From Models to Medicine: Understanding Stem Cell Differentiation in Regenerative Therapy

INTERVIEW WITH: DR. DAVID SCHAFFER

BY: LARA POTGIETER, HARJYOT KAUR, ALMA RAZAVILAR, and TANYA SANGHAL

***Dr. David Schaffer** is a Hubbard Howe Jr. Distinguished Professor of Chemical and Biomolecular Engineering, Bioengineering, and Neuroscience at UC Berkeley. He is also the Director of the Bakar BioEnginuity Hub, the Berkeley Stem Cell Center, and the California Institute for Quantitative Biosciences (QB3-Berkeley). After finishing his undergraduate education at Stanford in 1993, he earned his PhD from MIT in 1998 in chemical engineering, and completed his postdoctoral fellowship at the Salk Institute. He joined UC Berkeley's faculty in 1999.*

The Schaffer Lab's research focuses on employing engineering principles to tackle biomedical issues, such as gene therapy, genome editing, and stem cells in various tissues, particularly the nervous system. With several of their technologies in human clinical trials, they aim to optimize gene and stem cell therapies. Dr. Schaffer has also co-founded eight biotechnology companies. With numerous recognitions, patents, and publications, Dr. Schaffer is at the forefront of stem cell and gene therapy research.

BSJ: Can you share what drew you to this area of research? Was there a specific challenge or scientific question that motivated you to explore the role of mechanosensitive pathways in stem cell fate, or did your interest evolve over time?

DS: I come from a biomedical family. My mother was an MD who ran late stage human clinical trials for Novartis and Sanofi, while my father was an early stage biochemist academic. For me, I knew I wanted to work on something related to human health, but I felt my father's work was too basic or abstract: the connection to human health was not immediately apparent. My mother's work focused more on the clinical outcomes by getting drugs FDA-approved, which I am very proud of. However, I was drawn to a more mechanistic, molecular approach—working at earlier stages of discovery and invention, with the hope that my contributions might eventually make it into clinical trials. I ended up as their arithmetic mean, halfway in between the two of them, working at the interface between late stage academic research and playing a role in transitioning the research into companies.

When I started out my academic work, I gravitated towards therapeutics, specifically human therapeutic interventions that use DNA as the target. If you take a look at most pharmaceuticals that are used in the clinic right now, like Ibuprofen, they are small molecules that act on proteins as the target. But you can use messenger RNA itself as a therapeutic that can target at the level of RNA. I have always gravitated towards therapeutic interventions that act at the level of DNA. That's gene therapy. That's genome editing. That's cell therapy. The three of these offer the potential for a 'one-and-done' approach—a single dose that provides long-term therapeutic benefits, as DNA remains active for the duration of a patient's life. We are heading toward cures.

In the case of stem cells, the big challenge is, how do you control the cell? A stem cell has the potential to repopulate tissue devastated by disease and replace cells lost due to human illness.To achieve this, we need to guide the stem cell's transformation from an immature state into the specific cell type needed for tissue repair. What controls that process for stem cells? How do you direct its fate? How do you differentiate it into the right cell type? Signals from the environment surrounding stem cells teach them what to do. It is a blank slate waiting to be written on. For many years, biologists have made incredible discoveries investigating how biochemical cues, protein factors, and small molecules instruct the behavior of stem cells. But as an engineer, we started thinking about physics. Human beings are not bags of biochemicals. We have a solid phase. We have structural integrity. We have physical organization to us. Years ago, my colleagues and I, along with our collaborators, began to wonder, maybe stem cells are instructed not just by soluble proteins and small molecules but also by the solid phase, by the physical properties of the tissues in which they reside.

