New Innovations in Cell-Free Systems Technologies

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Biological manufacturing has become an increasingly common method of producing chemicals used in a variety of industries. Metabolic engineering and synthetic biology are fields that have been of great interest to scientists and engineers in recent years because of their potential to be utilized for manufacturing chemicals in an efficient and economically viable manner. However, as cells are highly complex systems with various intracellular processes, it can be difficult to alter one metabolic pathway without having undesired outcomes. Cell-free systems are *in vitro* tools that reduce these complex interactions by allowing researchers to study the reactions of interest in the cell apart from the full-cell system. In addition, they offer new strategies for on-demand biomanufacturing, molecular diagnostics, and education. Because of the tremendous potential of this technology to revolutionize biotechnology, the Society for Biological Engineering (SBE) hosted the Cell Free Systems Conference on December 4-6, 2019 in Boston, MA, and was funded by the National Science Foundation.

The field of cell-free systems has gained increasing prominence among researchers in academia as well as industry. The global market for cell-free protein expression/synthesis is estimated to have a value of $318.9 million by 2027[i]. The regional market was largest in North America, due to the increasing prevalence of diseases such as cancer that benefit from targeted and personalized medicine[ii]. However, cell-free systems have various applications beyond the medical field, including detection of environmental toxins as well as the synthesis of biofuel enzymes[iii].

Traditionally, metabolic engineering and synthetic biology tools have been used to produce various therapeutics, as well as fuels such as biohydrogen and precursors such as starches and lipids[iv]. However, these methods are known to have their limitations. Microorganisms utilize the energy from feedstocks for cell growth and maintenance, so less is diverted towards product production resulting in lower yields. Additionally, various coproducts can have a high degree of toxicity for microorganisms, resulting in lower titers due to microorganism poisoning when toxins accumulate[v]. Cell-free technologies are an emerging alternative method that may be more efficient. These systems are designed in order to perform complex biotransformations and chemical conversions using mixtures of crude or purified enzymes in a reaction vessel[vi].

However, various research challenges with cell-free systems remain. Cells contain regulatory systems and biochemical replenishing systems that are not present in cell-free systems, and so extra considerations must be taken to design systems that can self-sustain for sufficient periods of time[vii]. Additional challenges include adequate separation of product, mixing, inadequate knowledge of cofactors corresponding to the utilized enzymes, enzyme stability, and system optimization. The development of novel phase transfer catalysts can be utilized to improve product separation, and membrane reactor technology can be utilized to improve separation and selection of product removal[viii]. The Conference examined many of these challenges, as well as new technological innovations that have the potential of advancing the field.

Manufacturing

One underlying goal of developing biotechnology is economically feasible biosynthesis of added value products, such as fuels and pharmaceuticals. Advances in manufacturing using biological technologies include producing vital products with less resources, producing at a lower cost, and producing at a wide range of places. When manufacturing added value products, cell free-systems have several notable advantages to traditional cell culture systems. Cell culture bioreactors are large, expensive, complex, resulting in high cost of production without even accounting for the purification, packaging, and transport steps. Production is also more rapid with the absence of the cell barrier[ix].

While cell-free technologies allow for on-site production of various energy sources and therapeutics when there is such as demand, the issue of storing necessary reagents for production still remains. The proteins and RNA that are utilized in cell-free systems will deactivate at higher temperatures, creating a demand for thermostable reagents. Bundy *et al*. have worked to combat this issue by creating a lyophilized cell free system that is stable outside cold storage in elevated temperatures. It was shown that the use of antiplasticized sugar classes as lyoprotectants would result in significant improvements in the activity of biological molecules in elevated temperatures[x].

