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Cellular and Molecular Bioengineering: A Tipping Point

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Abstract—In January of 2011, the Biomedical Engineering Society (BMES) and the Society for Physical Regulation in Biology and Medicine (SPRBM) held its inaugural Cellular and Molecular Bioengineering (CMBE) conference. The CMBE conference assembled worldwide leaders in the field of CMBE and held a very successful Round Table discussion among leaders. One of the action items was to collectively construct a white paper regarding the future of CMBE. Thus, the goal of this report is to emphasize the impact of CMBE as an emerging field, identify critical gaps in research that may be answered by the expertise of CMBE, and provide perspectives on enabling CMBE to address challenges in improving human health. Our goal is to provide constructive guidelines in shaping the future of CMBE.

Keywords—Cellular and molecular bioengineering, Mechanobiology, Mechanotransduction, Molecular imaging, Tissue engineering.

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INTRODUCTION

The rise of engineering in the twentieth century led to profound changes for humankind. The advances in biology were equally amazing. As engineering and biology started to merge, the terms cellular engineering and tissue engineering emerged and became accepted, and technology furthered research at the molecular scale. The elucidation of the molecular basis of life became one of the great achievements of the twentieth century, culminating in the complete sequencing of the human genome. At the close of the twentieth century, therapeutic tissue-engineered products became a reality in the form of skin replacement products.

Today we stand in the midst of an amazing convergence, which has created a nexus for engineers, biologists, and clinicians. The Biomedical Engineering Society (BMES) realized the importance of research at this remarkable nexus: Cellular and Molecular Bioengineering (CMBE). In 2008, the BMES formed a new

society journal—CMBE—a first since its launching of the official society journal, Annals of Biomedical Engineering, 36 years ago.[12] The BMES and the Society for Physical Regulation in Biology and Medicine (SPRBM) also recognized the importance and great potential of CMBE and sought to cultivate the wealth of knowledge from researchers in the field. With the support of the National Institute of Biomedical Imaging and Bioengineering (NIBIB) and the National Institute of Musculoskeletal and Skin Diseases (NIAMS) of the National Institutes of Health (NIH), the National Science Foundation (NSF), and the United States National Committee on Biomechanics (USNCB), the inaugural joint BMES-SPRBM Conference on CBME was held in January 2011. The conference featured eight prominent keynote speakers and twenty-four distinguished invited speakers discussing their work in Molecular Imaging and Mechanotransduction. Together with presentations by rising stars and selected talks from students and fellows, the program demonstrated the strength and impact of CMBE research. All invited speakers were asked to contribute to a Round Table discussion entitled: The Future of CMBE. The goal of this Round Table discussion was to perform "Strengths, Weaknesses, Opportunities, and Threats (SWOT)" analysis of the CMBE field. Discussions and debates among attendees were intense and informative.[72] These discussions continued at the second combined BMES-SPRBM Conference on CMBE in January 2012, where over 30 keynote and invited speakers spoke alongside rising stars and students about their contributions to our understanding of Cell Motility, Matrix, Mechanobiology, and Regeneration (see this issue of CMBE journal).

The authors of this white paper on CMBE took on the challenging task of integrating the collective thoughts and opinions of the distinguished participants of these CMBE conferences. The goal of this paper is to emphasize the impact of CMBE as an emerging field, identify critical gaps in research that may be answered by the expertise of CMBE, and provide perspectives to enable CMBE to address challenges in improving human health. We hope that this paper will provide constructive guidelines in shaping the future of CMBE.

IMPACT OF CMBE

CMBE plays a central role in the analysis of human diseases. Disease often arises as a consequence of abnormal cellular and molecular processes, such as abnormalities in adhesion, migration, mechanics, cell division, or transport that are detectable with modern bioengineering tools. Analysis of cell and molecular phenomena with rigorous bioengineering principles and techniques is a powerful tool in understanding how molecular-level interactions give rise to cell, tissue and organ behaviors.

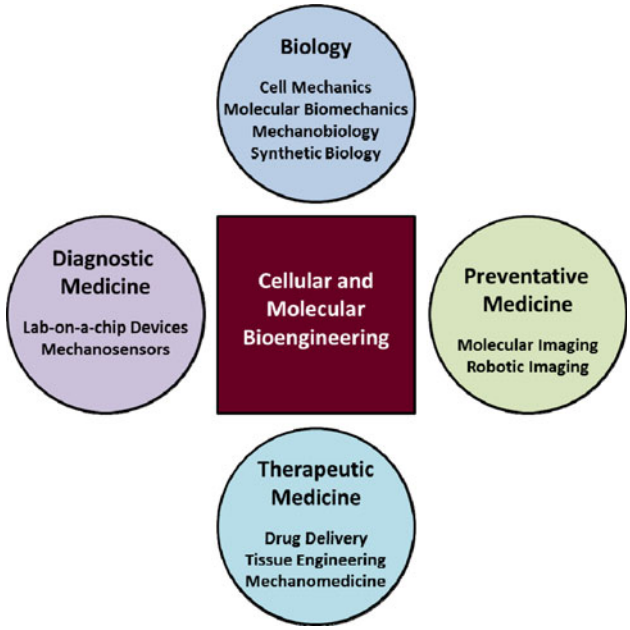

FIGURE 1. The field of CMBE has emerged at high time where many engineers in traditional engineering fields, physicists, chemists, and biologists join forces to attack the fundamental problems in biology, medicine, and public health and to solve urgent health-related problems.

Cell and molecular bioengineers are uniquely trained in the fundamentals of multiple disciplines; therefore, CMBE research is poised to play a major role in areas critical to human health (Fig. 1). CMBE probes new pathways and mechanisms in cell and molecular biology using novel bioengineering technologies. Furthermore, insights from CMBE studies drive technological advances that are pushing the boundaries of our knowledge of both basic and translational sciences.