In 2008, we published a paper that investigated whether stiffness is an important signal that regulates the behavior of stem cells. We found that if you place a neural stem cell in a soft environment and cause them to differentiate, they tend to become neurons; however, if you expose them to the identical soluble cues, but place them in a very stiff environment, they turn into astrocytes instead. The physical properties of an environment can in fact regulate cell fate. We started by asking: if stiffness is crucial, what other material properties matter? For instance, tissues in the body are generally elastic—they can deform and then return to their original shape, like when you press on the skin of your hand and it bounces back. But tissues also exhibit relaxation, similar to honey: if you press hard enough, they gradually give way, demonstrating viscosity. Recognizing the role of elasticity, we began to investigate whether viscosity is another key factor. To answer this,

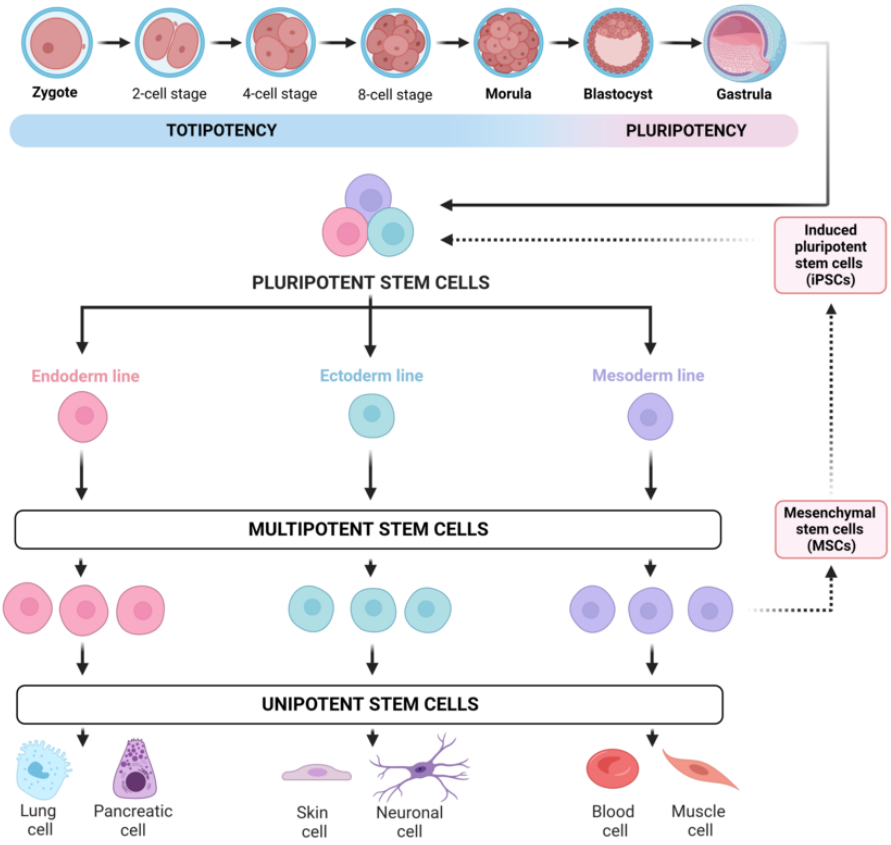

Figure 1: General Outline of Stem Cell Differentiation. Totipotent cells can differentiate into any cell type and stem cell potential becomes more rigid as they become pluripotent, multipotent, and finally unipotent.

we needed an experimental system that allowed us to manipulate these properties, so we developed a material that incorporates both elasticity and viscosity. We designed a system where we use DNA as a crosslink, and DNA dehybridization and rehybridization, which is reversible, as the relaxing component of it. This research led to the development of a new class of materials designed to answer our questions about viscosity's role in cell behavior. With these materials in hand, we began varying viscosity to see if cells responded—and they did. When we compared two materials with identical elasticity but different viscosities, stem cells behaved differently: they tended to become neurons on purely elastic materials, but differentiated into astrocytes on materials that could relax.

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This discovery reveals how tissue properties influence stem cell behavior, shedding new light on what governs these critical cells in the body. It also provides a novel tool for therapeutic applications—by controlling stem cell fate decisions, we can now tailor the production of specific cell types. This capability allows us to scale up in bioreactors and potentially mass-produce the necessary cell types to restore tissues depleted by disease.

