SIMULATING CELLS IN CONTEXT: Bringing Mechanics Into Play

By Sarah Webb, PhD

Like humans, cells are affected by their physical environment, their neighbors, the context in which they exist. Much research has focused on the chemical signals that control cell behavior. But increasingly researchers are finding important roles for physical interactions between cells and their surroundings as well. Recent computational models are helping researchers better understand this complex choreography and how it affects cell differentiation, tumor formation, and embryonic development.

"There is a lot that computational power can do for you to predict the behavior of experiments and also to really understand what is happening *in vivo*," says **Muhammad Zaman, PhD**, assistant professor of biomedical engineering and medicine at Boston University.

Context Guides Cell Differentiation

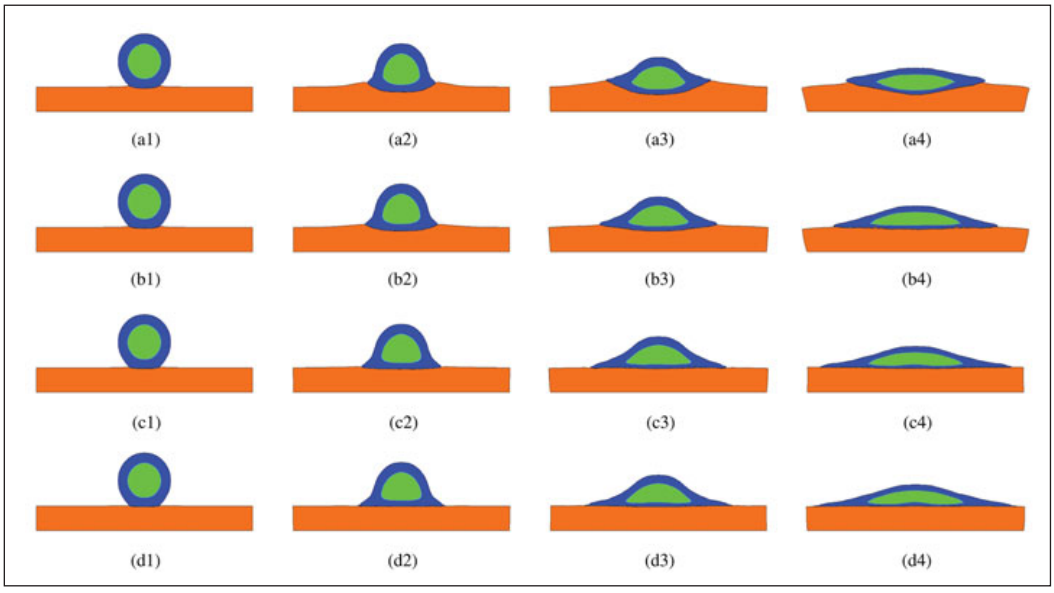
In a 2006 *Cell* paper, researchers at the University of Pennsylvania demonstrated experimentally that the elasticity of the matrix in which the cells were grown—without any other chemical signals—could determine the differentiation of mesenchymal stem cells. Soft surfaces prompted these stem cells to differentiate into neurons, stiffer substrates produced muscle, while the most rigid matrix gave rise to bone cells. These differences correlate with the elasticities observed in each of these tissue types.

This result prompted researchers at the University of California at Berkeley to build a novel computational model of the mechanical interaction between cells and substrates with differing elasticities. Previous cell models have treated the cell as a lipid bilayer—the so-called fluid mosaic model. **Shaofan Li, PhD**, professor of computational mechanics at Berkeley, and his colleagues instead treated the cell as two layers of bulk "soft matter" using mathematically derived measures of stiffness for the cytoplasm and the nucleus. The cells then move like a liquid but with a crystalline phase in it, which mimics the cytoplasm which is rich in F-actin filaments. Combining this soft matter model with an algorithm describing contact and adhesion, the team illustrated how cells flatten as they come in contact with increasingly rigid substrates. "If the stiffness of the extracellular substrate is different, then the spreading area will be different. Not only is the spreading area different, but the shape of the nucleus is different," says Li. These results mirror the experimental findings in the 2006 *Cell* paper. The work, published last year in the *Journal of the Mechanical Behavior of Biomedical Materials*, could help explain the mechanotransduction process between the stem cell and its environment and how the cell environment affects stem cell differentiation.

Context Guides Cell Clustering

Interactions between cells also help to explain their clustering behavior, which is important for tumor migration and wound healing. But this component of the process is poorly understood, Zaman says. So he and his colleagues combined modeling data from single cells with parameters developed from their experimental observations to simulate movement patterns in clusters of cells over a period of minutes to hours. The results, which were published in the *Annals of Biomedical Engineering* in July, provide a way to predict cell behavior based on factors such as the shape of cell clusters.

