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# From mechanobiology to developmentally inspired engineering

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The field of mechanobiology emerged based on the recognition of the central role that physical forces play in development and physiology. In this article, which is based on a lecture I presented at the 2018 Royal Society meeting on Mechanics of Development, I review work from my laboratory carried out over the 40 years which helped to birth this field. I will also describe how we are leveraging the fundamental design principles that govern mechanoregulation to develop new experimental tools and organ-engineering approaches as well as novel mechanotherapeutics.

This article is part of the Theo Murphy meeting issue 'Mechanics of development'.

## 1. Introduction

The Royal Society convened a meeting on the 'Mechanics of Development' in February 2018 at Chicheley Hall where scientists and engineers from around the world shared lessons they had learned about how mechanical forces impact developmental control. As someone who has worked in this field for many years, I was asked to present an overview of my contributions to this field and to provide examples of where I can see this field moving in the future. This article is therefore not a broad review of the fields of mechanobiology or mechanotransduction, which can be found elsewhere; rather, it simply summarizes my presentation. However, I do hope it will provide a historical context for those who are interested in exploring the role of physical forces in tissue or organ formation, physiological control or disease development, as well as those who are interested in leveraging the governing mechanobiological principles that have been uncovered to develop new engineering innovations.

## 2. Discovery of mechanobiological design principles

All of the work that has emerged from my laboratory over the past 40 years stemmed from a quest to address the fundamental question of how living cells and tissues are constructed. At the time I entered the laboratory in the mid-1970s, virtually all research centred on the role of chemicals and genes in developmental control. I suggested an alternative concept: mechanical forces may be equally important for regulation of cell and tissue formation, as well as for the development of diseases, such as cancer [1,2]. I raised this possibility on the basis of seeing early movies in an undergraduate developmental biology course taught by John Trinkaus; these showed how embryos develop and cells move, which revealed these processes to be associated with major changes in cell shape and tissue distortion that appeared to be mediated entirely by cell-generated forces. About the same time, the biologist Albert Harris published studies using flexible silicone rubber membranes as culture substrates, which showed that all types of cells generate contractile forces and apply traction to their substrate adhesions [3]. Judah Folkman had also published an article demonstrating a direct correlation between cell shape and growth, with more highly distorted cells proliferating most rapidly [4]. At about the same time,

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one of the first articles was published that showed that all living cells contain actomyosin filaments in their cytoskeleton which are responsible for generating contractile forces [5]. This led me to suspect that forces generated within the cytoskeleton might regulate tissue growth and development.

I also had another experience during this same period that had a huge impact on my vision of mechanoregulation and developmental control, as well as my future career. By chance, I was enrolled in a sculpture course where I saw a stick-and-elastic string model built using what is known as tensegrity (tensional integrity) architecture [6], which flattened when anchored to a substrate, and spontaneously pulled itself off the substrate and rounded when detached. I saw this the same week that I had my first experience in cell culture where I was taught to use trypsin to detach spread cells from the substrate by clipping their anchors. These cells popped off the culture substrate and rounded up much like the tensegrity models. Given the recent finding that all cells have an internal contractile cytoskeleton, this led me to assume that cells were also tensegrity structures.