BSJ: Could you explain the process of stem cell differentiation? Are there any common mistakes, misconceptions, or false simplifications about this process?

DS: There are a variety of different types of stem cells. If you think about it, we all started out as a single cell, a zygote, and that gave rise to so many things—to extraembryonic tissue, the placenta, and to the inner cell mass, which becomes the fetus. At the very beginning, the stem cells in these tissues are incredibly plastic. They can turn into everything and then become a little bit more restricted. As time goes on and tissues develop, like the central nervous system, muscle, and blood system, there are resident stem cells in each one of those tissues that can turn into the cell types within that tissue. So there is this hierarchy process where, at the very top, is a totipotent stem cell that can turn into anything. Pluripotent stem cells can turn into every cell type in the adult body. Multipotent stem cells, which are tissue-specific stem cells, like blood stem cells, skin, and neural stem cells, can turn into the cell types within those tissues. You undergo this process of gradual specification as you build out the differentiated functional cell types within those tissues. So, a lot of developmental biology and stem cell biology is focused on the question of how—what are the signals, processes, and mechanisms by which you become progressively more specialized or differentiated towards particular tissues and then ultimately, particular cell types? How does fate get specified, or how does a stem cell maintain itself as a stem cell, instead of getting

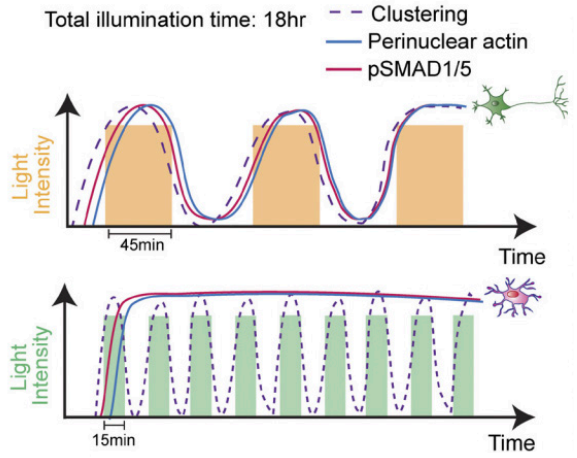

Figure 2: Frequency-dependent activation of Rho GTPases renders differential effects in neural stem cells. Cells exposed to high-frequency activation of RhoA showed promotion of astrogenesis compared to cells subjected to low-frequency stimulation of RhoA, which experienced higher neurogenesis.

differentiated? How is that balance between stem cell maintenance and stem cell division versus controlled differentiation? How does that balance get orchestrated in order to build out a full tissue and create all the differentiated cell types you need within that tissue? That's a set of questions that occupies the stem cell and developmental biology field.

Are there any misconceptions along the way? I would say that as you describe work to the lay public, there are some ethical considerations that come into play. For a while, stem cell biologists were thought to harvest stem cells from very ethically problematic materials, especially in the United States. However, a lot of the stem cells we use right now do not involve working with embryos at all.

Originally, embryonic stem cells were created from embryos, but there are nearly a half million frozen embryos in the US right now because people have been doing in vitro fertilization (IVF) for over 45 years. If you do IVF, a number of embryos are created and several of them are implanted into the uterus. But there are a lot of "leftover" embryos that are frozen down in vials of liquid nitrogen for years or decades. At some point along the way, it raises the question, what are we going to do with all of these? In many cases, parents opt to dispose of the embryos because they do not need them anymore. Alternatively, embryos have been donated to science to generate embryonic stem cells. Those embryonic stem cells are used for both research to discover fundamentally new findings about stem cell biology and human developmental biology, or they are used for therapeutic application, where companies like Bayer and Vertex are differentiating them into dopamine neurons or beta cells to try to cure patients with Parkinson's or Type 1 Diabetes. A lot of good in the world has come from the occasional donor who has decided to donate embryos to science or medicine rather than disposing of them. I think that is a big misconception, that all stem cell research involves manipulating embryos.

