Backstory

Interdisciplinarity and mechanobiology

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Cells in living tissues and organs continually sense and respond to both external mechanical stimuli and internal forces generated by other cells inside our bodies, and this phenomenon has become increasingly recognized as a key driver of physiological, developmental, and disease processes. Pursuit of the mechanisms that underlie these processes led to the emergence of the field of mechanobiology, which is an extremely interdisciplinary field of research at the interface between biology, physics, material science, and bioengineering. Researchers have been focusing on elucidating how cells sense and transduce mechanical forces, how changes in the mechanical properties of cells influence their behaviors, and how physical forces influence normal and diseased tissue development and function. Mechanobiology principles have been also applied to biomedical issues, and novel tools developed to explore these physical phenomena have helped to pave the way for the development of point-of-care diagnostics and the discovery of new mechanoresponsive therapies.

In the iScience special issue "Microsystems and Mechanobiology," guest edited by Donald Ingber

(Wyss Institute at Harvard) and Dino Di Carlo (University of California, Los Angeles), we highlight the latest advances in engineered microsystems employed to address major questions in mechanobiology and mechanomedicine.

Figure 1. Dino Di Carlo (left) and Donald Ingber (right), Guest Editors of the *iScience* special issue "Microsystems and Mechanobiology"

More content in the special issue can be found here:https://www.sciencedirect.com/journal/iscience/special-issue/10CKP1JBVHJ

In this backstory, the guest editors share their thoughts on the current challenges in the mechanobiology field, the importance of interdisciplinarity, the open questions that remain, and the exciting advances that will come in the next few years.

MOTIVATION

1. What originally interested you in the field of *mechanobiology*? Did you train in this area or are your research interests naturally aligned with the developing field?

Ingber: I majored in Molecular Biophysics & Biochemistry as an undergrad at Yale, which is where I first learned how physical forces determine molecular form and function, and hence govern activities that underlie all living systems. At the same time, I saw art students walking around the campus carrying large multifaceted polygonal structures that closely resembled the viral capsids I was learning about in my science classes. When I learned that the sculpture class they were taking was called, "Three Dimensional Design," I knew I had to get into it. After a tough time trying to convince the art professor why a science major should join his class, I got in, and this is where I was introduced to the tensegrity (tensional integrity) architecture principle first described by the architect R. Buckminster Fuller and constructed by his student, the sculptor Kenneth Snelson. In one class, the professor showed us one of these tensegrities that was composed of isolated rigid sticks that were stabilized and pulled up into a 3D spherical form by interconnection with elastic strings. When he pushed down on this with his hands, it flattened, and when he released it, it jumped up into the air. This was precisely what I observed when I had learned how to trypsinize cells the week before working in a cancer research lab at the medical school: the cells that had spread flat while growing on the rigid dish, rapidly retracted, rounded, and bounced up into the medium when I used the enzyme, trypsin, to clip its adhesive anchors. This was about 1976 when the first publications were coming out showing that all cells have an internal molecular skeleton or cytoskeleton, composed of contractile actomyosin filaments and not just muscle cells. This was my first 'aha' moment when I realized that living cells must be built this way. But more importantly, it led me back to Yale's science, art, architecture, chemistry, and physics libraries to learn more.

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Around the same time, I took a Developmental Biology course where the professor showed some of the first movies of cultured cells and the physicality of the process came to the fore. Healthy cells moved about the dish until they physically touched another cell, whereas cancer cells just kept on moving all over each other. In addition, when I started to read early developmental biology books and research articles, I learned that in the early days of the field, everything was explained in terms of mechanics because biochemistry and molecular biology did not exist yet. Amazingly, much of what these early biologists observed could be explained in mechanical terms. And again, I went back to the libraries.

Finally, about a year later, I entered the MD/PhD program at Yale, and there I started to learn about cell biology and cellular biochemistry. But in contrast to my classes on molecular biophysics, there was no mention of mechanical forces or deformations of molecular shape at all in these discussions. And when I would suggest that mechanical forces somehow came into play, people would laugh and say that idea went out at the end of the 19th century with 'vital forces.' But to me, it was hard to believe the physical forces that are so important at the molecular level, no longer play a role at the cell and tissue levels, especially because we all know our bodies react to physical forces (e.g., gravity, movement, and weight lifting) at the macro level. And back to the library I went. In the end, the last chapter of my PhD thesis presented the tensegrity cell theory and proposed that an entire tissue composed of many cells "may be coordinated as a single functional unit with morphogenetic changes in tissue form being guided through highly regulated alterations in architectural force distributions". And in many ways, this has been the guiding principle behind my work for the past 45 years.