Tensegrities are structural systems composed of multiple components that establish their three-dimensional form and shape stability (e.g. mechanical stiffness) through the establishment of a tensile 'prestress' (pre-existing state of isometric tension), rather than by continuous compression. Tensegrity sculptures commonly gain their shape stability by interconnecting multiple isolated compression-resistant struts (e.g. steel beams) with a continuous series of tensile cables that establish a mechanical equilibrium with an internal prestress. To explore the possibility that cells might be tensegrity structures, I built similar stick-and-string models using elastic tension cables, and I was able to visualize for others how tensegrity cell models can replicate cell spreading on a rigid substrate, as well as how they become rounded when detached; these models even pulled flexible substrates into wrinkles [2,7], much as had been observed with cells cultured on flexible silicone membranes [3]. I also built a large cell tensegrity model containing a nucleus that was built by similar tensegrity principles and attached to the larger cell model by addition of tensile elastic cables. This nucleated tensegrity cell sculpture exhibited coordinated extension of the cell and nucleus when the cell model spread across a substrate, again replicating behaviours observed in living cells [8]. Importantly, we later experimentally confirmed that cells behave mechanically like tensegrity structures, and that contractile (tensile) forces generated in actomyosin filaments and resisted by both internal compression-bearing microtubules and external adhesive tethers are responsible for stabilizing cell shape and mechanics [9]. We also demonstrated that intermediate filaments physically couple the nucleus to the cell surface [10], and developed a computational model of cellular tensegrity based on first mechanistic principles that effectively predicts both quantitative and qualitative mechanical behaviours of multiple types of living cells [11,12]. Using green fluorescent protein (GFP)-labelled cytoskeletal proteins, we also confirmed that actin stress fibres are tensionally prestressed [13] and that microtubules bear compression in living cells [14]. To accomplish this, we had to develop multiple new experimental manipulation and analysis techniques (e.g. cell magnetometry and laser nanoscissors), which have been useful for other groups in probing the role of mechanics in cell and developmental control.

The tensegrity model also provided a potential explanation for how physically deforming the shape of a tissue, as observed in the embryo, could influence cell growth and function. Studies with stick-and-string tensegrity models showed that when cells are deformed, their internal cytoskeletal filament networks rearrange in a coordinated manner much like how our muscles and bone change their relative positions when we distort our bodies. Importantly, work from many laboratories has revealed that much of the cell's biochemical machinery that mediates signal transduction, metabolism and protein synthesis as well as RNA synthesis and DNA replication involves 'solid-state' biochemistry: many of the participating enzymes and substrate are immobilized on molecular scaffolds at the membrane, in the cytoplasm or within the nucleus (reviewed in [15]). As physical deformation of individual molecules or molecular assemblies can influence their thermodynamic and kinetic behaviours, this provided a way to link changes in cell shape to alterations in intracellular biochemistry that mediate cell fate decisions [16].

As I mentioned above, Judah Folkman had shown that there is a direct correlation between cell shape and growth in the 1970s; however, critics argued that these findings could be explained by changes in cell-matrix adhesions or soluble factors. Thus, to test this idea directly, it would be necessary to devise an experimental system whereby we could vary cell shape in a controlled manner independently of changes in extracellular matrix contacts or soluble factors. We accomplished this working in collaboration with George Whitesides by using a soft lithography-based microcontact printing method he developed to microfabricate single cell-sized adhesive islands coated with a constant saturating density of extracellular matrix separated by non-adhesive regions [17]. Using this method, for example, it is possible to make round fibronectin-coated islands in progressively smaller sizes such that individual cells spread and take on a round pancake-like form on the large islands, exhibit a more compact cupcake-like shape on a mid-sized island and appear almost spherical like a golf ball-on-a-tee on the tiniest island. When we cultured various types of cells on these substrates in chemically defined medium containing a saturating amount of soluble growth factor, we found that spread cells proliferated, near spherical cells underwent apoptosis and moderately spread cells switched on a differentiation programme [17–19]. Later, by making square-shaped islands (and cells), we could show that when stimulated with motility factors, these cells preferentially extended motile processes from their corners in the regions of highest stress [20]. In short, we were able to confirm that physical distortion of cells does indeed switch them between different fates, even in the presence of an excess amount of soluble growth factors.

## 3. Molecular basis of cellular mechanotransduction

At the time I entered research, it was well known that physical forces due to blood flow, shear, weight-lifting and gravity influence tissue form and function at the macro-scale, and some groups had even demonstrated experimentally that mechanical forces can influence cell behaviour *in vitro*. However, no one could explain how cells sense and respond to mechanical forces at the molecular level, or what is known as 'cellular mechanotransduction'. One of the most important

insights that emerged from the cellular tensegrity model is that, if this building system is used to stabilize internal cell structure, then mechanical forces should be preferentially transmitted across the cell's surface via molecules that physically connect external adhesive scaffolds (e.g. extracellular matrix and cell–cell adhesion receptors) to the internal cytoskeletal framework of the cell. This led us to predict that transmembrane cell surface matrix receptors, such as integrins, should act as 'mechanoreceptors' in that they would provide a specific molecular pathway for sensing mechanical signals and transmitting them across the plasma membrane [2].

