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Review

Recent Advances in Cellular and Molecular Bioengineering for Building and Translation of Biological Systems

GUOHAO DAI,[1] ADAM W. FEINBERG,[2] and LEO Q. WAN [3]

[1]Department of Bioengineering, Northeastern University, 805 Columbus Ave, ISEC 224, Boston, MA 02115, USA; [2]Departments of Biomedical Engineering & Materials Science & Engineering, Carnegie Mellon University, 5000 Forbes Avenue, Pittsburgh, PA 15213, USA; and [3]Departments of Biomedical Engineering & Biological Sciences, Rensselaer Polytechnic Institute, Biotech 2147, 110 8th Street, Troy, NY 12180, USA

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Abstract—In January of 2020, the Biomedical Engineering Society (BMES)- Cellular and Molecular Bioengineering (CMBE) conference was held in Puerto Rico and themed "Vision 2020: Emerging Technologies to Elucidate the Rule of Life." The annual BME-CMBE conference gathered worldwide leaders and discussed successes and challenges in engineering biological systems and their translation. The goal of this report is to present the research frontiers in this field and provide perspectives on successful engineering and translation towards the clinic. We hope that this report serves as a constructive guide in shaping the future of research and translation of engineered biological systems.

Keywords—Engineering biological systems, Research translation, Cellular and molecular bioengineering.

INTRODUCTION AND BACKGROUND

In the field of cellular and molecular bioengineering (CMBE), engineering biological systems is one of the fastest-growing areas, especially with recent research breakthroughs simultaneously in multiple fields, including stem cell research, tissue engineering, gene editing, synthetic biology, omics, and biomanufacturing. The expanding toolbox of cutting-edge techniques enables transformative discoveries by the adoption of engineered biological systems for the modeling of a range of processes from development to diseases. However, there remain significant challenges: (i) how to integrate new technologies and novel biological findings to better mimic developmental and pathological processes, (ii) how to translate these engineered systems for applications in drug discovery and clinical practice, and (iii) how to foster new collaborations between scientists, engineers, clinicians, and industry.

To address these challenges, we co-chaired the annual BMES-CMBE conference in Puerto Rico on January 2–6, 2020, themed "*Vision 2020: Emerging technologies to elucidate the rule of life*" (see Appendix for the conference program). As such, we have recruited established and emerging research leaders who have performed innovative research, integrated engineering, and biology to solve complex problems and built a strong connection with the industry to translate research for diagnostics and therapies. The program focused on how novel and advanced techniques and model systems are applied to research in various physiological systems and diseases. To highlight both emerging research and emerging scientists, we honored a talented group of young principal investigators (as "Rising Stars"), postdoctoral fellows, and graduate students for their exciting research. In addition, we reached out to faculty and students at the University of Puerto Rico to encourage participation in the conference and incorporated two training modules on 3D bioprinting and molecular imaging, as well as extending opportunities to present their research and to connect with scientists from all over the world. At the conference, engineers, biologists, and clinicians shared research across disciplines towards the common goal of improving human health. Furthermore, the conference hosted a panel session dedicated to discussing strategies and best practices for translating novel platform technologies to industry and to the clinic in order to improve human health.

Address correspondence to Leo Q. Wan, Departments of Biomedical Engineering & Biological Sciences, Rensselaer Polytechnic Institute, Biotech 2147, 110 8th Street, Troy, NY 12180, USA. Electronic mail: wanq@rpi.edu

From these interactions, we increasingly believe that traditional approaches to answering outstanding biological questions will benefit from an integration of biology and engineering to model physiological and pathological processes. This includes utilizing genetic editing, single-cell analysis, multicellular emerging properties, novel biomaterial, advanced bioreactors, and organ/tissue-on-a-chip, to accurately and precisely drive assembly, formation, and maturation of biological systems. In addition, researchers can be inspired by and build from lessons learned from across diverse fields. Indeed, general principles and engineering approaches may be shared across a variety of biological systems and require venues for communication between researchers from these different fields in order to integrate and advance the field of engineered biological systems.

To highlight the observations and findings from the conference, as co-chairs, here we summarize the collective thoughts and opinions of the participants, including those experts who participated in the panel discussions at the conference (Drs. Nancy Allbritton, David Mooney, Doris Taylor, Valerie Weaver, and Kun Zhang). The goals of this paper are to highlight a selection of emerging research areas that define general principles and shift paradigms in the engineering of biological systems and to provide perspectives on translation. We hope that this paper will play a constructive role in shaping future research in this field.

EMERGING AREAS IN ENGINEERING BIOLOGICAL SYSTEMS

Engineered biological systems have become a hot topic in molecular and cellular bioengineering and have great potential to generate significant impacts in drug discovery and health care. First, engineered biological systems are often cost-effective and allow researchers to more quickly evaluate their hypotheses as compared to time-consuming and labor-intensive animal studies and/or carefully designed clinical trials and studies. Second, when properly implemented, engineered biological systems can allow for the screening of drugs and genetic and environmental factors that cause disease. Third, engineered biological systems can be used for mechanistic studies without systematic effects often associated with an *in vivo* system. Lastly, engineered biological systems may be a critical tool for research studies and scientific discoveries in certain fields, such as human embryonic development, due to ethical concerns.

Over the past few years, there has been significant progress in several frontiers in the engineering of biological systems resulting from advances in techniques and knowledge in several key fields. These new concepts/approaches are likely to continue to develop rapidly and further inspire new ideas on engineering a better biological system. Here we will review some of the important emerging research areas (Table 1). It is worthwhile to point out that there are several other fields of research that may be critical for the engineering of biological systems, including real-time measurement or monitoring of outcomes of the biological systems. We exclude them here as these techniques do not necessarily involve bioengineering at the cell and molecular levels, but they are certainly of great importance for developing efficient systems.