The work is just a first step and does not include many of the complexities of *in vivo* behavior such as chemical signaling. "The next step is to try to mimic the tumor microenvironment," he says, incorporating factors that are specific to a breast tumor or a brain tumor. Then they would like to add in hydrodynamics, factoring in the motion of blood vessels and angiogenesis. Those additional details, he says, will be critical steps toward building a tumor model that an oncologist could use to predict cancer behavior in individual patients.



Berkeley researchers modeled cell spreading over four substrates with different stiffness. Courtesy of Shaofan Li. Reprinted from Zeng, X and Li S, Multiscale modeling and simulation of soft adhesion and contact of stem cells,* J Mech Behav Biomed Mater, *4:180-189 (2011) with permission from Elsevier.

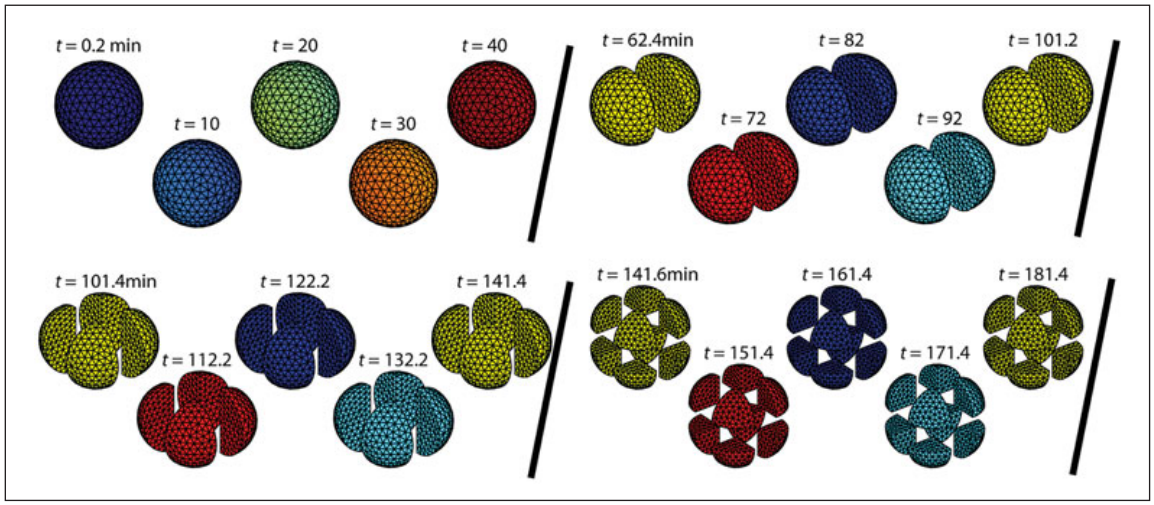
Context Guides Cell Division in an Embryo

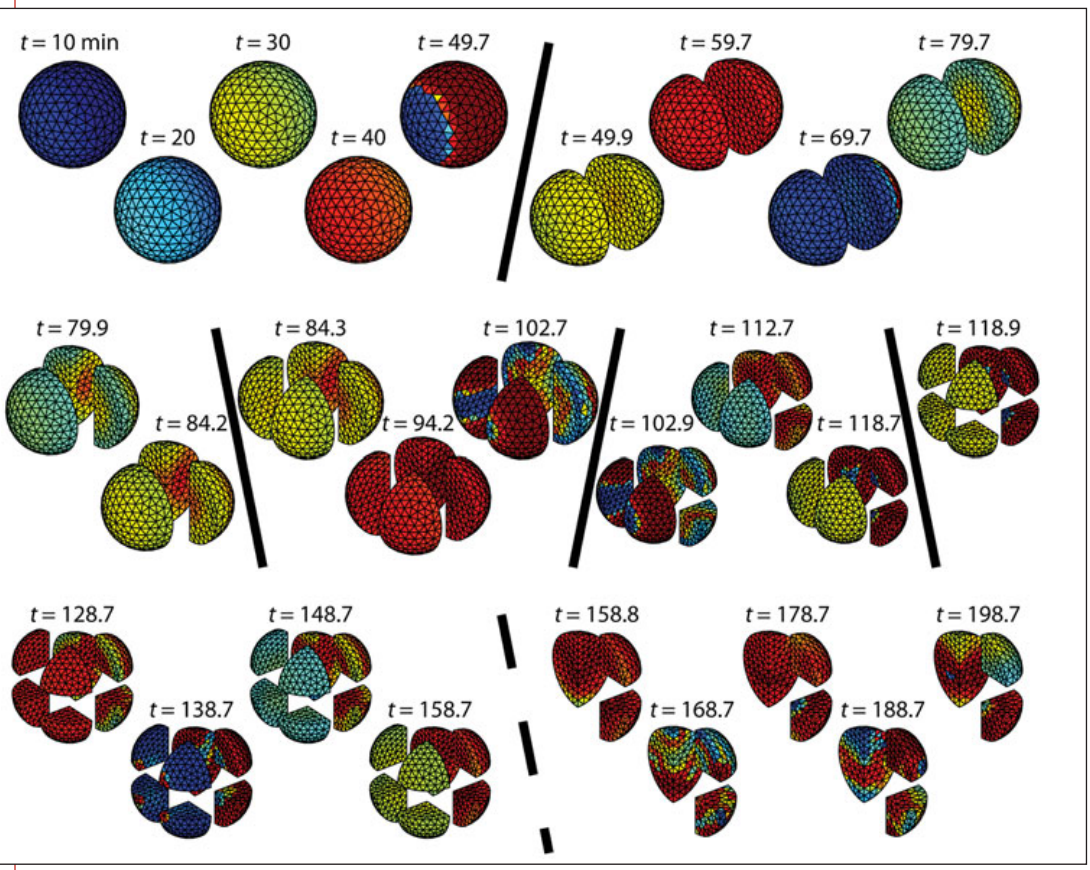
Another unique cellular environment, the embryo, relies on chemical and mechanical signals to develop from a fertilized egg. As this process begins, cell divisions are synchronized, but researchers haven't understood exactly how those divisions are