Cellular and Molecular Biology

Biomechanics and Mechanobiology

A defining contribution of CMBE efforts over the past decade to the broader scientific community has been to establish that mechanical and other biophysical signals in their environment strongly influence cell and tissue biology. Considerable effort has been directed at understanding how living systems function as mechanical structures (biomechanics) and how they sense and respond to their mechanical environment (mechanobiology). Thus biomechanics promises to solve many vexing problems that remain in biology, particularly those spanning lengths scales ranging from molecules to cells, tissues, and organs.

Some of the most challenging and important problems in the field of applied mechanics are related to biology. The grand challenges are no longer to be found in designing aircraft and buildings—the historical nursery of mechanics—but in understanding how a cell functions as a mechanical structure or how it senses and responds to its mechanical environment. There exists considerable motivation to work at the interface of biology and mechanics, due to both the challenges and the enormity of potential rewards.

The significance of biomechanics and mechanobiology to organ and tissue physiology are well recognized, and efforts to identify the cellular and molecular underpinnings of this relationship are ongoing. There are numerous examples where understanding human health and disease requires understanding of biomechanics and mechanobiology at the cellular level.[22] A large number of disease processes are known to involve mechanics, yet the detailed relationships between mechanical force and pathophysiology remain poorly understood in all but a few cases. When bone cells do not experience proper mechanical stimulation, bone formation ceases and bone resorption is initiated.[76] In coronary artery disease, changes in the temporal and spatial patterns of fluid shear stress on endothelial cells are linked to the formation of atherosclerotic plaques.[83] The pathogenesis of osteoarthritis occurs due to changes in physical loading that lead to altered mechanical signals experienced by chondrocytes.[36] Lung alveolar epithelial cells and airway smooth muscle cells are known to be regulated by cyclic mechanical stretch during breathing,[5] and exposure to airborne pathogens leads to airway smooth muscle hypersensitivity and hypercontractility that can cause asthmatic attacks. Viruses can mechanically disrupt cell membranes to facilitate entry and delivery of genetic material.[48] Biomechanical breakdown of the intestinal mucosal layer leads to autodigestion.[73] Metastatic cancer cells exert force as they migrate through tissue and attach at distant sites to invade.[44] Mechanical signals regulate fibroblast behavior during wound healing[37] and also critically regulate the tissue-specific differentiation of adult and embryonic stem cells.[26,38] Brain development, hypertension, heart failure, and angiogenesis all centrally involve the ability of the cell to interact with its dynamic mechanical environment.

Indeed, the importance of biomechanics at cellular, molecular, tissue, and organismal levels may extend to almost every area of biology and medicine. Even the most fundamental of cellular processes—such as membrane trafficking, endocytosis and exocytosis, microtubule assembly/disassembly, actin polymerization/depolymerization, dynamics of cell–matrix and cell–cell adhesions, chromosome segregation, kinetochore dynamics, cytoplasmic protein/vesicle sorting and transport, cell motility, apoptosis, invasion, proliferation and differentiation—have all been found to be regulated by mechanical forces.[29,89]

Furthermore, mechanical loading is increasingly important in the success of tissue and organ regeneration, and mechanical integrity is now being investigated as a key functional outcome for engineered tissues.[31] In addition to recreating supportive biochemical conditions, providing engineered tissues with the appropriate biophysical signals can improve their efficacy.

Mechanics is a critically important discipline in understanding the structure and function of biological systems. A rapidly growing body of literature describes the biology of mechanics at length scales from molecules to cells and beyond, aiming to leverage this information to enhance our understanding of human disease and improve the outcomes of tissue regeneration. Research into the biomechanical regulation of physiology has demonstrated the success of CMBE as a research discipline and affirmed its potential to improve the health and well-being of humankind.

Mechanotransduction and Mechanochemistry

Considerable efforts are being made by the CMBE community to understand not only what mechanical signals occur within cells, but also how those biophysical signals are converted to biochemical events at the cellular and molecular scale. With innovations in molecular biology, computational modeling, cellular and molecular imaging, and live cell biosensors, mechanotransduction has moved to the forefront of CMBE such that mechanical signals can be quantified and characterized at the cellular, subcellular, and molecular levels, and biochemical transduction can be visualized and quantified in real-time. Therefore, mechanotransduction gained a distinct flavor from general mechanobiology—the latter being more related to biological functions and consequences of mechanical loading—now with detailed, real-time, and 3D characterization and quantification of how mechanical signals are transduced into quantitative biochemical responses in a cell. For instance, cytoskeletal deformations can be correlated to biochemical outputs, such as mechanosensitive protein kinase activation, in osteocytes and other cells.[2] Just about all cells respond to fluid shear stress in a cell-specific fashion, and it has come to light that traditional membrane receptors are part of the mechanosensing mechanism. Thus traditional receptors known for their ability to signal into the cell cytoplasm may have a dual function, responding to both the binding of chemical ligands and mechanical forces.[52]

Cell–Cell, Cell–Matrix, and Cell–Material Interactions

The role of mechanical modulation of cell functions has been an intensive focus in modern biology and bioengineering, and cell–matrix and cell–material interactions have been identified as key players in these processes. Careful studies using surfaces engineered to manipulate cell adhesion, spreading, migration, cell contractility, and adhesion strength have revealed that cell function is highly dependent on extracellular matrix (ECM) mechanical properties and the transmission of forces across the cell membrane by the transmembrane and intracellular protein network.[42] Though the importance of cell–cell communication is well established, CMBE research is now framing this communication within a network of new information and interactions. These cellular interactions—and how they are controlled at the molecular level—have been active topics in CMBE.[43,50]

It is now clear that the geometry, elasticity, and dimensionality of the ECM can control polarity, motility, fate, differentiation, and other cell-defining behaviors. Stem cells cultured on materials of varying stiffness commit to different lineages based on the properties of the target tissue.[26] ECM stiffness also plays a role in the pathogenesis of cancers and cardiovascular diseases.[41]

Understanding how different cell types interact with their environment and with each other is critical for the formation, repair and maintenance of a distinct boundary or interface between various tissues found in the body. Furthermore, uncovering how the interactions at these interfaces may be altered in disease and injury will reveal mechanisms that can be targeted for treatment and repair.