BSJ: Your lab's research has found that Rho GTPase, a regulatory enzyme, signaling is involved with membrane protrusion frequency. Could you explain this phenomenon and its implications?

DS: We are trying to discover what signals outside the stem cell tell the blank slate what to do, regulate the behavior of the stem cell, and control their differentiation. Biochemical or soluble signals are interesting to us, but given our background in engineering and physics, we are really interested in solid phase and mechanical signals. If you take a mechanical signal and apply it to a cell, ultimately a stem cell makes a fate decision to execute one program of gene regulation versus another. So, gene regulation is kind of a biochemical event—this gene turns on, and that gene turns off. A mechanical signal eventually gets translated by the cell into a biochemical decision– it is going to express this set of transcription factors or that set of transcription factors. It is going to become a neuron or an astrocyte. There has to be some point along the way where that mechanical information is translated into biochemical information, and that transition is something that has been a fascinating question for our lab for many years.

As part of a collaboration between my and Professor Sanjay Kumar's labs, Dr. Rocío Sampayo* began to investigate this molecule called Rho GTPase, which has been known in the field for a number of years to be involved in mechanotransduction, the process of transmitting a mechanical signal into the cell. It was a molecule that we naturally started studying to see whether it played that role within our stem cells—with viscosity, stiffness, and elasticity cues. Dr. Sampayo found this really interesting observation that if you take a stem cell and place it on a stiff surface, it continuously sends out little protrusions from its membrane and samples the environment with a high frequency. On the other hand, if you stick it on a soft surface, it sends out these slower protrusions, which has a lot to do with the interplay of the mechanics of the substrate with the mechanics of the cytoskeleton inside the cell. Fast membrane protrusions were correlated with rapid oscillations in RhoA, a type of Rho GTPase, activity, and the slow protrusions were associated with slower oscillations in RhoA activation. That is a biochemical difference. That is, the mechanical difference gets translated into the frequency of oscillation of activity of an enzyme, RhoA. That is a correlation, but it does not establish causality.

How does fate get specified, or how does a stem cell maintain itself as a stem cell, instead of getting differentiated? How does that balance between stem cell maintenance and stem cell division versus differentiation?

Several years ago we created a way to control RhoA activity with light, as part of a field known as optogenetics. We showed for the first time that if you shine light on an engineered RhoA fusion protein, you can control the activation of RhoA with light. If we can do that, we can now take two stem cells in the exact same conditions, and with one of them we can oscillate light illumination really fast to drive fast RhoA oscillation, and the other one we can do it slower. Dr. Sampayo did that and showed that if you integrate the area under the curve, the total level of RhoA activation in both cells is the same because the light is on half the time and off half the time, only the frequency is varying. We discovered that fast frequencies cause differentiation into astrocytes, and slow frequencies cause differentiation into neurons. We essentially discovered that the ability of mechanics to regulate neural stem cell fate was frequency encoded at the level of RhoA activation. As engineers, we love words like stiffness, elasticity, frequency, and temporal response. These are ways in which physics can control or regulate life. So that was a particularly gratifying discovery for us.

BSJ: In your paper on how spectrin mediates 3D-specific matrix stress-relaxation, what is ECM stress-relaxation? What are its implications in development, cell behavior, and possibly disease?

DS: The extracellular matrix (ECM) is a set of typically high molecular weight molecules that self-associate to create a solid phase environment for tissues and cells inside our body. For example, collagen is both a solid phase material as well as the most prevalent protein inside the body. It creates what is in our knees and allows for the flexible joint materials and mechanical structures.3