Synthetic Biology and Gene Editing Approaches

Recent developments in synthetic biology and gene editing technologies have created new possibilities to control cell behavior through the engineering of genetic networks that respond to environmental stimuli. Synthetic biology involves the design and assembly of genetic circuits to create new biological functions. By understanding the native genetic circuits that are usually optimized via evolution and reconstructing them from modular design, bioengineers can use synthetic biology to build systems with novel activity.[4] In the early stage of synthetic biology, simple gene circuits could be built to be precisely executed to recapitulate patterns of certain natural biological systems in bacteria, such as oscillating gene expression networks, multistate toggle switches, logic computation, and intercellular signaling networks.[24,31,38] Following these early successes, significant progress has been made, such as new modular genetic parts with standardized design and connectivity principles to streamline the construction of novel circuits with greater complexity.[47,73] However, most of the early success of synthetic biology is performed in prokaryotic cells, while it remains difficult to directly transfer the methods into eukaryotic cells due to the complexity of the genetic networks. With the discovery of more precise genome engineering tools, such as CRISPR/Cas9, researchers have begun to interrogate and control gene function and network dynamics in eukaryotic cells.[75] CRISPR/Cas9 allows precise targeting of specific genome loci. When genetically engineered to pair with functional domains, this precise control has enabled the construction of multilayered gene circuits with higher-order functions in mammalian cells.[55,66]

Applying synthetic biology and gene editing in mammalian cells, researchers can now control the stem cell differentiation process and even reprogram somatic cells toward pluripotency or other cell types. Recent research has utilized tools and principles from synthetic biology to recreate natural developmental

TABLE 1. Technological approaches for engineering biological systems at various levels

Level	Scientific discipline	Engineering techniques and examples
Subcellular	Genetics, epigenetics	Gene editing and synthetic biology to alter cell gene expression and responses to stimuli
Cellular	Cell biology, cell heterogeneity	Single-cell profiling and -omics approaches for characterizing cellular properties
Multicellular	Cell-cell and cell-substrate interactions	Emerging and collective cell behavior through cell-cell interactions; Engineering of organoids and embryo-like structures through cell self-assembly
Tissue/organ	Tissue repair and regeneration	Tissue engineering, organ-on-a-chip, 3D printing, biomanufacturing
Whole body	Immunology	Immunoengineering using cellular engineering, biomaterial, and nanomedicine approaches

processes or engineer cell fate in precise ways. Previously, it was shown that ectopic overexpression of transcription factors could reprogram one cell type into another.[107] Transcription factors are considered the master regulators of cell-type specification and can be used to program cell fate decisions.[23] However, this method of overexpressing transcription factors for reprogramming relies on the ectopic expression of randomly inserted genes that can limit the efficiency and kinetics of generating the desired cell type. Consequently, several groups have utilized tools and approaches from synthetic biology to enhance native transcription factors and improve reprogramming efficiency and fidelity. Similarly, direct conversion of somatic cell types, such as the conversion of fibroblasts to skeletal myocytes by MyoD,[43] can also be achieved with synthetic biology combined with CRISPR/Cas9. In a recent study, multiplexed activation of three endogenous pro-neural genes using CRISPR/Cas9-based activators was sufficient to convert mouse fibroblasts to induced neuronal cells.[6] In this case, targeting the endogenous loci more rapidly remodeled the epigenome and induced transcriptional activation of the target loci than the ectopic overexpression of genes. In another study, a synthetic lineage control circuit in human cells was used to coordinate the kinetics of activation and repression of lineage-specific transcription factors by use of looped circuitry. The circuit directed the differentiation of iPSC-derived pancreatic progenitor cells into glucose-sensitive insulin-secreting β-like cells.[89] Similar circuits based on the same downstream signaling cascade were built to sense and respond to environmental pH level,[3] blood dopamine level,[83] and response to injury[44] for the treatment of diabetes, hypertension, or injury, respectively. Lastly, several synthetic circuits have been described to monitor and maintain aspects of metabolic homeostasis *in vivo*.[46,120,121]

Synthetic biology has also been used to control cellular signaling, gene expression, and phenotype at high spatial and temporal resolution in response to chemical, mechanical, and optical inputs. Natural biological processes often rely on the coordinated action of multiple different inputs to execute complex cellular behaviors. To this end, several groups have successfully built synthetic tools to link precisely controlled extracellular stimuli to intracellular signaling networks to regulate gene expression patterns and cell phenotypes. One example is the engineering of cells to respond to the mechanical environment. It is known that cells use mechanically-sensitive receptors to survey their extracellular microenvironment and generate a corresponding response. Thus, synthetic control of mechanical signaling could provide a means of programming cell behaviors. To this end, a mechanically controlled signal transducer in mammalian cells was developed.[94] Similarly, external inputs to cells have been controlled via light and ultrasonic pulses using synthetic biology approaches.[26,56] Genetically encoded actuators that respond to mechanical, chemical, magnetic, or optical inputs provide a diverse set of synthetic tools for control of cellular signaling and gene expression at unprecedented spatial and temporal resolution.

Beyond synthetic circuits in single cells, synthetic biology approaches could enable more elegant designs of engineered tissue constructs by programming logic circuits that assess cell fate and local environmental conditions and compute desired functional outputs or generate measurable signals.[35,62] The development of multicellular structures and tissues depends on cell-cell and cell-environment interactions and signaling. Thus, using synthetic biology to engineer the cell-cell interaction will allow controlling the multicellular structure and morphogenesis. For example, a synthetic Notch receptor has been engineered that is capable of mediating contact-dependent cellular signaling.[62] Toda *et al.*[109] used an engineered cell-communication system adapted from nature called synNotch[62] to mimic the native self-assembly during cell-cell interaction. The authors engineered the cells so that the synNotch sensors regulated the expression of cadherin proteins,

which mediate cell-cell adhesion, and so are essential for holding cells together and creating tissue boundaries during development. It was found that cell populations that have different patterns or levels of cadherins can sort themselves into separate groups after being mixed together and can self-assemble into a range of structures *in vitro*. Other types of synthetic-biology shape control can also be programmed. For instance, cells have been generated that can be artificially polarized such that asymmetric cell-cell contacts can be made.[45,58] These patterns—such as stripes, spirals, or the spots on a giraffe—arise during development as a result of biological signaling programs. In the future, these toolkits could be expanded to generate short- and long-distance cell-cell communication alongside a synthetic system that controls all of the shape-changing operations involved in making biological structures. This could eventually give engineers total control when designing shapes that have some of the properties of living multicellular organisms.

