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From Molecular Cell Engineering to Biologically Inspired Engineering

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Abstract—The field of Molecular Cell Engineering melds techniques from molecular cell biology, engineering and the physical sciences to quantitatively define mechanisms that govern the shape and function of living cells. This discipline offers a new and powerful approach to confront fundamental questions in the life sciences, such as how cells self organize through collective interactions among thousands of individ ual molecular components, and function physically as part of larger tissues and organs in our bodies. This approach has led to deeper understanding of the fundamental design principles that govern the mechanical behavior of living cells, and greater insight into mechanotransduction how cells sense physical forces and convert them into changes in biochem istry. This article briefly describes the history and current status of this field in context of the larger discipline of Cellular and Molecular Bioengineering, and discusses how new advances in this area can be leveraged to develop new 'biologically inspired' engineering approaches for cell and developmental control, as well as non medical applications, in the future.

Keywords—Mechanotransduction, Cell mechanics, Tenseg rity, Cell engineering, Biomimetics, Integrin, Cytoskeleton, Extracellular matrix, Tension.

"*If you drain the Pacific Ocean, don't be surprised to find that the islands are connected.*"—The late Judah Folkman (to whom this article is dedicated)

INTRODUCTION

In 1993, a Minireview published in the journal *Cell* described how molecular cell biologists, biophysicists, and engineers were beginning to join together and combine their knowledge and tools under the banner of a new discipline, called Molecular Cell Engineering, to attack the problem of how cells and tissues form from individual molecular components and function in the physical context of whole living organisms.[32] The convergence of these fields was driven, in large part, by recognition that the fundamental question of how structure governs function in biology cannot be solved by focusing exclusively on genes and biochemical mechanisms of cellular control because this problem is not based entirely on changes in chemical composition or local binding interactions; it also depends on mechanics, three-dimensional (3D) architecture, and system-level integration. Enormous advances have been made in this field over the past 15 years, which in combination with advances in biomechanics, biorheology, molecular biophysics, cell biology, and bioengineering, has contributed to the establishment of the larger discipline of Cellular and Molecular Bioengineering that inspired the creation of this new journal of the same name. In this article, I briefly review the early challenges that led to the emergence of Molecular Cell Engineering, and describe how pursuit of this interdisciplinary approach has resulted in deeper understanding of multiple cellular structures and processes. I also describe how insights into the principles that nature uses to construct and control living cells is inspiring bioengineers to create new 'biomimetic' materials, devices, and control technologies that may significantly impact medicine and industry in the future.

FROM MOLECULAR COMPONENTS TO BIOLOGICAL SYSTEMS ENGINEERING

The power and importance of the Molecular Biology revolution, which focused on the importance of genes and the polynucleotides and protein factors they encode, cannot be ignored. However, the same gene or chemical can produce completely different and sometimes antagonistic effects (e.g., growth or differentiation or death) depending on the cellular microenvironment in which they act.[9,38,59] The pioneering work of Judah Folkman suggesting that the physical shape of a cell—whether it stretches or retracts—controls cell

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growth[19] helped bring attention to the importance of physicality for cell regulation to mainstream biologists, and engineers as well. It is now clear that while individual genes and soluble growth factors can initiate tissue development, physical forces acting through the insoluble extracellular matrix (ECM) often govern tissue formation,[36] and many developmental abnormalities, diseases, and clinical problems result from changes in ECM structure or tissue mechanics.[35] Intracellular macromolecular scaffolds, such as the cytoskeleton and nuclear matrix, are also critical for cellular control because they orient much of the cell's metabolic machinery: many signaling molecules, enzymes and their respective ligands that mediate DNA synthesis, transcription, RNA processing, protein synthesis, glycolysis, and signal transduction function when physically immobilized on these insoluble scaffolds.[32,41,55] Thus, Molecular Cell Biology is severely restricted in terms of its ability to explain cell and tissue control because it only focuses on the role of one gene or molecule at a time, and it fails to incorporate the key contributions of mechanical forces and higher order structures to biological regulation.