Membrane Biophysics

The role of lipid membranes in controlling biological processes is well accepted by biologists and biophysicists. Over the past few years, a picture of membranes as highly compartmentalized and highly dynamic has been emerging. This compartmentalization is critical to biological functions, as lipids segregate proteins into lipid phase-specific signaling complexes, thus turning them on and off, and as the dynamics of lipids and their resident proteins permits rapid adaptation to environmental factors.[80] Clinically, nutritional supplements, anesthesia, certain drugs, and much pathology can be traced to the action on lipids with resultant changes in activities of proteins and protein signaling. Furthermore, nanoliposomes remain the tool of choice for drug delivery applications.[77] Understanding the molecular underpinnings of biological membranes is an apt example of the ability of CMBE research to apply classical engineering disciplines to basic biological and clinical problems.

Translational Sciences

CMBE principles have also aided in the investigation of translational sciences such as studies of disease, tissue regeneration, biomaterials, and other clinical problems. An example is inflammation in shock and multi-organ failure, a clinical problem associated with high mortality. Cellular bioengineering analysis has helped to trace the trigger for inflammation in shock to the digestive enzymes in the intestine. Synthesized in the pancreas as part of normal digestion, they degrade almost all biological tissues and molecules in the lumen of the intestine.[9]

CMBE research also intersects with tissue engineering and regenerative medicine and has made contributions to classical problems in these fields. Traditionally, the greatest obstacle that has limited the field of tissue engineering is the ability to recreate an anatomical and physiological microcirculation that can sustain an engineered construct. Early attempts at tissue engineering were limited to creation of avascular tissue or 2-dimensional constructs[47,82]; however, recently, there have been tremendous advances in the field with use of decellularized 3-dimensional scaffolds, which have demonstrated very promising results.[61,62] CMBE studies have demonstrated that free flaps (explanted vascular beds) represent a microcosm of the circulatory system, which can be harnessed as the scaffold for tissue engineering purposes.[8] Free flaps can be sustained *ex vivo* in a bioreactor and seeded with stem cells or engineered to express proteins in a targeted fashion.[8,20,32] Furthermore, the potential uses of free flaps are not restricted only to restoring form and function, but can also be used as a vector for targeted delivery of gene therapy.[33] The efficacy of the approach has been previously demonstrated in treating infections and cancer,[20,32] but the concept can be applied to the delivery of a number of proteins. For instance, delivery of HIF-1α and SDF-1 can ameliorate the ischemic effects of radiation or diabetes,[7] and VEGF-C can promote lymphangiogenesis for the treatment of lymphedema.[17]

Considerable efforts are also being directed toward understanding critical size defects—defects which would not heal on their own, given appropriate time, and that would result in functional and/or aesthetic disability—and toward developing methods for improving reconstruction. CMBE design opens opportunities for development of new interventions and prevention. For instance, bony reconstruction of the facial skeleton for defects caused by congenital anomalies, trauma or resections for neoplasms remains a difficult surgical challenge.[77] The dictum of replacing like-with-like, although theoretically ideal, is never possible as humans are not equipped with true "spare

parts". Many strategies are being investigated to meet this challenge, such as complete de novo tissue blocks created by 3-dimensional bio-printers or bioreactors designed to create anatomically-shaped bone grafts.[35] Many different scaffolds are being investigated with unique characteristics affecting their strength, resorbability and surface tissue interaction compatibility. Scaffolds are seeded with bioactive substances, cells, time release nanomolecules, and other materials in an effort to optimize the constructs biological integration and compatibility.

This last example alludes to another critical area in which CMBE is making considerable impact—technological innovations that change the way patients are treated and basic science is studied.

Bioengineering Technologies

A major strength of CMBE is the ability to transform the fundamentals of engineering, physics and mathematics into bioengineering technologies (Fig. 2). These technologies—ranging from molecular imaging to micropatterning further our understanding of basic science and improve the outcomes of clinical research.

Molecular Imaging

The explosion of molecular probes such as quantum dots and green fluorescent proteins (GFP) and optical imaging modalities *in vitro* and *in vivo* enable new fundamental discoveries at the cellular and molecular level. Molecular imaging now allows researchers to watch chemical and mechanical events in real-time that were previously unobserved.

Since the development of GFP and its relatives with different colors spanning the entire visible spectrum,[63,81] multiple molecules can be fused with different color fluorescent proteins (FPs) to monitor their

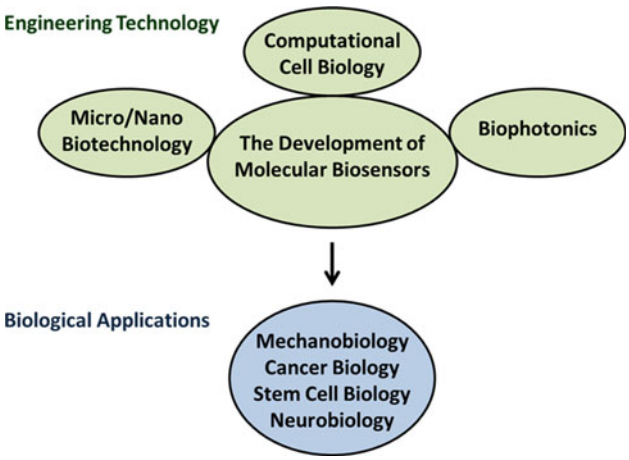

FIGURE 2. CMBE technology and tools for CMBE applications.

locomotion in a single live cell. Fluorescence Recovery After Photobleaching (FRAP) and Fluorescence Loss In Photobleaching (FLIP) with FP-fused target molecules can further allow the monitoring and quantification of the effective diffusion coefficient and motion kinetics of the target molecules.[16,19,60,68,71] The development of photoactivatable/photoconvertible FPs with fluorescence intensity (photoactivation) or color (photoconversion) tunable by light can also be fused to target molecules to track and measure their motion kinetics.[49] However, these strategies in general can reveal only passive properties of the molecules, such as their positions and motion parameters, without the capability of elucidating active molecular functions, such as enzymatic activity.