Omics, Single-Cell Profiling, and Big Data Approaches

The transcriptional, proteomic, and metabolic profiling of cells and tissues provides important information on the underlying molecular states of biological processes. Previously, the development of high throughput sequencing technology allowed the simultaneous acquisition of a large amount of molecular information. However, these efforts have relied on the bulk profiling of whole tissues, which reflect the averaged expression across a population of cells rather than individual cells. The specific 3D organization of different cell types, each with its unique molecular and cellular phenotypes, has a profound impact on normal function, natural aging, tissue remodeling, and disease progression. Recently, rapid advances in transformative technologies of single-cell profiling[68,92,106,108] and multiplexed spatial analysis of tissues have allowed us to interrogate this complexity at unprecedented scale and single-cell resolution.

These advances have motivated the extraordinary effort to build a high-resolution atlas and 3D maps of entire human tissues and organs. In particular, the NIH Human Biomolecular Atlas Program (HuBMAP) Consortium (https://hubmapconsortium.org/) was established to coordinate these efforts. To achieve spatially resolved, single-cell maps, researchers will use a complementary two-step approach. First, omic assays will be used to generate global genome sequence and gene expression profiles of dissociated single cells or nuclei in a massively parallel manner. The molecular state of each cell will be revealed by single-cell transcriptomic[9] and chromatin accessibility[8,14] assays. The transcription factor binding regions from the open chromatin data combined with the gene expression data will be used to construct a computational program to model the regulation of gene expression across the distinct cell types.[15] Second, spatial information will be acquired for various biomolecules such as RNA,[95] protein,[32] metabolites, and lipids in tissue sections, using imaging methodologies such as fluorescent microscopy (confocal, multiphoton, light-sheet), sequential fluorescence in situ hybridization (seqFISH),[25,59] imaging mass spectrometry (spatial proteomics),[104,110] and imaging mass cytometry (IMC).[11,12,76,91] The extensive single-cell and nucleus profiles obtained will inform in situ modalities, which will provide spatial information for up to hundreds of molecular targets of interest.

These data will allow the computational registration of cell-specific epigenomic or transcriptomic profiles to cells on a histological slide to reveal various microenvironmental states.[70,110] The powerful combination of single-cell profiling and multiplexed in situ imaging will provide a pipeline for constructing multi-omics spatial maps for the various human organs and their cellular interactions at a molecular level. To fully comprehend the human body atlas, the HuBMAP Consortium works actively with other ongoing initiatives, including the Human Cell Atlas,[78,84] Human Protein Atlas,[40] LifeTime (https://lifetime-fetflagship.eu/), and related NIH-funded consortia that are mapping specific organs including the brain,[21] lungs (https://www.lungmap.net/), kidney (https://kpmp.org/about-kpmp/), and genitourinary (https://www.gudmap.org/) regions). In parallel, NCI is sponsoring the Human Tumor Atlas Network (HTAN) (https://humantumoratlas.org) as part of the cancer moon shot project (https://www.cancer.gov/research/key-initiatives/moonshot-cancer-initiative/funding/upcoming/hta-foa-video).

During the course of these activities, innovative technologies will be developed to address the limitations of existing state-of-the-art techniques. For example, transformative technologies such as signal amplification in order to analyze molecules at low abundance by exchange reaction (SABER-Signal Amplification By Exchange Reaction),[51,88] seqFISH,[25,96,125] and lumiphore probes.[13] These technologies will be refined to improve multiplexing, sensitivity, and throughput for imaging RNA and proteins across multiple tissues. Furthermore, new mass spectrometry imaging techniques will enable the quantitative mapping of hundreds of lipids, metabolites, and proteins from the same tissue section with high spatial resolution and sensitivity.[122,124] To analyze a large amount of data that will be generated from these programs, new computational tools and machine learning algorithms will be developed for data integration across modalities.

In the end, a comprehensive, accessible 3D molecular and cellular atlas of the human body, in health and under various disease conditions, will be made available to the public. Moreover, these programs will produce an unprecedented volume and diversity of datasets for comprehensive data capture, management, mining, modeling, visual exploration, and communication. These data will be highly useful for the generation of new biomedical hypotheses, tissue engineering, the development of robust simulations of spatiotemporal interactions, machine learning of tissue features, and educational purposes. Ultimately, these data will catalyze novel views on the organization of tissues, regarding not only which types of cells are neighboring one another but also the gene and protein expression patterns that define these cells, their phenotypes, and functional interactions.

Emerging and Collective Behavior of Cells

During morphogenetic processes in embryonic development and tissue regeneration, the behavior of cells depends on not only the extracellular matrix (ECM) environment but also the interactions with neighboring cells.[29] The collective behavior and functions of a group of cells is, therefore, not simply a sum of individual cells. Instead, cell clusters may have spatial patterns of cell functions, depending on the locations of individual cells. The cells are known to coordinate and exhibit a swarming behavior in motion. Indeed, the emerging behavior of cells has been noted long ago and believed to underly the importance of the form (or shape) of biological tissues in their functions through the regulation of local cellular proliferation and differentiation. With microfabrication techniques, spatial differences in cell functions were observed and correlated to the distribution of mechanical forces as a result of cell-cell interactions.[63,85] The YAP/TAZ signaling may mediate the process.[1] These studies underscore the importance of the integration of biology and mechanics in understanding the self-assembly of biological tissues. A better understanding of the mechanobiology of the relationship between form and function may be critical in designing multicellular tissues or organoids.