Recognition of the importance of explaining how complex behaviors emerge from collective interactions among multiple components led to the emergence of the field of Systems Biology. But that discipline focuses primarily on information transfer and statistical relationships between gene and protein expression profiles and biological behavior. To fully understand biological control, we need to elucidate how nature has systematically assembled and matched component parts to carry out their biochemical and mechanical functions in the physical context of whole living cells and tissues. Thus, the challenge that Molecular Cell Engineering has confronted is a systems engineering challenge: to develop quantitative descriptions, theoretical predictive models and mechanistic explanations of how whole living cells form and function through self assembly and collective interactions among innumerable molecular components.

CELL MECHANICS AND MECHANOTRANSDUCTION

Although genes and chemicals dominated the biological literature over the past 50 years, physical forces have been known to play an equally important role in control of tissue and organ development for more than a century.[67] Adult tissues continually remodel themselves when mechanically stressed, whether it be compression in bone, tension in muscle, or fluid shear in blood vessels. Thus, to fully explain cell and tissue regulation, we must understand how cells sense and respond to these mechanical cues, so that they optimally integrate structure and biochemistry.

Cell mechanics and mechanotransduction—the process by which cells convert mechanical cues into changes in cellular biochemistry—are therefore two major research areas in the field of Molecular Cell Engineering. Initially, biologists viewed living cells as small bits of protoplasm surrounded by an elastic membrane, and thus engineers approached the problem of cell mechanics by modeling the cell as a mechanical continuum, such as a viscous or viscoelastic solid surrounded by an elastic cortex.[16,17] These models can provide useful descriptions of whole cell mechanical behavior, but they do not provide a way to link mechanics to specific molecular load-bearing elements inside the cell. This idea of molecular correspondence is critical because development of future means of therapeutic intervention in various diseases requires that we identify specific molecular targets responsible for regulation of the structural properties of living cells and tissues.

A molecular scale explanation of cell mechanics is also important because the cytoplasm of mammalian cells is not a viscous fluid; it contains a discrete, interconnected, filamentous framework composed of nanoscale molecular biopolymers (microfilaments, microtubules, and intermediate filaments), known as the cytoskeleton.[31] Many scientists assume that cells alter their mechanical properties exclusively via sol–gel transitions because cytoskeletal filaments can chemically depolymerize and repolymerize. Engineering models of the cell based on percolation theory can relate cell physical properties to these types of phase transitions.[20] However, cells can change shape from round to fully spread without significantly altering the total amount of cytoskeletal polymer in the cell, and individual actin stress fibers (microfilament bundles), intermediate filaments, and microtubules generally remain structurally intact for extended periods of time (minutes to hours), even though individual molecular components continually bind and unbind.[3,42,51] Recognition of the importance of cytoskeletal structure for cell shape control led to development of open-foam models of the cell in which stresses necessary to resist shape distortion arise in the cytoskeleton due to deformation (e.g., stretching, bending and torsion) of individual cytoskeletal filaments under the action of externally applied loads.[6,57]

But living cells are not passive materials; they are actively prestressed structures. Prestress refers to the pre-existing tensile stress that exists in the cytoskeleton before application of an external load. Cytoskeletal prestress results from the action of tensional forces generated in contractile microfilaments composed of actomyosin filaments, and resisted by adhesive tethers

to ECM and neighboring cells, and from the ability other cytoskeletal filaments (e.g., microtubules) to resist resultant inward-directed compressive forces inside the cell.[4,42] The cytoskeleton also may experience passive prestress generated by application of external loads, such as ECM distortion or osmotic swelling of the surface membrane and underlying cortical cytoskeleton, which physically link to the deeper (microfilament-microtubule-intermediate filament) cytoskeleton,[5] and from there, to nuclear matrix scaffolds inside the nucleus.[25,46,72]

These and other observations led to the development of an engineering model of the cell in which the cytoskeleton is organized as a 'tensegrity' structure.[31,33,39,40,54,55] A tensegrity is a stress-supported mechanical network that maintains its structural stability through the agency of tensile prestress. In a tensegrity, tension is transmitted over the discrete network that comprises the structure, and these forces are balanced by a subset of structural elements that resist being compressed, thereby establishing a mechanical equilibrium.