A popular approach for the detection of active molecular activities is based on fluorescence or Förster resonance energy transfer (FRET) technology. FRET occurs when two fluorophores are in proximity, with the emission spectrum of the donor overlapping the excitation spectrum of the acceptor. Any change of the distance and/or relative orientation between these two fluorophores can affect the efficiency of FRET and therefore, the ratio of acceptor-to-donor emission.[81] Because the two emissions can be obtained simultaneously and their ratio cancels out variations in the absolute concentration of the biosensors, the change in FRET biosensor ratio of acceptor to donor emissions are ratiometric and self-normalized to precisely monitor the molecular activities in live cells. Therefore, fusion proteins based on FRET and different FP pairs have been successfully developed to monitor various cellular events in live cells with high spatial and temporal resolution.[45,57,58,67,79,84,88,91,92] The most popular FRET pair at present is cyan fluorescence protein (CFP) as the donor and the yellow fluorescence protein (YFP) as the acceptor. To visualize multiple molecular events in a simultaneous fashion, biosensors with new FRET pairs, such as mOrange2 and mCherry, have been developed.[64]

Fluorescence Lifetime Imaging Microscopy (FLIM) is another technique capable of detecting molecular activities by obtaining lifetime information from every pixel of a fluorescence image.[15,74,85] Because FLIM is independent of the local concentrations of fluorescent molecules, this method can provide more reliable signals than those based on fluorescence intensity. In cases where FRET occurs, the lifetime of donors interacting with acceptors can change.[10,13,14] Hence, FLIM can separate the population of "FRETing" donors from those of non-interacting ones based on the lifetime distribution, thus enhancing the accuracy of FRET detection.[85] More importantly, because FLIM only monitors the donor lifetime to measure FRET signals without the need to measure the

acceptor lifetime, it can avoid the non-specific contamination of acceptor excitation/emission. Hence, FLIM is ideal for the simultaneous visualization of multiple FRET biosensors in live cells. As such, FLIM and FRET have been increasingly integrated for studies in CMBE. Indeed, FRET has been applied to visualize the initiation and transmission of mechanical force-induced Src activation.[59,86] The differentially distributed mechanical tension at subcellular regions was also successfully visualized by FRET-based biosensors utilizing the conformational changes of vinculin, spectrin, actinin, and filamin.[34,54–56] Furthermore, FLIM and FRET biosensors have been applied to visualize the subcellular activity of membrane-type 1 matrix metalloproteinase (MT1-MMP) for the assessment of the invasive potential of tumor cells.[24]

Recently, super-resolution imaging such as Spatially Modulated Illumination (SMI), wide-field Structured-Illumination (SI), Stimulated Emission Depletion (STED), Photo-Activated Localization Microscopy (PALM), and Stochastic Optical Reconstruction Microscopy (STORM) have been developed to provide sub-diffraction-limit resolution. Gene expression and transcription factor dynamics at the single-molecule level have also been achieved in live *E. coli*.[25,90] While these new technologies can provide exciting opportunities, the cost of advanced equipment and the high demand for expertise have hindered their broad application. It is expected, however, that these advanced technologies will be rapidly integrated with FRET and FLIM for the detection of molecular activities in live cells at super resolution and even single-molecule levels.

Biotechnology

A number of other technological advances are driving CMBE research and translation. For instance, micropatterned surfaces can be used to control cell adhesion and to study the molecular basis of cell–surface interactions.[11] This technology can also be applied more clinically to commit stem cells used in surgery to specific lineages.[38] With the advent of femtosecond lasers, nano-lasers, sophisticated micro-nano raman spectroscopy, nanowire-based evanescent wave sensors,[75] nano-mechanical resonator devices,[6,46] and the characterization of plasmon resonances of nanostructures,[66] CMBE is in a unique position to combine the known microstructural and molecular knowledge in cell biology and biophysics with these new technologies to build the next generation of devices that will help us learn the control switches of cells below the limit of our current understanding. In addition to making new research areas more accessible, advances in biotechnology also enable research to be performed more efficiently. For instance, lab-on-a-chip devices can process large amounts of data with ease and little time.[53] This knowledge can be translated into clinical applications for regenerative medicine and translational nanomedicine technologies.

CRITICAL GAPS IN CMBE RESEARCH

Due to the recent successes of modern biological tools in uncovering the genetic underpinnings of diseases, the future of biomedical research is increasingly focused at the cellular and molecular levels. The pace of this research is accelerating, and for CMBE to remain relevant in the decades to come, the field must sustain its impact and leverage its unique skillsets to address critical gaps in our understanding of biological systems in both health and disease at the molecular and cellular levels.

Cell and Molecular Mechanics

The field of biomechanics has made significant strides in the areas of organ and tissue mechanics. For example, we have a sophisticated understanding of tissue behavior under physiologic and pathologic conditions. The development of artificial joints, heart valves, stents, and many other highly successful medical devices owe their success largely to biomechanics. The nascent field of mechanobiology has focused on how mechanics affects and regulates biological systems. Relative to biochemical effects, the role of mechanics in biology is underappreciated.[27] It is clear that there exists a deficiency in the understanding of biology in terms of mechanical control of chemical behaviors, as evidenced by the relative dearth of research focusing on mechanics in mainstream biology. Although it is at the root of critical diseases like atherosclerosis, osteoarthritis, osteoporosis, and even cancer, a fundamental understanding of how cells sense and respond to mechanical signals remains unknown, except for a small number of specialized situations.