Collective cell migration is a very active research field. Using technologies such as particle image velocimetry, traction force microscopy, and monolayer stress microscopy, detailed migration velocity profiles and cellular stresses inside the epithelial layer have been obtained.[53] The cells can undergo laminar motion but also rotate and swirl. Cell proliferation (i.e., the addition of a cell) can lead to a bipolar flow field and propagate to a large field that is far away from the division site. Cell extrusion (i.e., removal of a cell) from the epithelial layer is typically associated with a coordinated movement of cells towards the extrusion site. The movement of the cells will be suppressed when the cell density increases. The change from the fluid-like behavior to the solid-like behavior is called the jamming transition, and the reversal is called the unjamming transition. The transitions are important for development and disease.[69,86] Biomechanical modeling has contributed to the understanding of the biophysical mechanisms. A cell vertex-based model predicts that the phase transitions can occur even when cell density remains constant.[5] A single parameter, the target shape index, or the preferred perimeter-to-area ratio, mediates the jamming and unjamming transition. The result suggests a strong cell-cell adhesion may promote a fluid behavior while a strong cortical tension enhances a solid behavior. A further study suggests that for an anisotropic tissue that is often found in development, a cell alignment index is also required to determine the status of the tissue.[114] With a self-propelled Voronoi model that takes into account cell-substrate interactions, the phase transitions can be further regulated by the self-propulsion speed that measures cell motility.[119]

Cancer cell collective migration is another interesting topic and has received much attention. For instance, cancer cells can form tubular structures in the tumor without endothelial cells, called vascular mimicry (VM). Multiple factors can affect VM, including hypoxia, matrix type, and the presence of the other cells (such as macrophages and fibroblasts). Recently, with engineered hydrogels, collagen matrices with small pores and short fibers were shown to change the transcription profile and motility of cancer cells and enhance the formation of a multicellular network.[112] Further studies suggest that ECM microstructure regulates cell adhesion possibly through mediating matrix degradability, which is necessary for the cells to attach 3D collagen fibrils.[111] In addition, the regulation of Snail 1 and Notch signaling was found, indicating the emergence of collective cell behaviors. The fundamental understanding of the collective behavior of the cells (i.e., VM and alike) may lead to the rational design of biological systems that will fully utilize the power within the cells and ultimately benefit the biological modeling of development and disease.

Engineering Human Embryo-Like Structures

The engineering of human embryo-like structures represents a great opportunity in the CMBE field that allows studies that are otherwise impossible due to ethical concerns.[30,79,117] In the United States, approximately 4% of infants suffer from congenital abnormalities, and these birth defects account for 20% of

infant deaths annually. Regulatory guidance often requires the use of animal models for teratogen testing, but they are often costly and labor-intensive. In addition, due to well-known human-animal differences, the findings from animals may not be representative of human development outcomes. Therefore, a humanized *in vitro* model that can recapitulate the developmental process is in high demand.

Earlier efforts, such as control of the clonal size of human pluripotent stem cells (hPSCs), have some success in guiding stem cells into specific lineages. It's not until recently that developmental structures can be recapitulated with *in vitro* platforms. One of the first early efforts used mouse embryonic stem cells to demonstrate the principles.[37] When mixed with extraembryonic trophoblast stem cells (TSCs) in a 3D Matrigel scaffold, artificial embryos were created with the formation of the pro-amniotic cavity and characteristic embryo architecture, patterning of the embryonic compartment, and specification of primordial-germ-like cells. Further studies show that without biochemical cues from maternal sources, the hPSCs alone, without the use of TSCs, were able to self-organize into human amnion-like tissue inside the biomaterial.[98] Later, with a microfluidic device, modeling human epiblast and amnion development with hPSCs was successfully demonstrated with high controllability and scalability.[123]

Human gastrulation has also been modeled with engineering techniques. These earlier studies using micropatterning techniques reveal that upon the BMP4 treatment, circular micropatterns of hPSCs have distinct three regions that represent the three germ layers found in early embryonic development.[115] With similar microscale patterns, WNT and ACTIVIN stimulation induced an organizer with the expression of transcription factor Goosecoid. These cells, when transplanted into chick embryos, induced and contributed to the formation of a secondary axis. Subsequently, using 3D biomaterials, the hPSCs treated with BMP4 form a luminal structure, which polarizes into regions expressing ectoderm and mesoderm markers, mimicking the anterior-posterior symmetry breaking found *in vivo*.[100] The full potential of these engineered embryonic-like structures is still actively explored, with great promise for impacting science and society.

Biomanufacturing: 3D Bioprinting and the Translation to Large-Scale Manufacturing

Biomanufacturing is emerging as a major research area within CMBE, as novel results and new technologies look to be translated to industry and the clinic. While the moonshot vision for the field has been the 3D bioprinting of organs for transplant, that reality is still at least a decade away and likely longer given the challenges of bringing tissue-engineered medical products to the market. Instead, 3D bioprinting has emerged as a powerful tool for building more complex *in vitro* systems that can mimic *in vivo* conditions and enabled hypothesis testing with the ability to systematically vary physical, mechanical, chemical, and biological properties of the microenvironment. Examples include organ-on-chip, small tissue disease models, and scaffolds for tissue regeneration that leverage the unique capabilities to combine cells, biological materials, and synthetic materials based on computer-aided design models.[61]