so perfectly coordinated.

Computational researchers at Princeton University recently addressed this question with a model that examined this process within embryos from African clawed frogs (*Xenopus laevis*). Cells in these embryos have relatively simple cycles of cell division, driven by oscillations in the activity of the protein Cdk1. One possible explanation for the synchronization is that cells pick up chemical timing cues from their neighbors. But when **Ned Wingreen, PhD**, professor of molecular biology, and his colleagues simulated this type of slow signal traversing a growing embryo, they observed that these Cdk1 oscillations were not perfectly coordinated as in a normal embryo. Instead cell division spiraled into a chaotic process, one that could not lead to well-ordered development.

"Why doesn't the real system become chaotic?" Wingreen asks. Their model showed that the synchronization is likely triggered is by a totally separate, one-time mechanism. To serve as the synchronization signal, this signaling wave has to be faster than the rate of diffusion. The calcium wave that is known to propagate through the embryonic system at the time of fertilization is the only known signal that's fast enough, Wingreen says. He and his colleagues propose that this one-time, fast release of calcium serves as the perfect chemical switch needed to set up the synchronized Cdk1-driven cell-cycle oscillation within a developing *Xenopus* embryo, much as electrical signals coordinate the contractions of the heart muscle. Their results were published in *PLoS Computational Biology* in July.





Ned Wingreen and his colleagues at Princeton University modeled the effects of a fast and a slow signal to prime the cell division oscillation in a developing* Xenopus *embryo. A fast calcium wave leads to ordered cell divisions (top), while a slowly diffusing Cdk1 signal from the cells' neighbors produces a chaotic system (bottom). Reprinted from McIsaac RS* et al.*, Does the potential for chaos constrain the embryonic cell-cycle oscillator?,* PLoS Comput Biol *7(7); e1002109 (2011).

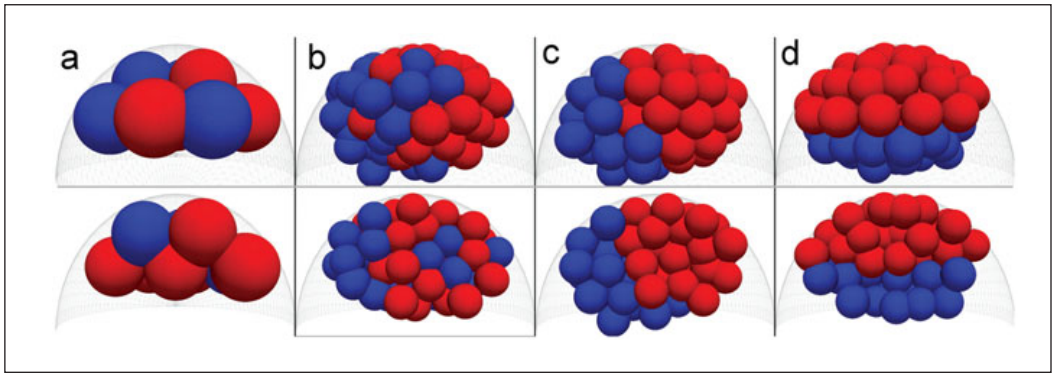
Context Guides Cell Orientation in the Embryo

In addition to timing cell divisions, a developing embryo has to orient cells to form appropriate patterns. One such pattern is the formation of the embryo's outer layer or trophectoderm, which encapsulates the inner cells (the endoderm) that eventually differentiate into developing tissues. **Carsten Peterson, PhD**, professor of computational biology and biophysics at Lund University in Sweden, and his colleagues have combined both genetic and mechanical factors to produce a model of blastocoel formation—the first 4.5 days of embryonic development in mammals.