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DiCarlo: I was trained as a bioengineer at UC Berkeley. This training was interdisciplinary but included a strong component in mechanical engineering systems and materials, which provided a context for understanding biological systems as physical systems in addition to chemical systems. As I started to be exposed to the dogma of molecular and cellular biology, it was clear that a number of the inputs into biological systems, such as stresses, forces, and physical confinement were largely ignored. In addition, this lack of depth extends to how cells output physical changes into the environment: e.g., adjust their stiffness, apply forces, move and interact with other cells, tune their extracellular matrix, etc. This gap really interested me. At the time it was starting to be filled by prominent researchers, such as my colleague Don Ingber. I was particularly interested in the quantitative tools that would drive improved understanding, and by making new tools, there was an opportunity to make an impact. One of the first tools I developed was a method to rapidly measure the mechanical properties of single cells, which opened up new avenues in understanding biology but could also be used in a diagnostic context.

2. What kind of scientific background do you have, and is this typical for other researchers in the field to have? Why does a scientist choose to work in this field?

Ingber: I received a BA/MA in Molecular Biophysics and Biochemistry but worked in the early part of my PhD research in a cancer pharmacology lab before carrying out my PhD dissertation in a cell biology laboratory. At the same time, I completed medical school training and received an MD. Although the range of my training is likely broader than some, I believe that a good number of people working in the mechanobiology field have cross disciplinary training. My medical education serves me well to ensure that I focus my work on clinically relevant problems that can have the greatest impact, even when pursuing basic mechanistic studies. I think scientists choose a field to work in based on following their passion and what brings them the greatest personal reward. In my own case, I felt that I saw something that others did not, and that I could explain things that were not possible using conventional approaches. So I had no other choice than to pursue this path and to stick with it even when the criticisms flew.

DiCarlo: In my studies as a bioengineer, I focused on tool development, gaining expertise in microfabrication, microfluidics, and single cell analysis. The rationale for choosing this direction is, overwhelmingly, advances in our understanding and use of biological systems is based on the underlying tools for measuring and manipulating these systems. From PCR, DNA sequencing to flow cytometers and now tools for measuring mechanical properties or forces that cells apply. Perhaps different from many scientists, I also spent two years as a postdoctoral scholar at the Center for Engineering in Medicine at the Massachusetts General Hospital. This environment gave me a practical connection to clinical problems that could be addressed with microscale tools that I developed.

INTERDISCIPLINARITY

3. What role does interdisciplinarity play in your research? Does this interdisciplinarity create any barriers for entering this field, e.g., training?

DiCarlo: All of our work is at the boundaries of disciplines. The challenge here is being able to speak the languages of the different disciplines to create connections and understand the needs across disciplines. One must be open to speak and learn from others and be fearless in making mistakes, similar to speaking a new language. As an example, to develop tools to measure the force that thousands of individual cells apply, my student needed to understand nuances of polymer chemistry and crosslinking and also the differences between synthetic and contractile phenotypes in smooth muscle cells.

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Ingber: I am a firm believer that all major disruptive advances in any field come from breaking down boundaries and changing frames of reference. This is what interdisciplinarity is all about. I think there is too much focus on didactic training when science is really based on apprenticeship and learning by doing and watching how others do what they do. I always would welcome trainees from diverse disciplines into my lab group as students and

fellows, not always knowing where we would go together, but always confident that something exciting would emerge. When these differently trained people would join the lab, in the beginning they would be unnerved and concerned because they would sit through group meetings and not understand what was being said. However, just like living with a family in a foreign country for an extended time, after about 6 months, everyone would be throwing out ideas and arguing with each other in these group meetings; they picked up the language of interdisciplinary science without ever having to read or study about it. Finally, I should note that I have never taken an organized engineering class in my life, yet this is a major part of what I do. This is because I learned that the best teachers are my outstanding collaborators and brilliant students and fellows.

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4. What suggestions would you give to a young scientist interested in *mechanobiology*?

DiCarlo: A young scientist entering the field should start with understanding their desire to make an impact. Do they want to understand a disease from a mechanobiology perspective to develop new treatments or diagnostic techniques? Do they want to dig deep into a fundamental biological question? Do they like to develop new tools and communicate with others and help them solve mechanobiology problems? There are many opportunities and understanding one's intrinsic motivation will inevitably maximize the impact and fun of your journey.

Ingber: The first thing I would say is that although mechanobiology is a burgeoning field, it is still in its infancy; therefore, there is so much you can contribute. The second is what I tell my own students: if you want to understand the future of any field, go back to the library even before PubMed and read as many of the major works (books as well as journal articles) that you can find. It's like analyzing a mathematical progression: once you get a feel for the flow of knowledge and exploration in the past up to today, the future becomes clearer. But I wouldn't only read about mechanobiology, I would try to identify the major questions and problems that scientists have confronted for the past 200 years, which are most relevant to human biology and health. If you do this, you will likely find that there were past controversies about the mechanisms that are responsible for clinically relevant physiological or pathophysiological processes (e.g., developmental control, cancer formation, etc.), and that there were multiple competing theories, each of which had some validity. But in the end, one of those theories won out and the rest were thrown out like a baby with the bath water. Go back and reevaluate those theories that related to problems are still not solved till today; with modern methods, you might very well find that some of those ideas were more correct and valuable than anyone recognized at the time; there was just no way to prove it. And this is precisely what I found in terms of the key role of mechanics in biological control, the central tenet of mechanobiology.