There are many other ECM proteins, such as laminin and fibronectin, as well as proteoglycans—protein-sugar complexes like heparan sulfate and chondroitin sulfate—that are also part of the ECM. These proteins associate with one another and with cells, forming the solid phase of a lot of tissue. This is important because they surround and support many of the cells within our body. All the solid phase cells inside of our body are exposed to ECM, and in the process a multitude of cues are conveyed. From cell receptors to adhesion receptors on the cell surface, they connect with the mechanical properties of the extracellular matrix. These adhesion receptors end up being part of the cell's ability to sense the solid phase, and in turn, to sense mechanics. In that sense, the ECM is incredibly important for both our integrity as three dimensional organisms and our ability to withstand stresses that come with mechanical movement. They also are really important components of the environment that surrounds the cell and regulates the behavior of a cell. As I mentioned earlier, these solid phase components have both elasticity, viscosity, or stress relaxation associated with them, and we are interested in how both of those properties regulate cell biology. Stress relaxation—related to viscosity—is the process of ECM molecules sliding past each other in response to an external force, analogous to how a material like honey changes shape in response to force.

BSJ: Could you elaborate on how the 3D-specific stress-relaxation response differs from 2D environments in terms of its influence on neural stem cell lineage commitment?

DS: Initially, the experimental systems that people developed to study the effects of mechanics on cell behavior were 2D. We typically create a cross-linked polymeric material of a certain viscoelastic set of properties, stiffness as well as a stress relaxation. By adding peptides or proteins on the surface of that material that enable it to bind to adhesion receptors in the cell, the cell can then engage with the mechanical properties of that material.

Initially, it was easier to do this in 2D because we had a certain set of materials like polyacrylamide that the field could work with, where you could vary stiffness. Working in 3D was challenging because it involved mixing the cells with monomers and polymerizing the gel around them. This process was difficult for researchers as it would constrain the cells, inhibiting their growth and division. They were not only experiencing the mechanical properties, but also the effects of confinement, as if the cells were trapped in tiny prison-like spaces. In contrast, in the body, these matrices constantly remodel to create space for cells to move and grow. In 2D, we are studying the effect of a cell sitting on top of this viscous material, but once you embed the cell inside of 3D, you expose the cell to mechanical cues and confining stress.

For several years, we studied behavior in 2D but eventually realized we were missing a significant factor: confining stress. In 2D, when we were studying viscosity, we started seeing that viscous materials tended to turn neural stem cells into astrocytes, and on non-viscous materials at the same stiffness, they differentiated into neurons. Whereas when we went into 3D we started seeing this confining stress behavior, and the confining stress was causing the cells to become astrocytes. This was a reversal where the viscous material that was stress relaxing were differentiating cells into neurons instead of astrocytes. This means

