentiation in a morphogen gradient. All images were adapted with permission.[10,42,44,59,65]

the main strategies the immune system uses to eliminate or inactivate self-reactive immune cells, preventing autoimmune diseases.

3.3. Appropriateness

This principle refers to the immune system's ability to tailor its response to the specific nature of the threat, balancing the response to effectively eliminate the pathogen or transformed cell without causing excessive harm to the host. The immune response is characterized by specificity; it can differentiate between different antigens and even between different strains of the same pathogen, ensuring that the response is appropriate to the specific challenge.

Regulatory mechanisms are in place to ensure the immune response is proportional to the threat, involving feedback loops that can amplify or dampen the response as needed. Memory cells play a crucial role in the appropriateness of the immune response, allowing for a more rapid and effective reaction to pathogens the body has previously encountered. Finally, these laws underscore the balance the immune system must maintain between being effectively vigilant against pathogens and potentially cancerous cells while avoiding overreaction or autoimmunity, illustrating the complexity and elegance of the immune network.

4. Synthetic Cell Approaches to Mimic and Study Universality in Immunity

Natural immune systems commonly achieve universality by introducing a component that integrates randomness on a genetic level such as somatic hypermutation in B cells[36,37] and genetic shuffling in T cells.[38] While randomness plays a crucial role in natural immune system universality, its application is challenging in the precise and quantitatively defined world of synthetic cell engineering. In this context, combining top-down and bottom-up approaches could offer a solution, e.g., by employing in vitro transcription-translation (TX-TL) systems and logical genetic circuits to recognize a broad array of structures.[39] Multiple types of synthetic cells with integrated TX-TL systems have been developed that also feature the production of membrane proteins and antibodies, entailing universal pattern recognition capabilities to the system.[20,40]

In a complementary approach, the direct integration of natural building blocks that confer universality, such as T cell receptor (TCR)-ligands and immunoglobulin antigens, has already enabled synthetic immune cells to exhibit a degree of universality and specificity. For instance, Jenkins et al. introduced a GUV-based synthetic cell that presents peptide-loaded major histocompatibility complexes (pMHC), capable of forming complex adhesive interfaces resembling immunological synapses with T cells, especially when costimulatory molecules like CD58 and ICAM-1 where included.[41] Furthermore, Hernandez et al. described a GUV-based system presenting cytotoxic proteins like FasL (CD95L) and TRAIL together with an antibody display module for interchangeable surface antibodies, making the system adaptable to any recombinantly expressed or purified IgG antibody.[42] This combination allowed to produce synthetic cells able to recognize a large, almost universal, spectrum of antigens and perform immune effector functions upon recognition.

Synthetic cells have also allowed to probe molecular components and mechanisms associated with universality in pathogen-immune interactions, where GUVs have served as mimetics of natural cell membranes. This has allowed to probe universal mechanisms associated with lipid membrane fusion of enveloped viruses. Rice et al. leveraged this model to explore how influenza viral fusion peptides interact with host cell membranes—a universal process in the fusion stage that is shared across all enveloped viruses.[43] In a related study, Haldar et al. employed GUVs to reveal the universal nature of these fusion mechanisms and their reliance on the lipid membrane's composition.[44] Additionally, Nikolaus et al. used GUVs to investigate the interactions of influenza hemagglutinin

proteins with lipid rafts, further illustrating the widespread principles that govern these viral interactions.[45]

Beyond the adaptive immune system, the innate immune system's almost universal recognition capabilities, albeit less specific, are crucial for identifying a wide range of pathogen-associated molecular patterns. Synthetic cells incorporating innate immune components, like a system developed by Mukwaya et al. that copresents hyaluronic acid and toll-like receptor agonist Pam3SK4, demonstrate the potential of synthetic cell technologies to mimic and harness this universality.[46] Such approaches offer promising new avenues for therapeutic strategies, leveraging the innate immune system's capacity for macrophage activation and broad pathogen recognition.