5. What are other disciplines that should look to this field with interest?

DiCarlo: The time is ripe for medicine to look at mechanobiology for new treatment and diagnostic modalities for diseases. Mechanical diseases, in which some aspect of a cell's physical properties is dysregulated, are widespread. Approaches to screen for changes in physical properties should be a fruitful area to find new drug targets. Disease processes that link to cell physical changes can also potentially be diagnosed by monitoring these changes, and treatments can be monitored to identify regimens that recapitulate base functional properties of cells.

Ingber: Dino said it well. I would just add that microengineering and artificial intelligence both have a huge amount to offer and to gain from working on mechanobiological questions. This relates to analytical tools already, but it also could provide ways to measure, apply, and control forces at the cellular level where they act and to devise novel ways to drive development, wound healing, and reverse disease states.

FUTURE

6. What are the most important/most exciting/interesting questions in the field currently, and why do they matter?

DiCarlo: Some of the most exciting questions relate to interfacing with cells physically to communicate with them and mitigate disease processes. For example, how can we apply forces locally to cells to activate specific cell types in a spatially precise manner without chemicals? We are now beginning to understand that different types of immune cells, such as antigen-presenting cells involved in our immune response to pathogens or macrophages involved in regenerative healing, are differentially activated based on the mechanical properties of the materials they interact with. How can we tune injected materials to recruit and activate the right cell types for vaccines or to regenerate injured tissues?

Ingber: I agree that the most important questions currently center around how mechanical forces acting at the cell level guide tissue and organ development and regeneration, as well as how when these mechanisms become deregulated as diseases emerge. Examples include the way in which local changes in extracellular mechanics can drive cancer formation and progression; how cell shape distortion governs cell fate switching and directs stem cells along one lineage or another; how physical changes in the stiffness of our tissues that accompany aging might be reversed; etc.

7. What do you think are the biggest challenges that the field is facing?

Ingber: Mechanobiology is challenging because one cannot easily see the underlying forces that guide pattern formation and govern biological systems. In addition, although mechanical forces influence cells, cells are the major generator of mechanical forces in our bodies. Besides, mechanical forces modulate cell responses to soluble factors (growth factors, hormones, etc.) and to adhesive cues (e.g., binding to extracellular matrix or other cells), but these soluble and insoluble cues also alter mechanical force generation. It is a complex multi-body problem in biophysics in which all systems are dynamic and reciprocal. Finally, forces exerted at the macro level trickle down through structural elements within the living hierarchy of biological systems (organs, tissues, cells, cytoskeleton, nuclei, and molecules) to impact cellular biochemistry and the forces cells exert through molecular actions are transported back up through this hierarchical architecture for us to move and interact with our environment. Developing new tools and approaches to describe, probe, and analyze these types of complex adaptive systems composed of hierarchical biological networks is the major challenge ahead.

DiCarlo: The trend in cell biology has been to collect more data, i.e., more "-omics" information to uncover new biological insights. In mechanobiology studies, the quantity of data generated has not been at the same level as single-cell sequencing or other –omic workflows. To be relevant, tools and approaches to collect ever more amount of data at the level of individual cells or cell-clusters are needed. Better approaches to tie this information to single-cell molecular information are also needed.

8. What's next? What breakthroughs do you imagine or hope to see in upcoming years?

DiCarlo: In the upcoming years, I expect to see techniques that can link molecular and biophysical properties of single-cells at unprecedented scale. Tools that allow understanding the influence of each gene on cell biophysical properties, adhesion, force generation, and more will underlie new breakthroughs in understanding mechanobiology and modulating phenotypes to mitigate diseases.

Ingber: The next wave will involve development of mechanotherapeutics in various forms. These will include small molecules, drugs, and biologics that target mechanotransduction pathways to reverse disease states, as well as wearables and robotics that apply forces in just the right place with precisely the correct timing and intensity to remedy a structural defect or promote healing. New mechanical imaging technologies also will need to be developed that can finally allow researchers to 'see' where forces are acting and how they impact biological systems at the subcellular and molecular levels; this could revolutionize the field just like optical and electron microscopy did for molecular cell biologists.

FINAL THOUGHTS

DiCarlo: It's an amazing time to be working at the boundaries of disciplines between engineering, physical sciences, and biology. Applying microtechnology tools is poised to amplify our understanding of

mechanobiology and enable improvements in human health, which is often overlooked while using molecular biology frameworks.

Ingber: As someone who has now worked in this area for almost 50 years, I am extremely excited to see the depth and breadth of interest by these younger basic researchers as well as clinical scientists and drug developers in the field of mechanobiology. But there is so much more to do as most biologists still focus on the parts and not on the forces that hold them together and govern how structures and functions emerge. So it will be up to the new generation of young scientists and engineers to design the precise experiments necessary to convince all of the naysayers as well as those who simply have no interest changing the way they do things now and how important it is to consider the role of hidden mechanical forces in their own work. Precisely, this mechanical perspective just might help them to answer questions and explain the phenomenon observed in living systems that conventional biochemical and genetic approaches cannot. Only this way can you convert critics into competitors.