To test this hypothesis, we developed a cell magnetometry technique in which we coated micrometre-sized magnetic beads with ligands for integrins or other cell surface receptors, bound them to cells and then applied twisting (torque) or pulling (tensional) forces to the cells over these specific molecular connections. This work led to the first experimental demonstration that integrins do indeed serve as mechanoreceptors, and that they transmit these forces across the cell surface and to the cytoskeleton [9]. By measuring how far the beads distorted in response to the applied stress, we were able to carry out stress–strain analysis on individual living cells. This analysis revealed that living cells exhibit a linear stiffening response by increasing their stiffness in direct proportion as the applied stress is raised, that this response is mediated by all three cytoskeletal filament networks (actomyosin filaments, microtubules and intermediate filaments) and that the overall stiffness is governed by the level of prestress (isometric tension) in the cell [9]. All of these results were predicted by both stick-and-string cellular tensegrity models [9] and a mathematical formulation of this theory [12].

Our group and many others went on to demonstrate that mechanical forces transmitted across integrins alter cellular biochemistry and gene transcription via multiple signalling mechanisms. For example, we demonstrated that pulling on integrins with ligand-coated magnetic beads activates the entire cyclic AMP signalling pathway from activation of heterotrimeric G proteins in the submembranous focal adhesion complex that forms at the site of integrin binding to stimulating adenyl cyclase and nuclear transport of phospho-CREB, to induction of gene transcription, whereas applying the same force to transmembrane metabolic receptors did not produce this effect [21,22]. However, the most rapid mechanochemical transduction response we could detect in non-sensory cells (endothelial cells) was within 5 ms after applying mechanical stress to integrin receptors [23]. This ultra-rapid mechanical signalling response was mediated by force transfer from integrin to a stress-sensitive ion channel in the membrane within the focal adhesion, known as TRPV4. Moreover, when we knocked down this ion channel in endothelial cells, they lost their ability to reorient in response to application of mechanical strain through their matrix adhesions [24]. Thus, these studies confirmed that integrin-mediated mechanochemical transduction is both biologically interesting and physiologically relevant for developmental control.

## 4. Mechanical control of embryonic organ formation

Based on our numerous studies that demonstrated the central role that mechanical forces and cell shape distortion play in control of cell growth and function *in vitro*, we decided to ask whether similar mechanobiological regulatory mechanisms are used *in vivo*. As we had done much of our culture work with capillary endothelial cells, we explored whether physical forces regulate angiogenesis (capillary blood vessel formation). In these studies, we showed that implanting extracellular matrix gels with different mechanical compliance resulted in different angiogenic responses, with optimal capillary ingrowth being observed in gels with moderate stiffness [25]. Interestingly, cultured capillary endothelial cells also exhibited higher expression of the angiogenic factor receptor, VEGFR2, on moderate stiffness gels, and this mechanical signalling mechanism was found to be mediated by the Rho inhibitor, p190RhoGAP, which modulates the balance of activities between two antagonistic transcription factors, TFII-I and GATA2, that govern gene expression of VEGFR2. By modulating the relative expression of these two transcription factors, we could also control mouse retinal angiogenesis *in vivo*. Thus, mechanical forces do indeed play an important role in control of the development of living tissues.

We then explored whether physical forces are important for embryonic organ formation, which is what first inspired me to pursue this path of investigation. We chose the embryonic tooth as a model system because, like many other organs, it is controlled through epithelial–mesenchymal interactions. However, in contrast to other organs that exhibit highly complex branching patterns (e.g. lung, pancreas and salivary gland), tooth forms as a result of the epithelium forming a single bud that extends down into the underlying stroma, which then folds back up on itself once to form the roots of the tooth. The tooth also is one of the most highly characterized model of organogenesis as many of the genes and morphogens that mediate odontogenic (tooth forming) signalling cascades have been identified; however, we still do not understand how a living tooth is physically constructed from living cells and extracellular matrix.