In terms of *in vitro* model systems, 3D bioprinting has seen rapid growth because of the ability to fabricate complex 3D microfluidics and engineered tissues using a wide range of cells and biomaterials.[27] For example, digital light processing (DLP) bioprinters have been developed that can use UV and visible wavelengths to photocrosslink synthetic and natural hydrogels with high resolution of ~ 5 μm.[64] Using well-established hydrogels such as polyethylene glycol diacrylate (PEGDA) and gelatin methacrylate (GelMA), these DLP bioprinters have been used to build complex 3D fluidic networks to replicate structures such as vascular networks and alveoli.[34] Multiphoton microscopes have also been modified into light-based 3D bioprinters to even achieve a higher resolution of < 1 μm to create true capillary-scale vascular networks.[77] Cells can be integrated into the material being printed, and/or be seeded after scaffold fabrication to create cellularized tissues. Extrusion 3D bioprinting has also seen widespread adoption because it has the flexibility to use many different kinds of biomaterials from thermoplastics, to hydrogels, to microparticle slurries, to photocrosslinkable polymers. Additionally, because the biomaterials and cell-laden bioinks are extruded out of syringes, it is straightforward to use multiple materials and cell types in the same print to increase complexity of the engineered tissues. One of the more recent advances has been extrusion inside of a yield-stress support bath using Freeform Reversible Embedding of Suspended Hydrogels (FRESH) 3D bioprinting inside a gelatin microparticle bath and Sacrificial Writing into Functional Tissue (SWIFT) inside a cell-spheroid support bath.[54,99,101] This has enabled relatively advanced tissues to be engineered, such as beating ventricle-like heart chambers and large vascularized cardiac tissues differentiated from human pluripotent stem cells.[52] These are just a few examples, and many researchers are working on innovative new biomaterials, printing methods, and engineered tissue constructs.

The growth of 3D bioprinting and the translation towards the clinic and large-scale biomanufacturing is being catalyzed by a combination of industrial, federal, and academic efforts. The US federal government has funded several center-level efforts through the NSF and NIH, primarily focused on academic research into 3D bioprinted tissues and organs. To bring in broader industry participation, the DOD funded the Advanced Regenerative Manufacturing Institute (ARMI) as a public-private partnership, part of the National Network for Manufacturing Innovation (NNMI) initiative. Rather than applied research, ARMI is focused on the technology, manufacturing processes, regulatory framework and work force training required to make tissue engineered medical products (TEMPs) a commercial reality. Direct investment by established companies in the 3D printing industry has also grown with 3D Systems investing in Lung Biotechnology and Desktop Metal acquiring a bioprinting franchise through its acquisition of EnvisionTEC. Many startups have also emerged based on university spinouts such as Aspect Biosystems, Volumetrix, and FluidForm, as well as 3D bioprinter companies including CELLINK, which is the first bioprinting-focused company to exceed a $1B valuation. These investments in research and development, manufacturing, and clinical translation are strong indicators that 3D bioprinted tissues will soon be commercialized for a range of biopharma and medical applications.

Immunoengineering

The field of immune engineering, termed immunoengineering, is progressing at a fast pace, owing to rapid advances in several fields, including immunology, nanomedicine, biomaterial, and tissue engineering. The native immune system is the defense system of our body against pathogens, and it also regulates tissue repair and regeneration. Immunoengineering uses engineering principles and techniques to establish models for the immune system and develop therapeutic solutions for a variety of diseases, such as infection, cancer, diabetes, and inflammatory diseases.[33,116]

To fight against infectious diseases, scientists have immunoengineered new types of vaccines and constructed them with lipid nanoparticles carrying genetic material (such as mRNA and DNA). The recent development of mRNA vaccines for SARS-CoV-2 is based on intracellular delivery of mRNA encoding a harmless version of the spike proteins present on the surface of the virus. Such vaccines evoke and train the immune system against the virus.[41,72] Cancer vaccines are another exciting area of immunoengineering.[81,116] This type of therapeutics intends to train the immune system to activate and exert a systemic cellular immune response against cancer. Cellular engineering for adoptive T cell immunotherapy is a hot field and shows great promise in the clinic.[42,82] In particular, chimeric antigen receptor T cells can be created through gene editing approaches for treating blood cancers.

Inflammation involves the natural process of wound healing as well as the host tissue responses to implanted tissues and biomaterials. Therefore, modulating inflammatory responses in tissue repair and regeneration is of great interest. One area of research focuses on understanding the role of immune responses on the outcomes of regenerative medicine therapies. Macrophages play a central role in wound healing and host tissue responses. The effects of those cells and their different phenotypes have been evaluated on cells of interest, such as human bone marrow-derived mesenchymal stem cells and hPSC-derived cardiomyocytes. For example, cardiomyocytes co-cultured with macrophages activated with lipopolysaccharide (LPS) and interferon-gamma (IFNγ) or those with interleukin 4 and interleukin 13 had decreased expression of cardiac-related genes.[118] In particular, the expression of BMP2, BMP4, and GATA 4 was affected by the exposure to macrophages and inflammatory signals (i.e., LPS and IFNγ). This study highlights the potential impacts of modeling inflammatory responses on the efficacy of tissue-engineered products or regenerative strategies.

Careful choice of biomaterial properties can harness the power of innate immunity.[102] The responses of macrophages depend on scaffold material and structure (e.g., fiber size and scaffold porosity).[87] The increase of pore size seems to associate with an elevated expression of M2 phenotype or anti-inflammatory responses of these macrophages.[105] A soft substrate results in lower amounts of inflammatory cytokines, while a stiff cell-adhesive surface increases the foreign body response.[7] Modification of the surface topography has been shown to modulate the function of macrophages as well. The elongated cellular shape on the nano- to micro-scale patterns skews macrophages towards an M2 phenotype.[10,60,113] Therefore, engineering biomaterial properties such as fiber size, pore size, stiffness, and surface topography can serve as a simple means to modulate the immune response. Detailed mechanisms and design principles are, however, unclear and worthy of further investigation.