Similar to biomechanics, mechanobiology has largely been established at the organ and tissue levels. However, both disciplines have reached a critical impasse in their application at the cellular level. Due to the revolution of modern biology, the future of biomedical research is increasingly focused at the cell and molecular levels.[27] For biomechanics and mechanobiology to remain relevant in the decades to come, it is critically important that mechanics make the leap to the cell and molecular levels.

Fundamental questions about how the expression of specific genes is regulated inside the nucleus of a living

cell[18] are at the center of current and future cell biology, covering physiological processes from embryogenesis, development, and pattern formation to differentiation and many other biological processes. A most recent finding on the sensitive responses from a subnuclear organelle protein interactome to a local surface force of physiologic magnitudes highlights the vital roles of force and mechanics in subnuclear functions.[70] We envision that genomics biomechanics or mechanoepigenomics could play a distinct but vital role in this largely untapped area to elucidate mechanisms on how the "command center" of the cell is controlled.

Furthermore, as smaller and smaller scales are considered, biomechanical observations are eventually made in the regions surrounding individual cells. Physical signals occur in this pericellular region to which cells sense and respond. Pericellular mechanics, then, becomes critical in understanding what physical signals cells experience and how they are transduced.[40] Despite this, our understanding of pericellular mechanics is limited. Cellular and molecular mechanobiology not only raises fascinating scientific questions, but also ones with profound implications for human suffering and disease.

Coordination of Biophysical and Biochemical Signals—Cells as Engineers

CMBE is important to the understanding and engineering of biological systems across length and time scales, including the organ, tissue, cellular and molecular levels. The basic unit of life is the cell, with biomolecules as its building blocks. Besides providing structural support, biomolecules also work as nanomachines with moving parts, convert energy and materials from one from to another, and transport energy and materials in space and time. All of these processes can be treated by CMBE principles. The integrated and collective behaviors of these nanomachines give rise to cellular structures, properties, and functions such as cell–cell communication and signaling. Grand challenges in CMBE are to understand how cells function as engineering structures and sense and respond to their physiochemical environment in both healthy and diseased states.

Despite the tremendous advancements in our understanding during past decades on the role of mechanical/physical/chemical environmental cues in regulating cellular pathophysiology, it remains unclear how cells sense the spatiotemporal characteristics of stimuli, transduce and process such information, and coordinate the molecular hierarchy at subcellular levels to produce functional responses. In biology, it becomes increasingly clear that the framework of causal cascades starting with genes at the top and scenarios playing out according to solution chemistry is being replaced by a framework in which a web of causality with cell structure, physical forces, and epigenetic factors playing indispensable roles. We are poised to make significant contributions in this paradigm shift because CMBE analysis facilitates understanding of important cellular and molecular events, and CMBE design opens opportunities for development of new interventions and prevention.

Coupling biochemical with biophysical understandings is important for a number of diseases. For instance, increasing evidence suggests that most cardiovascular diseases, tumors and other ailments are associated with an inflammatory cascade.[28,30] Inflammation is accompanied by activation of cells in the circulation and fundamental changes in the mechanics of the microcirculation, expression of pro-inflammatory and down-regulation of anti-inflammatory genes and activities, activation and attachment of leukocytes to the endothelium, elevated permeability of the endothelium, thrombosis, mast cell degranulation, apoptosis, growth factor release, and many other events. An adequate understanding of this process is unattainable without studying its coordination.

By looking at big picture ideas such as the ability of cells to coordinate responses to multiple signals, CMBE can identify new important components to these complex biological cascades. There is recent evidence that some mechanotransduction processes occur due to modulation of lipid organization and dynamics.[65,69] Although there has been representation in bioengineering for membrane-based research, the scope of the research pales in comparison to fields such as biophysics and chemical engineering.

Another area for CMBE to lead is the study of the mechanical regulation of intracellular molecular interactions. The cell is a collection of protein machines,[1] and machines have moving parts that interact with each other and with those of other machines, such as the molecules in a signaling network. Currently, intracellular molecular interactions are mainly studied by biochemical means; studies of mechanical regulation of intracellular molecular interactions are limited to a small number of structural proteins, such as actin and microtubules. The CMBE community has developed expertise in studying the mechanical regulation of intercellular molecular interactions and thus is in an ideal position to lead the study in this new area. The tools of genetic engineering and synthetic biology are now being used to control these mechanoregulatory phenomena. For example, inducible/repressible expression of mechanotransductive genes was recently used to quantitatively "tune" cellular mechanobiological properties and downstream tissue-scale behaviors, including cell–cell adhesion and matrix compaction.[51]

This gap in particular highlights the ability of CMBE to drive technological advances. As the

questions brought forth by CMBE researchers push the boundaries of biological understanding, new technologies must be developed to answer those questions. For instance, the improved understanding of the lifetime and turnover of molecular and cellular systems will enable an improved understanding of the biological aging process, which is increasingly important in an aging society. The visualization of molecular locations and functions with high spatiotemporal resolution in live cells at subcellular levels should advance our fundamental understanding of molecular functions. Thus, biosensor-based imaging techniques must be designed or adapted to meet these criteria.