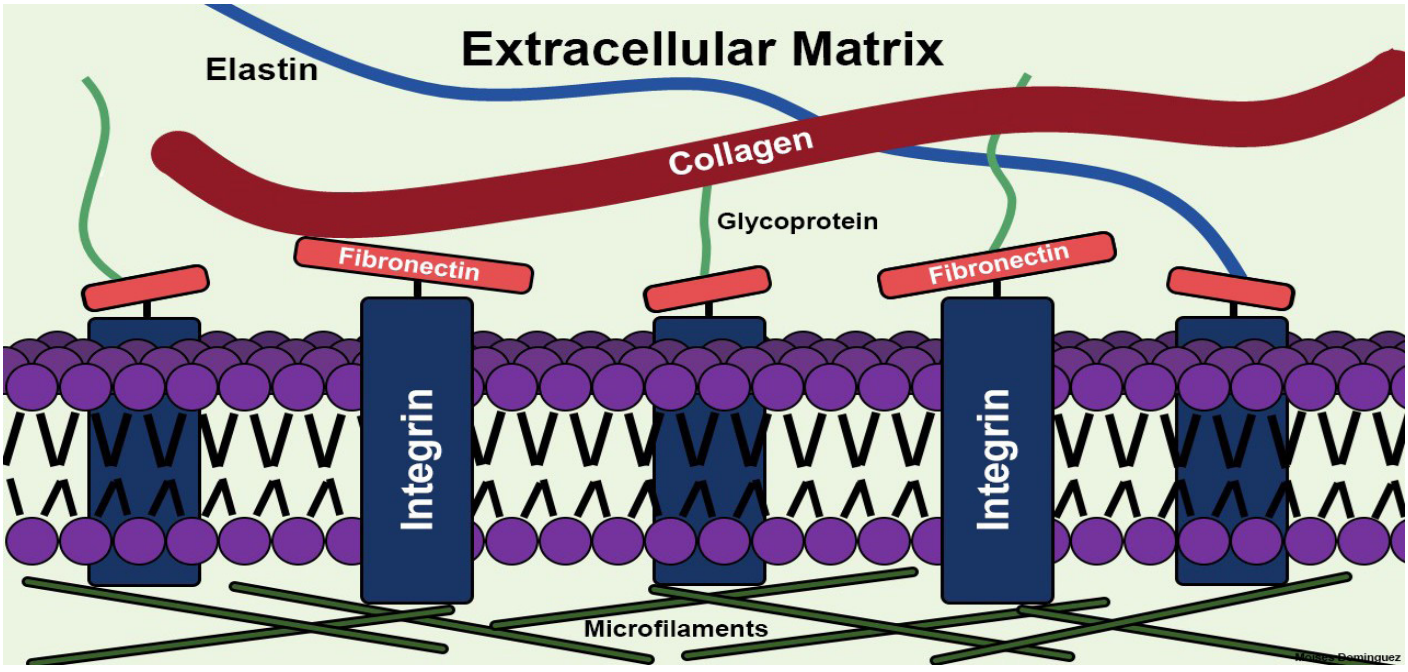

Figure 3: Extracellular Matrix with Collagen Structure. Collagen is shown forming strong fibers providing tensile strength, while the elastin allows tissue to stretch and return to its original shape. The depicted glycoprotein and fibronectin, plays a crucial role in connecting collagen and elastin within the extracellular matrix (ECM).