5. Synthetic Cell Approaches to Mimic and Study Tolerance in Immunity

The challenge of achieving tolerance in a synthetic immunological system is comparably demanding as the approaches to universality. Tolerance, unlike universality which is an inclusive concept to integrate a broad range of targets, operates on principles of exclusion. This distinction requires the implementation of selection mechanisms and affinity maturation processes akin to those seen in natural immune systems, often facilitated by complex tissue structures like the thymus,[47,48] which are intricate to replicate synthetically. In this context, incorporating DNA and RNA nanotechnology into synthetic cell systems emerges as a promising strategy to equip such systems with the necessary selectivity and nuanced levels of affinity essential for tolerance.[49] Through precise control over DNA/RNA binding sequences, it could be possible to quantitatively adjust selection processes through established microfluidic pipelines for aptamer selection.[50–52] This approach offers a pathway to replicate the sophisticated mechanisms of natural tolerance within synthetic constructs. To realize this concept of tolerance in synthetic cells, a pivotal element involves the selective expansion of cell populations that are tolerant to self-antigens. The ability to undergo controlled division and proliferation is central to this effort. Current research explores various mechanisms for regulated synthetic cell division and growth, with self-replication seen as a crucial factor for incorporating a fitness criterion into the selection process.[53] Dreher et al. have explored a simpler process based on physical chemistry principles in phase separation and subsequent growth of protocells, relying on the physical properties of lipid membranes in GUVs.[54] Meanwhile, other strategies employ proteins derived from bacteria to facilitate spatially precise constriction of the synthetic cell structure.[14,55] Parallel to these developments, methods for sorting and eliminating non-tolerant synthetic cells must be devised. One intriguing possibility is leveraging self-selective predatory behaviors, which have already been demonstrated in synthetic cell communities, to perform this task.[56] This multifaceted approach underscores the complexity and innovation driving the field toward replicating the dynamic processes of natural tolerance within synthetic systems.

The effectiveness of these systems would likely be maximized through the implementation of sorting and spatial segregation behaviors within a tissue-like environment that fosters close interactions between synthetic effector cells and cells presenting self-antigens. This strategy could be specifically successful as vertebrates employ the same principle in the thymus and lymph nodes. The development of self-sorting mechanisms, facilitated by chemical communication, has already been achieved in various synthetic cell constructs, including proteinosomes and coacervates.[57,58] These advancements suggest a promising direction for creating synthetic environments that replicate the intricate dynamics of natural immune tolerance training and selection processes.Together with these strategies, a system for the differentiation and maturation of a tolerant subpopulation is essential. Vertebrates utilize a process of sequential maturation, where a naïve cell population, not yet fully equipped with effector capabilities, undergoes selection and subsequently differentiates into a mature state. This process has been mimicked in synthetic cells, where morphogen gradients induced within coacervate protocell communities, successively differentiate subpopulations into effector cells with specific enzymatic activities.[59] Future directions could involve adapting this methodology and its underlying chemistry to a tissue-like setting where intercellular interactions are enhanced by spatial confinement. Toward this goal, various methods for creating synthetic tissues have been introduced, such as using droplet-based compartments combined with DNA aptamer cross-linking or employing direct in-solution 3D printing technologies.[60,61]

Moreover, incorporating an active migratory capability within the naïve cell population would be crucial, enabling these cells to scan the selecting tissue environment for a broad spectrum of self-antigens. This aspect is again central to synthetic cell engineering, as self-powered movement and directed migration are essential characteristics of living systems. In this line, a system that closely mimics a molecular mechanism of cellular translocation has been integrated into GUVs. Benk et al. produced GUVs presenting surface-integrated ligand-sensitive integrins that are coupled to cytoskeletal elements through a talin-head domain.[62] Upon encountering a surface functionalized with RGD-containing fibronectin, the synthetic cells undergo spreading and initiate signaling pathways from the outside to the inside, mirroring the critical process of T-cell navigation and homing observed in lymph nodes. In pursuit of even more precise control and enhanced migratory capabilities, Bartelt et al. have applied more artificial methodologies, such as light-guided GUVs.[63] These systems have been engineered to allow for directed migration under external light stimuli, offering a level of extrinsic control that opens new possibilities for manipulating synthetic cell movement and interaction within an environment selecting tolerant synthetic effector cells.