The formation of the tooth, like other epithelial organs, is initiated through a process known as a 'mesenchymal condensation' in which loosely distributed mesenchymal cells suddenly group together to form a compact cell mass directly beneath where the first epithelial bud will form. Interestingly, the size and shape of the condensed mesenchymal cell mass also dictates the final three-dimensional form of the organ [26], and past embryonic tissue recombination studies have shown that the source of the mesenchyme governs tissue patterning (histodifferentiation), whereas the epithelium determines which specialized functions will be expressed by the lining cells (cytodifferentiation) [27]. Given that mesenchymal condensation drives the cell fate switches that drive development in multiple organs, we explored whether this process could be governed by a physical signal associated with cell compaction.

Using mouse embryonic tooth as a model system, we showed that mesenchymal compaction occurs because the overlying embryonic epithelium secretes two opposing motility factors—the motogen FGF8 and the repulsive factor Semaphorin 3A [28]. FGF8 is present in a shallow gradient and reaches deep into the underlying stroma, stimulating all mesenchymal cells to migrate towards the basement membrane. At the same time, Semaphorin 3A appears in a steep shallow gradient that pushes back against the approaching cells, causing them to 'condense' and form a tightly packed

cell mass directly beneath the epithelium. We could visualize these gradients using immunostaining in histological sections of developing tooth, and impressively, we could stimulate artificial mesenchymal condensation *in vitro* by culturing embryonic dental mesenchymal cells within a microfluidic channel where we established similar opposing gradients of both factors. Interestingly, using micro-engineered adhesive islands to control the shape of individual cultured mesenchymal cells, we could show that it is the rounding of these cells that results from cell compaction, rather than increased cell–cell contact formation, which induces expression of odontogenic transcription factors, such as Pax9. More importantly, however, we could induce whole tooth formation by mechanically compressing isolated, undifferentiated (loosely packed), embryonic dental mesenchyme tissue between two pieces of silicone rubber, and then recombining it with isolated undifferentiated dental epithelium and implanting it under the kidney capsule in the mouse. Thus, while growth factors and morphogens (e.g. FGF8 and Semaphorin 3A) mediate organogenesis, they act by inducing mechanical changes in cells and tissues that drive cell fate formation; once these physical alterations are induced, there is no longer a need for these soluble factors.

We recently leveraged these fundamental mechanobiological design principles to engineer synthetic materials that potentially could be used to induce organ formation. For example, we created biocompatible, temperature-sensitive, 'shrink wrap-like' scaffolds that compact in three dimensions when warmed to body temperature [29,30]. When embryonic dental mesenchymal cells or adult mesenchymal stem cells are plated within these scaffolds and cultured at 37°C, they round to a size similar to that observed in condensed mesenchyme *in vivo*, and this is again accompanied by induction of odontogenic transcription factors. More importantly, when these compacted cell scaffolds are implanted *in vivo*, they form tooth-like tissues that express dentin. To create a whole tooth, it will be necessary also to integrate an epithelium; however, this finding suggests that this developmentally inspired approach to organ engineering is worth pursuing in the future.

## 5. Engineering cells into organs *in vitro*: organs-on-chips

About 9 years ago, I founded the Wyss Institute for Biologically Inspired Engineering at Harvard University which I still lead. When we formed this Institute, we were challenged with focusing on problems that if solved could bring about transformational change for the better. The biggest problem I could see in medicine is that the drug development model is broken: the costs are huge, the timeline is glacial and the likelihood of success is extremely low. One of the major underlying problems is that preclinical animal studies, which are required by regulatory agencies, often do not predict results in humans. Thus, when we started the Institute, we started a major programme to address this challenge.