Importantly, tensegrity models predict that changes in cytoskeletal prestress will alter cell static mechanical properties, as well as dynamic cell rheological behaviors, and these predictions of cell elastic and frictional moduli have been confirmed in various types of living mammalian cells.[4,6,7,13,42,60 66,68,69,71,72,74] Prestress is also critical for synthetic gels composed of natural cytoskeletal polymers and molecules to exhibit mechanical properties that are also displayed by living cells.[22] In tensegrities, changes in this internal force balance alter cell deformability by promoting rearrangements of components located throughout the structure, and by altering the dynamic mechanical behavior of individual elements. Tensegrities also may be organized as multimodular hierarchical structures such that destabilization in the force balance results in shifts in force between discrete internal and external load-bearing elements in different structural modules.[33,37,66] Experiments with living cells have similarly confirmed that physical disruption of a single actin stress fiber results in rearrangements of the remaining elements of the actin cytoskeleton, as well as force transfer to external ECM adhesions.[42] In contrast, disruption of ECM adhesions or enhanced contractility results in increased compression within microtubules.[4,26,42,72,73] Tensegrity also effectively describes the organization and mechanical behavior of subcellular components (e.g., submembranous cytoskeleton, mitotic spindle, actin microfilaments, lipid micelles, viruses, etc.) as well as well as larger multicellular tissue and organ structures.[33,37]

One of the most important outcomes of studies with the cellular tensegrity model is that it suggests that the physics that controls cell rheology occurs at a high level of structural organization (i.e., at the whole cell level), rather than being governed by any individual molecule or structural component. Work showing that the dynamic mechanical behavior of living cells scales with a weak power law similar to that exhibited by soft glassy materials over a relatively wide frequency range[18] is consistent with this idea that mechanics is governed at the whole system level. But the most important feature of the tensegrity model is that it provides a mechanism to link these integrative system-level properties to changes in forces transferred between distinct load-bearing elements (e.g., microfilaments, microtubules, cell–ECM adhesions, nuclei) at the molecular level,[33] which is not possible using continuum models of cell mechanics or soft glass theory. Moreover, even the dynamic power law-like mechanical behavior of living cells is governed by prestress in the cytoskeleton.[65] Thus, at present, tensegrity appears to be the most generalizable model of cell mechanics, in addition to being useful to describe the mechanical behavior of living materials at multiple other size scales, from individual molecules to whole organisms.[37]

CELLULAR MECHANOTRANSDUCTION

While cell mechanics focuses on how cells generate internal stresses necessary to stabilize cell shape when mechanically stressed, cellular mechanotransduction describes how cells respond biochemically to these physical cues. To better understand this process, it is helpful to define the path by which mechanical stresses are transmitted across the cell surface and to the load-bearing cytoskeleton. As in any 3D structure, mechanical loads will be transmitted across structural elements that are physically interconnected. Thus, forces that are applied to the entire organism (e.g., due to gravity or movement) or to individual organs or tissues will be transferred to individual cells via their adhesions to ECM that link cells and tissues throughout the body. Other forces, such as those to fluid flow in blood vessels, air flow in lung, and fluid pressure in the bladder are exerted on the apical pole of cells; however, because the cell responds mechanically as a tensionally integrated structural network, the ability of the cell's basal adhesions to result shape distortion in response to these stresses also can contribute to the cellular mechanotransduction response.[2,14,25,33] Some cells have specialized mechanosensory structures, such as apical primary cilia that stimulate transmembrane ion flux when they are deformed by flow or other mechanical stimuli.[52] However, even the sensitivity of these apical mechanosensors are governed by the overall mechanical state of the prestressed cytoskeleton and basal ECM adhesions,[2] which also controls the permeability of apical cell–cell junctions.[45]