6. Synthetic Cell Approaches to Mimic and Study Appropriateness in Immunity

The concept of appropriateness in immunity, which demands sophisticated regulatory mechanisms for precise control, highlights an area where synthetic cell engineering could be particularly impactful. The field of bottom-up synthetic biology has been dedicated to enhance control over molecular systems, a focus that becomes especially beneficial when synthetic cells interact with natural immune cells or those adversely affected by immune

responses. To facilitate these interactions, several strategies for engineering the interface between natural and synthetic cells have been advanced, showcasing the potential for synthetic cells to integrate with biological systems and contribute to regulated immune responses.[64]

For instance, investigations into the interface between synthetic and natural immune cells have been conducted to study the exchange of information among key T cell types, including helper T cells (T_H), cytotoxic T lymphocytes (CTL), and regulatory T cells (Treg). At the molecular level, Cespedes et al. reconstituted a lipid bilayer systems displaying specific densities of coregulatory proteins to study how extracellular vesicles mediate transfer and regulatory signals from antigen-presenting cells to T cells, a vital process in ensuring the precision of adaptive immune responses.[65] In a similar approach, GUV-based synthetic cells have been utilized to investigate the coregulatory signals involved in T-cell activation. Jenkins et al. demonstrated the application of in vitro reconstitution methods to study biophysical mechanisms of immune signaling.[41] This approach enables the examination of the interplay between biochemical and biophysical signals, specifically how the diffusive properties of lipid layers and the segregation of proteins contribute to T-cell activation.

Soluble cytokines play a crucial role in regulating the appropriateness of immune responses. These powerful ligands for membrane receptors modulate immune signaling through paracrine, juxtacrine, and autocrine mechanisms. Given their role in cell communication, cytokines are prime candidates for emulation or augmentation by synthetic cells. Andersen et al. have developed a zymogen-based system capable of transmitting chemical signals through a receptor into a synthetic cell model.[66] This system induces regulated transcription from DNA within the synthetic cell and demonstrates the critical aspects of chemical communication and cellularity in immunity, emphasizing how the spatial separation of biochemical entities is vital. In a parallel development, aimed at addressing autoimmune diseases like diabetes, a system involving soluble-factor sensitive synthetic beta cells has been designed. Chen et al. crafted a synthetic cell, structured around large vesicles, capable of detecting glucose levels and triggering an enzymatic response to oxidize glucose.[26] This design emulates key features of soluble factor sensing and processing found in natural immune cells, showcasing a system that is both highly responsive and dynamic.

However, also synthetic cell systems with sensitivity to actual immune-associated cytokines have been developed. Drawing inspiration from the mechanism of natural effector T cells, which target and kill cancer cells via Fas-receptor activation, synthetic versions of cytotoxic effector cells have been developed by Hernandez et al. These cells incorporate signaling pathways involving interleukin-2 and tumor necrosis factor-alpha. Hernandez et al. demonstrated that incorporating these cytokines into GUV-based synthetic cells significantly boosts the ability of these synthetic effector cells to eliminate cancer cell lines, showcasing a novel approach to harnessing synthetic biology for cancer therapy.[42] In a pioneering effort to apply synthetic cell engineering principles in clinical contexts, synthetic cells have been combined with natural erythrocytes.[67] This approach aimed to remove cytokines in COVID-19 treatment. While this strategy has a lower degree of biomimicry and does not fully mimic the compartmentalized complexity of natural immune cells, it underscores the transitional area bridging synthetic cell engineering and biomaterial sciences.