Various manufacturing technologies have been presented to utilize cell-free systems to create therapeutic products. When synthesizing added value products using cell-free techniques, *E.coli* is often chosen as a host organism due to its simplicity, robustness, fast growth, and cost effective production, yet there are issues such as a narrow range of peptides and proteins that can be produced as well as toxic coproducts[xi]. Guo *et al.* have utilized an alternative approach, using leukocytes from human blood for expressing recombinant protein[xii]. In past studies, many groups have used CHO or *E. coli* extracts in the manufacturing process, but human blood is easier to obtain and thus lowers the barriers to production. This technology can allow for patients' blood to be taken, the medication created, and injected back to the patient.

Despite its promise, there remain various issues, primary regulatory barriers and hurdles to implanting these novel manufacturing methods to the market.

Prototyping and Characterization

Cell-free systems also offer a powerful tool for uncovering fundamental knowledge that could be imperative for accelerating the engineering field. The development of genetic libraries for various species, development of gene regulatory networks, and biosynthetic pathways can play a role in advancing engineering and manufacturing capabilities.

Cell-free systems can be used to re-create and study bio-assembly process such as macromolecular self-assembly. Technologies such as microfluidics and chips are often used to study such complex processes. One example is re-creating ribosome biogenesis, which is a very complex process difficult to recreate outside the cell. Bar-Ziv *et al.* recreated this process in *E. coli* by synthesizing, capturing all ribosomal proteins and RNA on a chip allowing for autonomous stepwise assembly which can reveal assembly, interactions, intermediates and binding. This data can be used to engineer a self-replicating artificial cell[xiii].

Researchers have also used cell-free systems in conjunction with microfluidic devises to uncover genetic parts such as promoters or repressors. These devises offer reductions in sample volume, low cost, low devise footprint, ability for quantitative detection, and precise manipulation of the sample, rendering them more effective in controlling cell-free reactions[xiv]. For instance, Maerkl *et al.* used a cell-free system with a microfluidic devise to study tuning mechanisms of synthetic zinc-ginger repressor library. This allows for rationally engineering gene-regulatory networks and studying transcriptional regulation[xv]. Zinc-finger domains are utilized in DNA-binding motifs in eukaryotic transcription factors, and are involved in tumorigenesis, cancer progression and metastasis formation, and alterations in zinc finger domains are involved in diseases such as neurodegeneration, skin disease and diabetes[xvi]. Therefore, the ability to understand such mechanisms has the potential of revolutionizing the treatment of many diseases increasing in prevalence.

Additionally, prototyping synthetic organelles allow for the improvement of artificial cells. For instance, polymers with separated compartments can act as templates for transcription and translation. Devaraj *et al.* used this technique to enhance understanding of the effects of spatial organizations of biochemical reactions, as well as to study the communication of cells through diffusive protein signals[xvii]. Developments such as this can help scientists and engineering with bottom-up engineering of increasingly sophisticated systems.

Biosensing and Diagnostics

For biological engineers to use a bottom-up approach to building complex biological systems for applications in agriculture, energy, and healthcare, the complex interactions within the cellular system must be fully understood. Compartmentalization allows engineers to create boundaries in a cell free system where processes (such as growth and metabolism) are carried out and allow researchers to observe changes in the environment[xviii]. This technique not only allow for scientific inquiry, but allows for enhanced functionality in synthetic and natural systems[xix]. Simmel *et al.* utilized quorum sensing signals to establish communication between compartmentalized gene expression systems and bacteria within structures made with a droplet-interface-bilayer technique[xx].

Cell-free technologies allow for advanced methods of detecting various molecules, from pharmaceuticals to heavy metals and other contaminants. The ability to measure or detect biomarkers quantitatively with high specificity is crucial for many medical diagnostic tools. For instance, Jewett, Lucks, *et al*. have developed a portable biosensor for detecting cyanuric acid, which is an interest for human and environmental health[xxi].

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The conference featured five sessions: (1) Diagnostics and circuits, (2) Building Biology, (3) Circuits and Measurement, (4) Prototyping and Characterization, (5) Manufacturing. It also featured poster session, where students could share their work and interact with professionals in their field of interest. The Conference was a success, which will allow SBE to host the 2nd Cell-Free Systems Conference in Spring 2021.

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