The biomaterial has also been used for the delivery of immunomodulatory molecules and cells.[18] As biomolecule carriers, biomaterial scaffolds can be designed to physically entrap biomolecules, to change their diffusion properties, and to alter the scaffold degradation profile to determine when the biomolecules are released. The cargo release dynamics can be tailored by using different chemical reactions that affect the cargo-carrier affinity. The release can be de-

signed to be triggered by internal or external factors, such as pH, temperature, and magnetic fields. Biomaterials-assisted cell delivery can provide the cells with an artificial microenvironment to support the survival and function of immune cells. The delivered cells are encapsulated locally, but the secreted therapeutic factors can diffuse out and reach the rest of the body. For example, islet encapsulation in Type 1 Diabetes can allow the insulin to diffuse out the biomaterial system while protecting the cells from the attack of the host immune systems.[17,19,103]

Efforts have also been focused on engineering lymphoid cells and organs, including bone marrow, thymus tissue, and lymph nodes.[49] For instance, recreating the bone marrow niche allows for the maintenance and expansion of the CD34+ cell population.[28,65] Recapitulating the interaction of stromal cells (genetically engineered to express DLL1 for Notch activation) and human hematopoietic stem cells (HSCs) enables the long-term maintenance of lymphoid progenitors and improves the efficiency of differentiation and positive selection of human T cells.[93] Activated B cells can be produced from engineered immune organoids mimicking the germinal center.[2,67,74,80] The development of these *in vitro* systems provides an opportunity for investigating the physiology and pathology of immune systems and for designing and developing novel immunotherapies.

PERSPECTIVES ON BUILDING BIOLOGICAL SYSTEMS

How to build biological systems and how to translate the engineered systems have been two central topics towards developing platforms and strategies for improving human health (Table 2). For the former, it often requires deep knowledge in human physiology and disease pathology. The engineering of a system has to capture the main characteristics of human organ or tissue physiology and closely mimic diseased processes at the molecular, cellular, and tissue levels. Due to the variety and complexities of biological systems, there are many different strategies that have been utilized by researchers. Below are a few major considerations in building a sound biological system, highlighted during the panel discussion.

There is no doubt that the understanding of biological and engineering principles may be critical in designing systems that recapitulate the essence of the physiology of biological tissues/organs and the pathology of associated diseases. This highlights the importance of basic research in engineering biological systems. The knowledge may include the genetic mutations relevant for specific diseases, epigenetic processes that regulate gene expression, biomechanical mechanisms that may involve cytoskeleton and morphological processes, and the crosstalk between various signals and different cell phenotypes. Successful engineering can be achieved when the essential aspects of human physiology and diseases are incorporated in the engineered systems through the appropriate inclusion of factors such as cells, biomaterial, biomechanical constraints/signals, and genetics. In the absence of any critical element, the engineered systems are deemed to fail from the beginning.

Interpretation of the results from an engineered biological system has to be careful. When we utilize the strengths of *in vitro* biological systems, such as the increased throughput that can accelerate research discovery, please note that the *in vitro* systems may miss certain characteristics *in vivo* and/or bring artificial features that do not exist *in vivo*. Therefore, when designing *in vitro* systems, the minimal essential requirements for *in vitro* systems need to be identified so that they can be useful for studying physiology and pathology. When implementing a specific design, be aware of possible drawbacks that may complicate experimental results and data interpretation. Often verification of an *in vitro* system is accomplished by comparing to *in vivo* situations (e.g., with an animal model) to examine its physiological and pathological relevance. This verification process is usually per-

TABLE 2. Challenges and suggestions for building and translating biological systems

Challenges	Suggestions
How to build a biological system relevant to physiology and diseases?	• Out-of-box thinking for maximal impact • Exploit what is known from basic research • Use a template from nature or an existing model • Keep it simple if possible • Verify it earlier with animal models
How to accelerate the translation of engineering biological systems?	• Familiar with governmental policies • Collaborate with clinicians • Develop engineering solutions that are marketable. • Manage time and efforts with different paths towards translation

formed prior to a large-scale study or screen using the *in vitro* system. Importantly, any substantial findings from *in vitro* systems have to be fully verified by animal models and subsequent clinical trials.

Out-of-the-box thinking or being innovative is necessary to make impactful or transformative contributions to the field. The progress of a field becomes stagnant when researchers only focus on trying different parameters under the same framework of thinking. A new idea, concept, or design may bring drastic changes that may be desired. These new thoughts and techniques can be paradigm-shifting and disruptive to the current research practice. When they are widely adopted, they become new standards. In this regard, the engineering of biological systems is, in fact, to bring such a change to existing practice in the field. Therefore, the incorporation of new thinking and new techniques may be the key to generate significant impacts.

The engineered systems don't have to be overly complicated. If the essential part of biology can be recapitulated, the engineered biosystem can be simple yet effective compared to native biosystems. There are some examples of well-established assays. A transwell assay can establish epithelial monolayers with well-connected junctions as seen *in vivo*, and a scratch assay simulates cell polarization and migration occurring in the wound-healing process. Recently, hPSC- based organoids, including the human embryo-like structures discussed above, utilize the self-assembly capabilities of differentiating stem cells and demonstrate great similarities in gene expression and tissue structures as native tissues/organs.[48] These organoids provide tremendous opportunities for us to optimize approaches for stem cell differentiation, to understand organ development, and to explore the genetic and environmental factors in defects and diseases.

Mimicking biology or physiology doesn't need to start from scratch. Using a template from nature is sometimes helpful. One example is the success of using decellularized material or other biomaterial derived from human or animal tissues. These materials preserve the components and structure of native tissues that promote proper cell-ECM interactions and normal cellular and tissue functions. Another example is using animal models (or their modifications) and their cells that may be sufficient to model human physiology or to create biomedical products that will treat human diseases.

Finally, ethical concerns on engineered biological systems shall be properly addressed before any *in vitro* experiments and translational studies can be conducted. In particular, when we deal with the culture of human pluripotent stem cells,[50,57] genetic editing,[16,36] synthetic biology approaches,[20,90] and development of embryo-like structures,[97] potential ethical and societal impacts have to be carefully evaluated by professional committees. For instance, while the development of embryo-like structures can reveal valuable insights into human embryonic development and birth defects, researchers shall know the existing guideline that may not allow for the culture of human embryo beyond 14 days[97] and ongoing debates whether such a rule shall apply to engineered embryo-like structures.[39,71]

PERSPECTIVES ON TRANSLATING ENGINEERED BIOLOGICAL SYSTEMS

For clinical translation, one has to understand government policies regarding possible products. For tissue/cell engineered products that will be used in human bodies, the Regenerative medicine advanced therapy (RMAT) designation by the FDA (Food and Drug Administration) may be highly relevant.[22] RMAT designation was created under the 21st Century Cures Act at the end of 2016 and provided ways for patients who have a serious or life-threatening disease or condition to receive innovative treatments. RMATs have a wide scope and may include human cell and tissue products, cell therapies, tissue-engineered products, and any combination products. Only around 37% of all applications (55 out of 149) for the RMAT designation have been approved as of October 2020. This highlights the importance for the researchers who work on the bench side to have those policies in mind so that the impact of their research can be maximized and that the products can reach their intended patients in a timely fashion.