Integrins are ubiquitous transmembrane cell surface adhesion receptors that mediate cell anchorage to ECM.[1] Integrins also mechanically link to the internal actin cytoskeleton by promoting assembly of an anchoring scaffold or 'focal adhesion' that contains various actin-associated proteins (e.g., talin, vinculin, paxillin, α-actinin, zyxin) as well as many biochemical signaling molecules (e.g., tyrosine kinases, inositol lipid kinases, ion channels, small and large G proteins, growth factor receptors, etc.).[23,50,56] Integrins are therefore outstanding candidates for acting as mechanoreceptors that represent the first molecules on the cell surface that sense mechanical signals, and then convey them across the cell surface where they can be converted into changes in intracellular biochemistry.[30] In fact, their key role in mechanotransduction has been confirmed in innumerable experimental studies in a wide range of cell types.[1,11,12,23,25,30,46,48,49,70 72] Clearly, other receptors and molecules also contribute to mechanosensation; however, the key point is that force transfer to the cytoskeleton and mechanical distortion of molecules located within load-bearing structures and scaffolds in the cell mediate this response.[37] The state of the art in the mechanotransduction field now focuses on identification of molecular connections between integrins, other structural components of focal adhesions, and the signal transduction molecules that mediate mechanosensation, as well has how structures located throughout the cell, and even at different size scales in cells, tissues and organs, are mechanically regulated as one.[37] Because the mechano-chemical conversion process effectively occurs in a 'solid-state' on insoluble scaffolds in the cell, and because cells use tensegrity to mechanically stabilize these structures, prestress in cells, tissues, and organs modulate this mechanotransduction response at virtually all size scales.[37]

In this context, it is interesting that cell fate switching—the mechanism by which individual cells decide whether to grow, differentiate, move or die—is also controlled mechanically.[9,15,28,38,53,59] Apparently, cells switch between different phenotypes as a result of collective interactions at the level of the genome-wide gene and protein regulatory networks, which effectively transition between different stable 'attractor' states.[8,27,29,34] This type of complex systems control requires that multiple nodes in the regulatory network alter their activity simultaneously. The cytoskeleton orients multiple signaling molecules that govern gene expression and other forms of biochemical regulation, and thus, this may be how structural and information processing networks integrate in living cells.[34]

BIOLOGICALLY INSPIRED ENGINEERING

There is much more to Cell Mechanics, Mechanotransduction, and Molecular Cell Engineering than I described above. I primarily reviewed my own experience in this area to provide a sense for how those of us who helped to develop this field approached the challenge of understanding cellular regulation. However, perhaps even more exciting is what lies before us for the future. As a result of recognition that the isolated islands of biology, physics, and engineering are indeed connected (now that the oceans are drained), the boundaries between living and non-living systems are breaking down. We are beginning to identify fundamental design principles that govern the form and function living cells and other biological materials, such as tensegrity and solid-state mechanochemistry, which may lead to development of entirely new engineering principles that could transform medicine as well as industry. As a result, the new discipline of Biologically Inspired Engineering is emerging, in which the goal is to create biomimetic materials, devices, robots, and control technologies that emulate the way in which nature builds living things. These fully programmable materials and devices inspired by biological design might provide powerful ways to promote regeneration and reboot complex disease processes. A deeper understanding of how living cells build, manufacture, and recycle materials also could lead to more efficient and environmentally friendly ways to generate energy and produce materials, and thereby create a more sustainable world.

This is a big vision, however, we have already started to move down this path. The emerging area of Synthetic Biology provides ways to engineer complex gene circuits that include integrated networks of sensors, processors, and actuators.[21,24] The potential of engineering an entire genome is now within the realm of possibility, and this could lead to a vast array of applications from cellular devices that function as implantable drug factories to new forms of bioenergy production. Nanotechnologists and bioengineers are building tensegrity-based materials from the bottom up using synthetic DNA molecules and polymeric materials.[43,44,58] Cell growth, differentiation, movement, and apoptosis in endothelial and epithelial cells[9,15,53,59,77] and neural circuitry in brain cell networks can be changed by physically restricting their size using microengineered substrates.[76] Biomimetic polymer scaffolds that mimic the spatio-temporal delivery dynamics of various growth factors induce growth and maturation of spatially organized tissues.[10] Non-invasive 'man-machine' interfaces also have been developed using magnetic fields in combination with magnetic nanoparticles bound to single receptors; when the beads are magnetized, they pull the receptors into clusters and activate intracellular signal transduction in a dynamic and reversible manner.[47] And computer scientists have invented bioinspired

algorithms to control swarms of robots that build structures and control systems, much like cells do in the embryo.[75,78] But this is only the beginning, as more and more young people are drawn to the exciting world of Cellular and Molecular Bioengineering where imagination is the only limit. So it will be interesting 15 years from now, to look back and see how much closer we will be to a complete unification of the biological, physical and engineering sciences, and how it will impact our world.

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