Appropriateness is crucial not only in adaptive immunity but arguably holds even greater significance within innate immunity. A key process in innate immunity involves the recognition and uptake of foreign pathogens or tumor-derived antigens by professional antigen-presenting cells, such as macrophages, encompassing the initial capture and breakdown of these materials. Synthetic cell engineering has begun to explore this area, with Kostina et al. developing dendrimersomes capable of engulfing and endocytosing bacteria.[68] Similar to biomimetic porous capsules that degrade bacteria upon uptake[18]—a pivotal step for antigen presentation—these strategies rely on physical principles for capture. Such methodologies have been applied across various protocellular systems, including lipid droplets,[69] GUVs,[70] and coacervates.[71] Notably, while current uptake mechanisms primarily leverage physical rather than biomolecular principles, they have facilitated the modulation of biochemical activities and behaviors within the protocells. Rodriguez-Arco et al. have shown that colloidosomes can exhibit unique properties like buoyancy, membrane reconstruction, or hydrogelation upon synthetic phagocytosis.[72] Other approaches draw on signaling pathways characteristic of innate immunity, specifically targeting effector mechanisms of neutrophils to combat bacteria. Netosis, a critical mechanism in this response, has been replicated in synthetic systems by Walczak et al. who established a complex DNA-based signaling network.[73] This network is responsive to chemical interactions between synthetic cells and bacteria, leading to netosis, bacterial immobilization, and subsequent destruction.

These studies collectively demonstrate the potential of synthetic cells as tools to investigate the principles of appropriateness in immunity. The focus has primarily been on the physical principles that govern signal integration and effector functions within both adaptive and innate immunity. Furthermore, they have highlighted the complexity of the molecular mechanisms that underpin appropriateness in immune responses. Delving into these regulatory networks is challenging, given the immune system's reliance on a complex array of molecular components to ensure robustness and sensitivity. For leveraging the full potential of synthetic cells and their applications to understand appropriateness in immunity, the application of artificial intelligence (AI) could be particularly beneficial.[74,75] Synthetic cells, with their capacity to generate highly precise and clean data in well-defined experimental setups, are ideally suited for this purpose. Their quantitative tunability and the ability to test numerous parameters in a high-throughput manner, owing to the miniaturized nature of synthetic cell systems, are significant advantages.[76] It has been recognized that leveraging automation in conjunction with AI could significantly enhance our ability to derive deeper insights from such datasets.

7. Application Cases

To further highlight the applicability of synthetic cell research in immunology, in the following, two application cases are introduced in greater detail.

7.1. Application Case 1

Walczak et al. aimed to develop a synthetic signaling network mimicking a complex innate immune response, specifically netosis[73] (**Figure 4**A,B). This process involves neutrophils excreting neutrophil extracellular traps composed of genomic DNA and antimicrobial proteins to trap and disrupt pathogens. The synthetic pathway designed by Walczak et al. includes two artificial-cell-like agents: responsive DNA-based particles and antibiotic-loaded liposomes. These agents work together in response to bacterial activity. The DNA particles sense a decrease in pH due to *E. coli* glucose metabolism, leading to the formation of a synthetic DNA NET that traps the bacteria and permeabilizes the liposomes, releasing antibiotics to inhibit bacterial growth. The study involved fabricating pH-responsive DNA particles with a core-shell structure, using cholesterol-functionalized DNA nanostars. These particles self-assemble into framework-like materials, responding to pH changes by forming a sticky network. This network traps bacteria and disrupts liposomes, releasing their antibiotic payload. The study characterized the pH responsiveness, assembly, and functionality of these particles using various techniques, including UV absorbance, circular dichroism, dynamic light scattering, and microscopy. The findings demonstrate how advanced life-like behaviors can be engineered from the bottom-up, utilizing a relatively small number of molecular and nanoscale components. The study provides a proof-of-concept for the development of biomimetic antimicrobial solutions and synthetic-cell therapeutics. The synthetic netosis pathway showcases the potential for creating complex innate immune responses in artificial cells, paving the way for innovative applications in in vivo therapeutics.

7.2. Application Case 2

A critical step, not only for providing adaptive immunity but also for adoptive immunotherapy, is the antigen-specific activation of cytolytic T cells. In an immunotherapeutic context, this is typically achieved through the ex vivo activation of patient-derived T cells. The process of T-cell activation is highly orchestrated, involving major shifts in the cells' biochemistry and a multitude of different triggers. These triggers can be biochemical, such as soluble cytokines binding to cell surface receptors or stimulatory antigens and costimulatory ligands binding to the T cell receptor. However, in vivo, T cells also receive biomechanical triggers that