Molecular Technologies

FRET technology and genetically encoded biosensors have provided powerful tools for visualizing active molecular events with high spatiotemporal resolutions in live cells. Indeed, previous studies have shown that genetically encoded FRET biosensors with interacting peptide partners sandwiched between two different fluorescent proteins (FPs) are capable of monitoring various cellular events in live cells with high spatial and temporal resolution.[87] FRET biosensors with distinct colors will also be particularly important to allow the visualization of the signaling coordination at subcellular levels in a single live cell, as it becomes clear that molecular interactions and their biological functions in live cells are largely dependent on their subcellular location/environment.[3,78] Molecular engineering and high throughput screening technologies will be crucial for the development and optimization of these molecular biosensors. At the same time, technologies of biophotonics, such as laser tweezers and laser ablation, and micro-nanotechnologies, such as microfluidic channels and micropatterning technologies, can allow the development of tools to manipulate the mechanical/physical/chemical microenvironment and deliver stimulations for cells at subcellular levels.[39] The integration of these different technologies should advance our systematic understanding of how intracellular molecular networks are coordinated in space and time to respond to the cellular microenvironment and shed new lights on the underlying mechanisms governing disease development. Furthermore, the information obtained will provide a solid foundation for the development of new disease diagnostics and therapeutics.

Physiologically and Pathologically Relevant In Vitro Models

Discoveries from research in the field of CMBE have generated new questions that bioengineers can address, but designing studies to investigate these questions is often limited by the complexity of the biological environment or the simplicity of current *in vitro* tools, particularly in problems involving cellular-environmental interactions. How do cells transduce these chemo-mechanical cues from the material surface into intracellular signals that ultimately determine cell function? Why do various cell types respond uniquely to different surface characteristics? Much can be learned from the native tissue environment from which these cells are derived, but the complexity of these environments precludes identification of specific features that dominate the response. Furthermore, the fact that classical understandings of cell–material interactions are predicated on model systems, which are largely macroscale and often with little relevance to the physiological environment limits the application of current understandings to tissue engineering and the formation of complex tissues.

Cellular and molecular bioengineers have the capacity to build sophisticated instruments to make measurements at the cell–surface interface that will accelerate the pace of this research. With the advent of nanotechnology, high resolution micro/nano-fabrication and material characterization methods can be developed with high fidelity and biomimetic model systems of the ECM can be further refined to systematically investigate the individual and collective contributions of relevant ECM parameters.

ECM-Inspired Biomaterial Design

The field of regenerative medicine holds great promise for fabrication of tissues *ex vivo* and *in vivo*. There is still much to be learned about how cells contribute to the assembly of living tissues with physiological function. During tissue reassembly following a wound, cells sense physical and biochemical cues and respond by dynamically changing the neighboring ECM from a wound healing state to a remodeling state, which later reaches homeostasis. An understanding of this complex process will contribute to therapies that promote normal healing and drive the development of new biomaterials, which could accelerate healing or generate tissues *ex vivo* for implantation. Biochemical parameters such as growth factors and other growth-regulating cytokines are important. Mechanical cues such as rigidity and physical cues such as topology and adhesive patterning at the micro- to nano-scales are also significant controllable parameters.[23] Since these features are all found in the cells' native tissue environment, they should motivate the rational design of future biomaterial surfaces.

Bioengineers have developed creative approaches to quantify changes in biomaterial characteristics that affect cell behavior, but new techniques with

nano-scale resolution need to be developed to study and design biomaterials at the cell-biomaterial nano-interface. The physiological environment must be replicated by biomaterials consisting of 3D scaffolds/surfaces composed of fibrillar matrices with optimized anisotropy, mechanical properties, topological features, and biochemical ligand distributions to regulate cell morphology and function in a controlled manner. These materials must be engineered at macro- to nano-scales in order to present these features to subcellular structures at the cell–material interface.[39] It is expected that the nano-scale characteristics of the material surface will profoundly affect the nature of focal adhesion protein interactions at the interface to regulate intracellular signaling and gene expression.

Research into the mechanism of cell–cell and cell–matrix interactions has significant impact from two perspectives. In addition to augmenting and, in many cases, revolutionizing our understanding of biological systems, these efforts will also lead to the formulation of critical design criteria (cell-related and biomaterial/scaffold-related) that will enable the formation of functional and integrated complex tissue systems for regenerative medicine.

Theoretical and Computational Models

Complex proteins, such as motor proteins, are often referred to as ''biological machines,'' with an ability to generate force and perform autonomously complex mechanical and chemical operations. However, our understanding of their design is still rooted in biophysical perspectives with an emphasis on forces, springs, and oscillators. In contrast, machine design in engineering is critically concerned with reliability, lifetime, wear and fatigue. It is hypothesized that these concerns also apply to biological machines and critically shape their design and utilization within larger systems. Similarly, our understanding of the cell as a ''nanoscale factory'' does not yet leverage the decades of engineering expertise accumulated in logistics and operations research. It is hypothesized that a full understanding of cellular complexity requires advanced modeling of mass and energy flows.

As a field, CMBE must also stress the quantitative and modeling aspects that engineers are uniquely trained to deliver. Increases in the amount of quantitative information provided by new instrumentation will necessitate theoretical models to interpret data from various sources under a general framework, allowing more efficient identification of relationships between biochemical and biomechanical signals at cellular and molecular levels. Model predictions can also drive the design of experiments, which further elucidate the molecular influence over cellular behaviors.

The limited capability and efficiency of human power in analyzing imaging data has also hindered our ability to obtain comprehensive biological information. There is an emergent need to develop automated and high-throughput computational tools for analyzing live-cell imaging data and obtaining biologically significant information.[4] Therefore, it is crucial to tightly integrate cutting-edge live-cell molecular imaging with the development of micro-nanotechnology, biophotonics, and computational tools for the investigation of molecular hierarchy at subcellular levels.[2]

The next 10 years of development in CMBE will witness many important advances driven by the power of engineering computation and modeling. The massive data sets produced by high-throughput genomics will be used to construct quantitative models of biological cells and tissues based on quantitative molecular reactions. This will have an enormous impact on the predictability of drug development efforts, which currently take 10–14 years, thus increasing the ability to develop multiple drug therapies and to enable new strategies of individualized medicine. It will be critically important to continue to train students and investigators within realms of both biology and mathematics to realize the potential of biological computation.