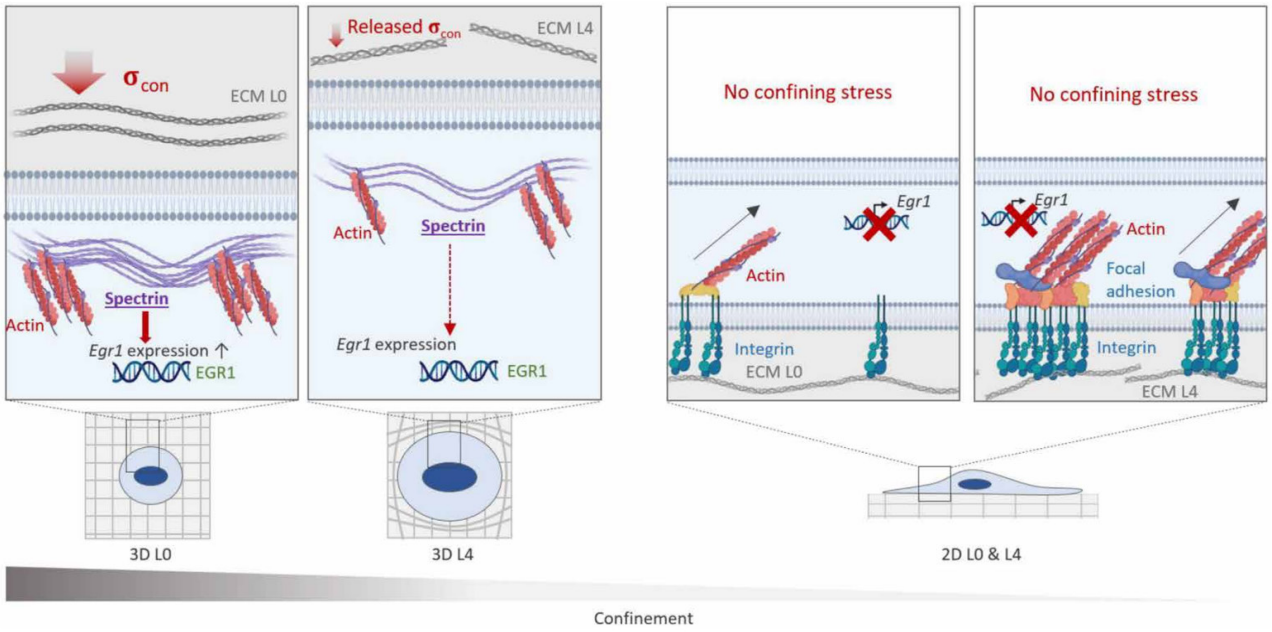

Figure 4: Model of 3D and 2D Cell Behavior Under Presence of Confining Stress. Depicts how in 3D environments, where confining stress cannot be relaxed over time, there is a build up-regulation of spectrin and EGR1 expression, as opposed to 2D environments where there is not appreciable confining stress.

that the 3D outcome was exactly opposite to what was observed in 2D, as a result of the absence of confining stress behavior in 2D models. Nevertheless, I do not think that 2D results were necessarily incorrect. Rather, we were studying how different environmental factors impact cell behavior, and both models provided valuable insights. With the inclusion of the 3D models we can see additional effects that are a realistic part of our bodies. Due to us being three dimensional organisms, we were unable to fully understand how the material cues regulated cell behavior in 2D models.

With the inclusion of the 3D models we can see additional effects that are a realistic part of our bodies. Due to us being three dimensional organisms, we were unable to fully understand how the material cues regulated cell behavior in 2D models.

BSJ: In the context of developing more accurate disease models or designing biomaterials for regenerative medicine, how do you envision leveraging these mechanosensitive properties to control stem cell behavior in therapeutic settings?

DS: Developing more accurate disease models or creating cell replacement therapies are two potential applications of stem cells. For example, Type 1 Diabetes involves the loss of β-cells or insulin producing cells. We can use stem cells as a renewable source of β-cells, which can be implanted into patients with Type 1 Diabetes. The other important application of stem cells is modeling human diseases. For example, for Huntington's disease, you can derive stem cells from a patient's cells, then differentiate them into neural cells or neurons to study the disease's mechanisms.

BSJ: What do you see as the next steps for your research? Are there any gaps or unexplored areas that you believe future studies should focus on to fully understand how mechanical cues drive cell fate decisions?

DS: My father's research was fundamental, focused on uncovering new insights into how biology works. In contrast, my mother's work was highly translational, aimed at getting a drug approved for osteoporosis. In my lab, I strive to ensure that every project moves in both directions—advancing our understanding of fundamental biological mechanisms while also bringing us closer to clinical development and drug treatments for disease. We recently uncovered how the mechanical properties of tissues can influence cell behavior, a mechanism not previously known in the brain. We aim to further explore this pathway, which likely involves signals traveling from the extracellular matrix (ECM) to a cell adhesion protein, then to the cytoskeleton, leading to RhoA activation, and finally, impacting a transcription factor we have identified.

The next questions that Professor Kumar, our students and fellows, and I are exploring include which transciption factors are involved, what genetic programs they activate, and which promoters they bind to. These fundamental, mechanistic questions will deepen our understanding of how mechanical properties of the ECM instruct cell behavior. At the same time, one of my companies is focused on how the environment and the signals from the environment regulate cell behavior for the purposes of scaling in a bioreactor to mass produce cells to treat human disease. The idea is that if we can learn enough about how those signals work, we should be able to incorporate those signals into a controlled bioreactor so stem cell differentiation can be

scaled into something that's going to be useful in the clinic. Those are the two directions that I see for our future, and I am very excited about them.

ENDNOTES

* Dr. Rocio Sampayo is a Post Doc at University of California, Berkeley in the Department of Chemical and Biomolecular Engineering. She is the first author of "Mechanosensitive stem cell fate choice is instructed by dynamic fluctuations in activation of Rho GTPases," one of the two articles this interview centered on.

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