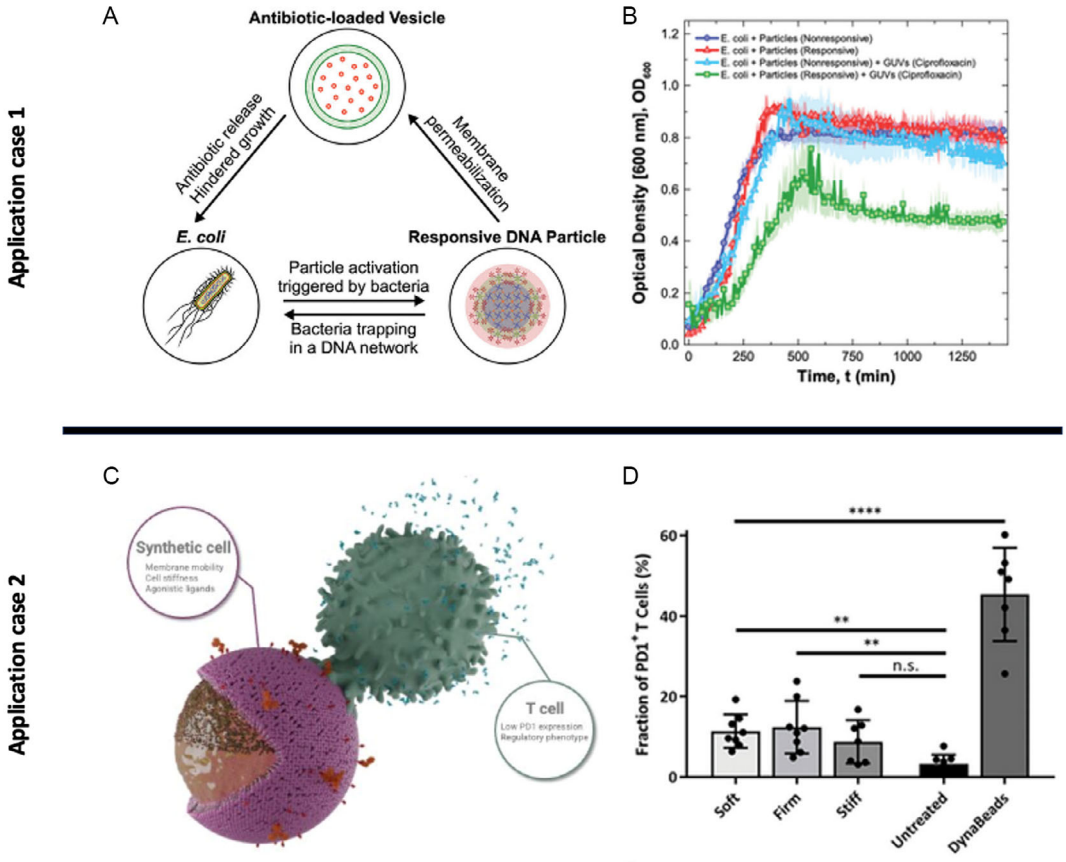

Figure 4. Application cases for synthetic cells in immunology. A) Schematic illustration of the main components in the synthetic neutrophile cells developed by Walczak et al. capable of netosis. B) Measurement of bacterial growth by optical density analysis of *E. coli* cells cultured with the synthetic neutrophile system (green). C) Schematic illustration of the synthetic antigen-presenting cells developed by Burgstaller et al. integrating a mechnomimetic approach in a dispersed supported lipid bilayer system. D) Quantification of PD-1 positive primary human T cells after expansion with synthetic antigen-presenting cells of varying stiffness and with the gold standard of the field (DynaBeads). Adapted and reproduced with permission.[21,73]