PERSPECTIVES ON CMBE

In the decades to come, it is envisioned that biological research will strike a balance in understanding between chemistry and mechanics and bridge the gap between biology and engineering. This will be critical to overcome some of the most difficult and expensive—both in terms of money and human suffering—diseases we currently face. A concerted effort is required to achieve this balance, and CMBE is well positioned to take on this role.

Investigators in CMBE bring training from basic science, physical science, engineering, and clinical disciplines. With great advances in mapping the human genome, understanding protein–protein interactions, molecular imaging, and new cell and tissue culture technologies, it is increasingly evident that mechanical and chemical factors at different levels (molecular, subcellular, cellular, tissue, organ, and organismic) play unique and vital roles in the structure–function of life. However, due to a lack of coherent efforts in the past, researchers in this emerging field scatter around different areas, and no attractive locus exists that can amplify the great efforts by various scientists and drive research further into the field. Furthermore, scientific opinions expressed at conferences, at study sections, and in journal reviews tend to self-segregate along lines

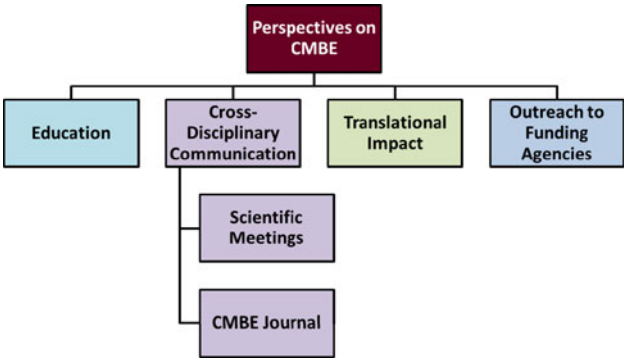

FIGURE 3. CMBE can better serve its investigators and the general scientific community through several parallel efforts.

based on traditional disciplines or training backgrounds. This lack of concerted efforts hampers potential major advances in biology and medicine. For CMBE to sustain its impact, it must actively address critical challenges that face the field.

CMBE can better serve its investigators and the general scientific community through several parallel efforts (Fig. 3). The current knowledge of CMBE must be incorporated into mainstream biological thinking and teaching. CMBE should promote collaboration and the exchange of ideas across disciplines at conferences and in journals. An emphasis should be placed on translating research advances in CMBE to improved clinical results. And finally, CMBE must attempt to establish stronger relationships with funding agencies to keep its research at the forefront of biological discovery.

Education

A major goal of CMBE researchers should be to educate scientists, clinicians, and the business community about quantitative approaches and engineering vocabulary in cellular and molecular biology and medicine. For example, CMBE review articles and conferences should include primers on the mathematical language and data analysis methods that will guide the field. The field of biology typically focuses on the roles of biochemical stimuli (e.g., cytokines) and intracellular signaling, and considers matrix structure mainly from a pathology standpoint. The role of mechanical and physical stimuli is largely ignored by biologists due to the lack of training in biomechanics and bioengineering. Efforts should be made to make engineering concepts more amenable to students and researchers less comfortable with quantitative analysis.[21]

Biomechanics and mechanobiology are also absent from most cell biology textbooks. Educational materials highlighting the importance of mechanics in biology must be developed and disseminated to cell biologists, so that this material gets into textbooks, both at the high school and undergraduate levels. A highly effective way to address this would be to make an effort to incorporate some engineering and mechanics into the biology "Bible" books. This would not only improve exposure of students to these fields, but would also provide biology faculty with tools to integrate biology and mechanics. The teaching of mechanics would have to be adapted to accommodate the variable mathematics backgrounds of biology students, but many powerful mechanics concepts are accessible without formal training in calculus (e.g., stress, strain, Young's modulus, stretch, shear, etc.). Curricular advances are also needed in teaching mechanics to biomedical engineering students. It is no longer acceptable to have our students exposed to mechanics in another department focused on mechanics of traditional engineering structures. CMBE can take a leadership position in developing teaching materials to broadly cover mechanics—solid, fluid, computational, experimental, and statistical—using the cell as a mechanical paradigm.

Cross-Disciplinary Communication

The research of a number of clinicians, disease-oriented scientists, biophysicists and biochemists naturally interfaces with that of CMBE. As more researchers from the clinical and basic science community join the translational research effort, the CMBE community could foster communication across disciplines by including multiple vocabularies in authored articles, conference speaker lists, and invited reviewers for manuscripts and proposals. By promoting the intersection of CMBE with other disciplines at scientific meetings and collaborative research in CMBE and related journals, this approach could open the communication of pioneering designs of devices, molecular reagents, imaging tools, and cell engineering approaches.

Scientific Meetings

CMBE efforts are frequently lost in the "noise" of large life sciences meetings, such as ASCB, BPS, and FASEB, but more specialized society meetings, such as SPRBM, currently lack the broad attendance base needed to cross-fertilize the CMBE, biophysics, and cell and molecular biology communities. Thus, a mechanism for leveraging the strengths of each scientific meeting format is required.

Members of the CMBE community have sought to achieve this by merging SPRBM into BMES as a special interest group (SIG) on CMBE. The current style of the annual meeting (Gordon research

conference-style) would be maintained under this merger, and the CMBE community would have an enhanced ability to reach out to broader disciplines with the support of a larger society. This CMBE division within BMES will provide a leadership consortium for CMBE research, education, and outreach, and a liaison with other biomedical engineers within BMES, scientific communities outside BMES, industries, government, and the general public. At the time, this whitepaper appears, we are pleased to announce that the first SIG, CMBE, has been officially formed in the BMES. We welcome all CMBE authors and researchers to join this exciting community of CMBE.