During the process of clinical translation, communication between experts in different fields is the key. It may be ideal for engineering-trained researchers to work with clinicians at the very beginning of the projects. The importance perceived by engineers may not be what is really needed in clinical settings. By talking with clinicians, the engineers are ensured to work on real problems and gain insights into pathological conditions. In addition, the collaboration helps engineers create treatment solutions eventually translatable into clinical practice, therefore making impacts on the health of patients. Early collaboration between engineers and clinicians on a project driven by clinical needs may be the best path towards more fruitful outcomes of research translation.

It is important to note that developing medical products or any commercial products may be very different from doing lab research for scientific discoveries. For a commercial product that will be viable in the market, we have to consider the cost of the product and the possible ways to manufacture it in a large quantity. For instance, a human heart has 2-3 billion cells. To obtain cells in this order of magnitude may

require extensive cell culture and the use of growth factors to a level that one has to question financial feasibility. Techniques or ideas overcoming these challenges can be valuable. The marketability as an important criterion of the feasibility of an idea shall be in the mind of engineers on day 1.

For academic PIs, there are several considerations of choosing paths toward successful translation. For technologies developed in a bioengineering lab that may have many biomedical applications, it may be wise to choose the direct path that takes the least effort and time so that the technology can generate immediate impact. Also, depending on interests and availability, there are different levels of involvement: licensing, conducting academic clinical trials, and building your own company. Licensing requires much less time involvement but gives you less control of the outcomes of invented technology. Starting your own company gives you great control, but it may take a lot of time and energy, and most startups fail.

CONCLUSION

The field of engineering biological systems has grown drastically in the past few years, thanks to the rapid progress in several related fields, including gene editing, omics, biomanufacturing, and tissue and cell engineering (including stem cell biology, organoid engineering, and immune engineering). Engineers, biologists, and clinicians are joining forces to tackle the most challenging questions on building such biomimetic systems for drug discovery and clinical translation at several frontiers. The success of the efforts will rely on not only the close integration of biological insights and cutting-edge techniques but also the familiarization of governmental policies and the effective communication between scientists with different expertise as well as among all players in research and development. In the decades to come, we hope to see the tremendous scientific and social impact of this field of research.

APPENDIX: SESSIONS AND SPEAKERS AT THE 2020 BMES-CMBE CONFERENCE

BMES-Cellular and Molecular Bioengineering (CMBE) Conference

"Vision 2020: Emerging Technologies to Elucidate the Rule of Life"

Thursday January 2nd, 2020

14:00-20:00 **Registration**
19:00 **CMBE Council Meeting**

Friday January 3rd, 2020

7:00 **Continental Breakfast**
7:45 **Welcome/Introduction**

8:00-10:10 **Session 1: Molecular, Genetic/Epigenetic Engineering**
Chairs: Drs. Timothy Downing and Deborah Leckband
8:00 **Keynote:** Ron Weiss, PhD, Professor of Biological Engineering, MIT
Mammalian Synthetic Biology -- Foundations and Applications to Programmable Organoids
8:40 Li Qian, PhD, Associate Professor of Pathology and Laboratory Medicine, UNC
Reprogramming Approach for Heart Repair
9:00 Peter Yingxiao Wang, PhD, Professor of Bioengineering, UCSD
Engineering Controllable Immune Cells for Cancer Immunotherapy
9:20 Selected talk from the abstracts
9:40 Postdoc or student talk selected from the abstracts
9:55 **Poster viewing I, networking, with coffee break**

10:40-13:20 **Session 2: Rising Stars**
Chairs: Drs. Chelsey Simmons and Leo Q. Wan
10:40 Keyue Shen, PhD, University of Southern California
Understanding Hematopoietic Stem Cell -- Niche Interactions on Supported Lipid Bilayers
11:00 Timothy Downing, University of California, Irvine
Cell-generated forces contribute to bottleneck during somatic cell reprogramming
11:20 Madeleine Oudin, PhD, Tufts University
Engineering ECM gradients to study ECM-driven cancer metastasis
11:40 Matthew Fisher, PhD, North Carolina State University
New Approaches to Improve Translation of Fiber-based Scaffold Approaches
12:00 Xiaojun Lian, PhD, Pennsylvania State University
Wnt signaling controls mesoderm and endoderm bifurcations in a dose-dependent manner

12:20 Eun Ji Chung, PhD, University of Southern California
MMP-1 binding nanoparticles for inhibiting plaque rupture in atherosclerosis

12:40 Stephanie Seidlits, PhD, University of California, Los Angels
A biomaterials-based approach to investigate how regional tissue mechanics influence glioblastoma tumor invasion

13:00 Gregg Duncan, PhD, University of Maryland
Novel strategies to design synthetic mucus

13:30-15:00 **Lunch with leaders (Keynote speakers, Rising stars, Graduate awardees, and postdoctoral awardees)**

15:00-18:00 **Afternoon Break**

18:00 **Welcome Reception**

Saturday January 4th, 2020

7:15 **Continental Breakfast**

8:00-10:10 **Session 3: Cell Atlas**
Chairs: Drs. Dennis Discher and Kwonmoo Lee

8:00 **Keynote:** Kun Zhang, PhD, University of California, San Diego
Constructing single-cell maps of human organs

8:40 Nikolai Slavov, PhD, Northeastern University
High-throughput single-cell proteomics quantifies the emergence of macrophage heterogeneity