fine-tune this activation process. For instance, the stiffness of the antigen-presenting cells is crucial to initiating TCR signaling. Additionally, the mechanical properties of the membrane on which antigens are presented are essential. Burgstaller et al. developed a synthetic cell technology specifically focused on mimicking these biomechanical cues to T cells in ex vivo culture[21] (Figure 4C,D). They produced an emulsion-based supported lipid bilayer model that features comparable stiffness to natural antigen-presenting cells as well as their crucial membrane properties. On the membrane, ligands were immobilized to activate primary human T cells. They demonstrated that the expansion was comparable to that achieved with the current industry gold standard (DynaBeads) but produced T cells with lower expression of immunosuppressive receptors (e.g., PD-1). This demonstrated that biomimetic approaches, which incorporate both the biochemistry and biophysical properties of antigen-presenting cells, can produce a better-quality T-cell phenotype. This showcases how advanced synthetic cell engineering can open new avenues in immunotherapy and be applied in fundamental research to study essential signaling mechanisms. This is exemplified by the discovery of the fundamental role of the lipid membrane in the expansion of a PD-1 low phenotype in this study.

8. Conclusion and Outlook

Synthetic cell technologies represent an expanding frontier in immunology to complement and expand a toolbox for our understanding and manipulation of immune functions. These technologies also offer a promising pathway to sustain, rescue, and expand natural immune functions for biomedical purposes, including the integration of genetic engineering approaches for synthetic receptors. The dynamic and adaptive nature of immunology, characterized by complex feedback loops, control mechanisms, and hierarchical organization, calls for equally sophisticated synthetic cell systems that can adapt and respond in a time-dependent manner to immunological challenges. Therefore, to fully harness the potential of synthetic cells in immunity, systems that can accurately reflect the temporal dynamics of immune responses are essential. Recent advancements are paving the way for such developments, aiming to integrate synthetic cells within the temporal framework of natural immune functions. This development would mostly rely on developing out-of-thermal-equilibrium synthetic cell systems that are able to dynamically respond to immunological triggers.

Furthermore, establishing an interface between synthetic cell technologies and natural immunological systems will be crucial.[77] This integration can be most effectively achieved through the integration of natural immune components—such as protein-based receptor ligands, recombinant antibodies, or cytokines—as intermediaries between living and synthetic cells. Additionally, this integration could extend to incorporating synthetic receptors, engineered cytokines, and synthetic genetic circuits that offer faster response times and enhanced signal processing capabilities.[78,79] A key challenge lies in integrating the myriad of individually developed approaches and chemistries into a cohesive, complex system capable of functioning in physiological contexts. While many synthetic cell technologies remain conceptual, a growing number of applications demonstrate direct applicability and offer glimpses into the potential for these systems to tackle long-standing questions in immunology. Questions such as the quantitative relationship between molecular complexity and system robustness, or the mechanisms by which misdirected self-amplification of nontolerant structures leads to systemic destructive behavior, are within reach. Synthetic cells provide minimal models that quantify biological responses otherwise obscured by the complexity of biological milieus.

As with any synthetic material foreign to the host organism, applying synthetic cells in immunotherapeutic procedures presents challenges related to stable integration and immune evasion. The innate immune system, with its ability to recognize various building blocks used in synthetic cells (such as synthetic DNA, recombinant proteins, and lipids), could pose significant challenges. Although methods to reduce the immunogenicity of each of these components have been developed,[80,81] combining them within a single structure might produce additive effects, necessitating new solutions. These solutions could involve using autologous building blocks or humanized proteins to mitigate the innate immune response. Another significant challenge in applying synthetic cells in immunity is navigating regulatory restrictions. Current guidelines and standard procedures exist for small molecular compounds, many biologics, and even cell-based therapies. However, integrating all these elements into one therapeutic compound is unprecedented and will require new methods for assessing safety and applicability, along with revised approaches for personalization and manufacturing. This complexity will be further amplified if bottom-up synthetic biology achieves its goal of creating synthetic, yet living, components. Such a breakthrough would introduce a completely new class of self-replicating and functioning compounds, presenting regulatory challenges that are currently unimaginable.

In conclusion, synthetic cell technologies stand at the crossroads of innovation in immunology, offering novel tools to dissect and manipulate immune processes. As these technologies evolve, they promise not only to augment our arsenal against diseases but also to illuminate the fundamental principles underlying immune system function. The integration of synthetic cell systems with existing biological knowledge and techniques will undoubtedly open new avenues for research and therapy, bringing us closer to solving some of the most enduring puzzles in immunology.

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Conflict of Interest

The author declares no conflict of interest.

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