The first two CMBE meetings were extremely successful in strengthening the CMBE community by inviting leaders across fields and providing a forum for discussion and exchange of ideas, and these meetings should certainly continue. While early meetings may feature cell biology and bioengineering investigators with pre-existing CBME efforts, future meetings could be expanded to target specific subsets of basic biologists, clinicians, disease-oriented scientists, biophysicists and biochemists, particularly those outside areas of existing synergy, such as the cardiovascular and orthopaedics fields. Furthermore, CMBE meetings could serve as a forum for scientists in subfields for which BMES has been an inadequate meeting place, such as biophysicists interested in the role of membranes in cell biology and clinical medicine.

Exposure of the CMBE within the general cell biological community can also be enhanced by inviting program directors from the NIH and NSF, as well as editors of broad-impact scientific journals such as Nature, Science, and Cell. High-throughput approaches to CMBE could be advanced by inviting representatives from the pharmaceutical and biotechnology sectors, where much of this technology is developed. In addition, tracks or sessions should be organized at cell biology venues such as ASCB, Experimental Biology, and Keystone Conferences. Joint meetings with other scientific groups and organizations, such as those at the Janelia Farms campus of the Howard Hughes Medical Institute who have instruments to study cellular processes at sub-molecular scales, should also be organized.

CMBE Journal

Outcomes of improving communication across communities are difficult to describe exactly, but metrics may be developed using the journal CMBE. Overall, outcomes should be designed to evaluate the true interdisciplinary nature of the CMBE community. For example, published articles should represent a balanced mix of authors from clinical, basic science, and design engineering laboratories.

To increase the visibility of CMBE studies, in addition to making efforts to publish CMBE studies in major biological journals and journals of general readership, publications in the CMBE journal should be made more accessible to general biomedical scientists. Placing a thematic emphasis and editorial features in each issue can highlight research in exciting areas of CMBE. Visibility may also be enhanced by encouraging discussions of published studies. For example, articles in CMBE could include follow-up discussions as invited editorials, or the CMBE journal could host online discussions of hot topic articles that are marketed to the clinical, business, and basic science communities as well as biomedical engineers.

Finally, the CMBE journal can better serve its membership with special issues on select topics such as cell–material interactions and mechanobiology of membranes and invite specialists in these areas to write articles, maintaining an emphasis on applications to biology and medicine.

Translational Impact

While CMBE ideas are slowly beginning to percolate into the mainstream cell biology community, one senses that this body of work is still not on the radar screen of many clinicians and disease/translation-oriented basic biological scientists. This is quite ironic, particularly for mechanobiology, as a physician's clinical evaluation and a surgeon's demarcation of tissue boundaries are based in no small part on manual palpation of tissue. This disconnect impairs progress in the field, because we need these talented scientists and clinicians to work with us to understand the importance of mechanotransduction and other CBME principles in human disease and to translate our findings to eventual clinical and biotechnological use. A stronger interface of the CMBE field with clinical and the disease/translation-oriented research would accelerate the clinical and technological translation of findings in this field and would spin-off methods that could prove valuable for fundamental CMBE studies. Thus, the translational impact of CMBE is contingent upon improving dialogue and collaboration among these fields.

The interface between CMBE and the disease/translational sciences could be improved through several parallel efforts. First, the CMBE SIG of BMES should make an effort to invite clinicians and disease-oriented scientists who might naturally interface with CMBE to the annual meeting. It would be particularly valuable to expand this list beyond professionals in the cardiovascular and orthopedic fields who already have a strong investment and interest in cell and tissue biomechanics. Oncology and neurosurgery are areas of high potential.

Second, the field needs to put significant effort into developing high-throughput, user-friendly mechano-biological platforms (e.g., 96-well plate format) to make CMBE studies more accessible to disease/translational scientists and provide a viable route toward drug screening. These technologies would also accelerate our fundamental understanding of mechano-transduction because they would open this field up to genetic and pharmacological high-throughput screening methodologies that are often a first step toward elucidating molecular mechanisms. A way to start this could be to invite pharmaceutical and biotechnology company representatives to the CMBE meeting with the goal of eliciting seed funding for academic investigators to develop such assays.

Improved awareness of CMBE findings in the clinical/translational arena could improve the prospect of developing a long-term funding foothold at the NIH and NSF, stimulating interest from the private sector, and publishing in general interest journals. All of these factors are keys to ensuring the long-term success of the field.

Outreach to Funding Agencies

In the current climate where federal support of biomedical research is uncertain, the CMBE community needs to improve its outreach efforts to the NIH and other funding agencies and educate the general public regarding the relevance of CMBE to biology and medicine. We should work with NIH and NSF program directors who are enthusiastic about CMBE research and bring them together with leaders in the field to discuss potential funding opportunities, such as cross-institutional efforts, roadmap-type programs from the director's office, and center grant or program project grant-type initiatives similar to a recent NCI Physical Sciences-Oncology initiative. "Proteins as machines and cells as factories" could be the topic of an Emerging Frontiers in Research and Innovation (EFRI) solicitation by the NSF, which would jump-start the interdisciplinary collaborations critical for paradigm changes. Inviting funding officials to CMBE functions will be an important mechanism to help ensure this outcome.

We have reached a critical mass and the science is progressing rapidly. Now the key is to convince funding agencies, especially NIH, that CMBE represents an exciting, sustainable, and unique field that can significantly impact biology, medicine, and public health.

CONCLUSION

The field of Cellular and Molecular Engineering has emerged at a time where many engineers in traditional engineering fields, physicists, chemists, biologists and clinicians are joining forces to attack the fundamental problems in biology, medicine, and public health and to solve urgent health-related problems. In the decades to come, we hope to build upon the technical and biological foundation of CMBE to achieve a more thorough biological understanding from the molecular level to that of the cell. In order to achieve this balance, CMBE must expand across disciplines and encourage changes in public policy, research investment, and education. CMBE is well positioned to lead the efforts to realize these changes.

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