Our approach was to leverage the soft lithography-based microfabrication approach we had adapted from computer microchip manufacturing years earlier [17] to engineer microchips containing living human cells that reconstitute organ-level structures and functions, or what are now known as human 'organs-on-chips' (organ chips) [31]. Our organ chips are optically clear, microfluidic cell culture devices with separate parenchymal and vascular microchannels lined by living human cells that recapitulate the tissue–tissue interfaces and physiologically relevant mechanical micro-environment of key functional units of living organs, while providing dynamic vascular perfusion *in vitro*. For example, the first device we created was a human lung alveolus chip [32–34]. It is the size of a computer memory stick, made of optically clear and flexible silicone rubber, and it has three parallel channels along its length, each less than a millimetre wide. The central channel is split into top and bottom channels by an intervening silicone membrane that has multiple small (approx. 5 µm diameter) pores, which we coat with extracellular matrix.

To recreate the alveolar–capillary interface, we culture human lung alveolar epithelial cells on the top of the membrane and human lung microvascular endothelium on the bottom surface of the same membrane in the lower channel. We then introduce air into the upper channel to create an air–liquid interface, and perfuse the vascular channel with medium, with or without immune cells [32,33], or even with human whole blood [34]. To recreate the relevant mechanical micro-environment, we apply cyclic suction through the remaining two hollow chambers on either side of the central cell-lined channel, which causes the porous membrane and attached tissue–tissue interface to rhythmically extend and retract at the same rate and degree as lung alveolar cells experience when we breathe. We have used similar approaches with different organ-specific cell types to build models of the human lung airway [35,36], small intestine [37–40], kidney proximal tubule [41], kidney glomerulus [42] and blood–brain barrier [43], among others, always attempting to recreate the relevant physical micro-environment. When we created a human intestine chip, we similarly applied cyclic mechanical strain to mimic effects of peristalsis-like motions [37–40], and when we created the kidney glomerulus chip [42], we applied cyclic deformations the living glomerulus experiences due to pulsatile blood flow.

Most importantly, we have shown that these human organ chips faithfully model key features of various human diseases and disorders (e.g. pulmonary oedema, asthma, chronic obstructive pulmonary disease, inflammatory bowel disease, ileus, and viral and bacterial infections) and replicate drug toxicities as well as or better than animal models [32–42], but only if we recreate the relevant mechanical micro-environment. For example, in the lung alveolus, mechanical breathing motions were found to be required to model nanoparticle absorption [32] as well as pulmonary oedema induced by the FDA-approved cancer drug interleukin-2 [33]. This drug toxicity response mimicked the pulmonary vascular leakage at the same dose and over the same time course as seen in patients who experience this toxicity. Peristalsis-like motions in the human gut chip were also discovered to be responsible for suppressing the growth of commensal bacteria, rather than this being due to decreased fluid flow as was assumed in the past, thus providing new insight into the mechanism of the ileus (bacterial overgrowth resulting from cessation of peristalsis) [39]. Cyclic vascular pulsations were also shown to be required for optimal podocyte differentiation and reconstitution of urinary clearance functions in the kidney glomerulus chip [42].

Finally, in our past work on mechanotransduction in endothelium, we had found that one of the first mechanochemical transduction mechanisms activated by force application to these cells was stimulation of the mechanosensitive TRPV4 ion channel that induces calcium influx into cells [23]. I was aware that the pharmaceutical company GlaxoSmithKline had developed a chemical inhibitor of this channel, and they kindly provided some of this compound for our use. When we tested it in the human lung chip, we completely inhibited pulmonary oedema induced by interleukin-2 [33]. Researchers at GlaxoSmithKline then carried out similar studies in dog and rabbit models of cardiogenic pulmonary oedema and obtained similar results [44]. This 'mechanotherapeutic' drug is now in phase II human clinical trials.

## 6. Conclusion

In summary, my experience in science over the past few decades has reaffirmed my belief that mechanical forces play as important a role in cell, tissue and organ development as chemicals and genes. I described how living cells use tensegrity architecture to stabilize their shape and mechanics through modulation of internal prestress, which tunes cellular responses to external signals much like changing the tension in a violin string conveys different information in the form of different tones. As a result, the key determinants of tensegrity—three-dimensional architecture and prestress—appear to be critical governors of the cell's biochemical response to stress. However, tensegrity is used to stabilize biological structures at other size scales in the hierarchy of life, from individual molecules to multimolecular complexes and organelles, to whole cells, tissues, organs and organisms [45–47]. For example, we stabilize our bodies by interconnecting multiple compression-resistant bones with a continuous series of tensile muscles, tendons, ligaments and fascia, and it is the level of 'tone' (isometric tension) in our muscles that governs whether we rigidify our arm or allow it to flex. In addition, our bodies, like cells, are multi-modular tensegrities in that they are composed of multiple subsystems (e.g. arm, leg, nucleus and cell process) that can be disrupted individually without compromising the whole. If the Achilles tendon is cut, that leg cannot be flexed normally; however, the other leg, torso and arms can all still stabilize their forms. Yet, normally all of these individually self-stabilized structures function as one tensionally integrated unit.