9:00 Eric Darling, PhD, Brown University
Proteomic Characterization of Cell Types, Subtypes, and Phenotypes

9:20 Selected talk from the abstracts

9:40 Selected talk from the abstracts

9:55 Postdoc or student talk selected from the abstracts

10:10 **Poster viewing II, networking, with coffee break**

10:40-12:50 **Session 4: Multicellular Emerging Behavior**
Chairs: Drs. Eun ji Chung and Shelly Peyton

10:40 **Keynote:** Ali H. Brivanlou, PhD, Robert And Harriet Heilbrunn Professor, Rockefeller University
Deconstructing the Human Brain: The Emergence of Neuruloids

11:20 Stephanie Fraley, PhD, Assistant Professor of Bioengineering, UCSD
Engineering Cancer Cell Migration

11:40 Lisa Manning, PhD, Associate Professor of Physics, Syracuse University
Predicting the material properties of cell collectives

12:00 Selected talk from the abstracts

12:20 Selected talk from the abstracts

12:35-18:00 **Afternoon Break**

16:00-18:00 **Workshop: Grant Writing**
Moderator: Leo Q. Wan
Presentation: Dr. Laurel Kuxhaus, Program director of BMMB, CMMI Division, NSF
Panelists: Drs. Timothy Downing, X. Edward Guo, Roger Kamm, Laurel Kuxhaus, Eunji Lee

18:00 **Gala Dinner Shu Chien Achievement Award (Awardee: Roger Kamm)**
Chris Jacobs Award for Excellence in Research and Leadership (Awardee: X Edward Guo)

Sunday January 5th, 2020

7:15 **Continental Breakfast**

8:00-10:10 **Session 5: Engineering Cell-ECM interactions**
Chairs: Drs. Yi Hong and Ngan Huang

8:00 **Keynote:** Valerie Weaver, PhD, Professor & Director, Center for Bioengineering and Tissue Regeneration & Co-Director Bay Area Center for Physical Sciences and Oncology, UCSF
The reciprocal interplay between ECM stiffness and tumor immunity

8:40 Adam Engler, PhD, UCSD
Improving cardiovascular "diseases-in-a-dish" with active materials

9:00 Jeffrey Ruberti, PhD, Northeastern University
Making Bones from Soup: Mechanochemical Force Structure Causality in the Matrix

9:20 Selected talk from the abstracts

9:40 Selected talk from the abstracts

9:55 **Poster viewing III, networking, with coffee break**

10:40-12:50 **Session 6: Engineering Cell/Tissue Models**
Chairs: Drs. Nadeen Chahine and Keyue Shen

10:40 **Keynote:** Nancy Allbritton, PhD, Kenan Distinguished Professor, Chair of UNC/NC State Joint Department of Biomedical Engineering
Large Intestine for Basic Physiology and Drug Assays

11:20	Danielle Benoit, PhD, Associate Professor of Biomedical Engineering, University of Rochester **Engineered Salivary Gland Tissue Chips**
11:40	Jianping Fu, PhD, University of Michigan, Ann Arbor **Synthetic Human Embryo-like Structure: A New Paradigm for Human Embryology**
12:00	Mara Domenech, PhD, Assistant Professor of Chemical Engineering, University of Puerto Rico **Engineering culture substrates for enhanced drug and cell potency assays**
12:20	Selected talk from the abstracts
12:45-13:30	**Panel Discussion: Biological Systems Engineering and Translation** **Moderator:** Dr. Adam Feinberg **Panelists:** Drs. Nancy Allbritton, David Mooney, Doris Taylor, Valerie Weaver, and Kun Zhang
13:30-18:00	**Afternoon Break**
15:00-16:00	**Educational Outreach: 3D Bioprinting & Biomanufacturing** **Instructors:** Drs. Adam Feinberg and Peter Yingxiao Wang
16:00-18:00	**Family Friendly Social Event**

Monday January 6th, 2020

7:15	**Continental Breakfast**
8:00-10:00	**Session 7: Engineering Immune System** Chairs: Drs. Michael Mitchell and Rebecca Pompano
8:00	**Keynote:** David Mooney, PhD, Robert P. Pinkas Family Professor of Bioengineering, Harvard University **Building immunity with biomaterials**
8:40	Ning Jenny Jiang, PhD, Associate Professor of Biomedical Engineering, UT Austin **Systems immunology enabled immune engineering**
9:00	Donald Freytes, PhD, Assistant Professor of Biomedical Engineering, UNC-CH & NCSU **Role of Inflammatory Cells During the Design of Tissue Engineered Constructs**
9:20	Wandaliz Torres-Garcia, PhD, University of Puerto Rico, Mayaguez **Multi-omics characterization of CAR-T cells through an integrative computational pipeline**
9:40	Selected talk from the abstracts
10:00	**Poster viewing IV, networking, with coffee break**
10:40-12:30	**Session 8: Engineered Tissues/Organs and the Path to Translation** Chairs: Drs. Hossein Tavana and Feng Zhao
10:40	**Keynote:** Doris Taylor, PhD, Director of Regenerative medicine Research, Texas Heart Institute **Engineering Tissues and Organs and the Path to Translation**
11:20	Warren Grayson, PhD, Associate Professor of Biomedical Engineering, Johns Hopkins University **Point-of-Care Cell-Based Strategies for Treating Large Craniofacial Bone Defects**
11:40	Lexi Garcia, PhD, ARMI-Advanced Regenerative Manufacturing Institute **ARMI:Driving the Future of Tissue Engineering to Achieve Scalable, Modular, Automated and Controlled Production of Tissue Engineered Medical Products (TEMPs)**
12:00	Selected talk from the abstracts
12:15	Selected talk from the abstracts
12:30-13:00	**Closing remarks, awards, and Survey**

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CONFLICT OF INTEREST

Guohao Dai, Adam W. Feinberg, and Leo Q. Wan declare that they have no conflicts of interest.

ETHICAL STANDARDS

No human studies or animal studies were carried out by the authors for this article.

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