Living organisms are also hierarchical tensegrities in that when we move or experience a mechanical force, the stresses are transmitted across physically connected load-bearing elements (tissues, fascia, extracellular matrix, integrins, cell–cell adhesion receptors, cytoskeletal filaments, nuclei and individual molecules) from the macro-scale to the nanoscale [47]. Forces channelled over stiffened elements in this hierarchical tensegrity system result in either rearrangements or deformations in structures at smaller size scales. Physically distorting a molecule will change its shape and chemical activities (e.g. binding on/off rates, kinetics and thermodynamics) and thereby alter intracellular biochemistry and gene expression [16,47]. Moreover, it is precisely because individual molecules stabilize themselves through use of tensegrity principles that binding of a ligand to a small region of a cell surface receptor can result in propagation of global structural rearrangements throughout the length of the molecule, thereby changing its three-dimensional molecular conformation on the inner surface of the plasma membrane. This is the essence of transmembrane information propagation triggered by chemical and mechanical signals. Recent multi-scale computational molecular dynamic simulations confirm that tensegrity is indeed used to integrate structure and function across multiple size scales, and from atoms to whole cells [48,49].

Importantly, while there is a great focus on identifying specific mechanotransducer molecules, the reality is that the whole cell is the mechanosensor. The same cell will respond to a chemical signal or a mechanical tug on its integrins differently depending on the overall shape of the cell. This is because the cell integrates signals across genome-wide regulatory networks that are highly connected. If a cell wants to turn on a growth programme, it also must turn off other fate programmes (e.g. differentiation, motility and apoptosis). Thus, although signal transduction is usually discussed in terms of deterministic linear and branching pathways, cell fate is actually a result of the architecture and dynamics of its underlying gene and protein regulatory networks. In these types of dynamic regulatory networks, multiple targets in different pathways must be simultaneously perturbed to switch the network between a limited number of different stable end-programmes (attractor states), such as growth, differentiation and apoptosis [50]. Cell shape distortion switches cells between the same discrete cell fates (e.g. growth, differentiation and apoptosis) as does binding of specific growth factors and matrix proteins to their respective cell surface receptors. The cellular tensegrity model suggests that it is precisely because stress-induced deformations and rearrangements in the tensionally integrated cytoskeleton and nucleus alter the activities of many signalling components at once that cell distortion can produce these same discrete changes in cellular phenotype [51].

I also hope that I have conveyed the importance of appreciating the potential clinical relevance of mechanobiology by describing how we have leveraged understanding of physical control mechanisms to engineer new materials, devices and mechanotherapeutics. Developmentally inspired materials offer an entirely new approach to tissue and organ engineering, which could also be combined with other exciting methodologies that are currently being explored (e.g. three-dimensional printing and stem cell engineering) to produce even more effective and functional tissue structures. The organ chip technology has the potential to revolutionize drug development by replacing animal testing with human-relevant preclinical models. They also can be created using patient-specific primary or induced pluripotent stem cells, which should provide a powerful new approach to personalized medicine.

None of this work would have been possible without recognition of the importance of mechanical forces for biological regulation. Interestingly, this was the accepted dogma in the field of developmental biology at the beginning of the twentieth century, but the idea fell from attention with the advent of molecular biochemistry and genomics. The time has come to recognize that all of these factors—biochemical, genetic and mechanical—are equally important for biological control, and that to truly understand living systems, we must break through our silos of knowledge and create a fully integrated explanation of how our bodies work.

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