During the first few days of development, the volume of the early embryo cell mass is largely fixed. Although gene expression exerts most of the control over the sequence of events during that time, Peterson and his colleagues proposed that the mechanics of several key cell types interacting in an increasingly crowded space would be important as well. To test that theory, they modeled each cell as a single entity with mechanical and elastic properties within the defined volume, says **Pawel Krupinski, PhD**, postdoctoral researcher in Peterson's lab and the first

author of the paper. On top of that, they included a handful of genes, concentrations of expressed proteins and the interactions between those genetic products. They found that geometric organization, a mechanical factor, can influence the direction of division or the polarization of the cell. At the same time selected genes can affect the elasticity or adherence properties of the cell. "This connection works both ways: the genes can influence the mechanics and the mechanics can influence the genes," he says. This model, which was published in *PLoS Computational Biology* in May, provides the most inclusive simulation of these factors developed to date.

Even with this progress, researchers are just at the initial stages of building models of how cells respond to their environments. Further work will rely on close collaborations between computational and experimental researchers. "We're just scratching the surface," Zaman says. "I hope more people start to look at modeling complex behaviors of *in vivo* environments and go beyond what we already know." □



Carsten Peterson and his colleagues at Lund University in Sweden have combined genetic and mechanical signals to accurately simulate the layering of cells in the early embryo. Here, two different views (external above and cross-sectional below) show various simulations including (a) the pre-set "salt & pepper" pattern of the two cell types that determine cell orientation (GATA6 in red; NANOG in blue) next to the blastocoelic surface (gray); (b) the effect of random movements alone; (c) the effect of setting different adhesion properties for each cell type (a layer forms but positioning isn't stable or in the right place); and (d) the addition of stronger adhesion between the NANOG cells and the surrounding trophectoderm, which stabilizes the endoderm in the correct position next to the blastocoel. Reprinted from Krupinski, P, et al., Simulating the Mammalian Blastocyst—Molecular and Mechanical Interactions Pattern the Embryo, PLoS Comput Biol 7(5)1-11(2011).

DE NOVO PROTEIN DESIGN: Designing Novel Proteins that Interact

By Sarah Webb, PhD

By stringing together amino acids in a prescribed sequence that then folds into a defined structure, nature designs proteins to perform specific functions. Nowadays, computational researchers are doing some protein designing of their own—and it's bearing some valuable fruit.

The goal is to come up with new proteins to perform specific functions and recognize or bind to specific substrates, says **Jeffery Saven, PhD**, professor of chemistry at the University of Pennsylvania. "What matters is what they can do and what they can recognize," Saven says.

In nature, proteins acquire changes to their sequence of amino acids that lead to new functional forms. In the lab, researchers make chemical changes to an amino acid sequence and test it to see whether it functions in ways they can understand. But by taking the initial design work *in silico*, researchers can simplify the experimental workload by honing in on candidates worthy of laboratory work. Essentially, researchers computationally create a multitude of novel amino acid sequences, predict and build models of the new proteins' likely structures, and model or simulate how they will interact with other molecules.

Although this process is no easy matter, progress is being made, as described in a recent review by Pantazes *et al.* in *Current Opinion in Structural Biology*. Most notable, perhaps, are the efforts aimed at modeling binding to other proteins and designing new enzymes.

Designs For Binding With Hot Spots

Protein design requires overcoming the difficult challenge of getting a novel protein to bind to another protein at the correct site, in the correct orientation, and with high affinity. To address that problem, **David Baker, PhD**, professor of biochemistry at the University of Washington, and his colleagues developed a new and generalizable approach that focuses on a specific patch on a defined target. They computationally place disembodied protein side-chains next to the patch to determine how they interact in the hotspot. Only when they are satisfied with those interactions do they attach it to a protein scaffold with a shape that is complementary for anchoring the hotspot proteins. They then use computational methods to recalculate the energies and make other adjustments aimed at ensuring the appropriate "hotspot" contacts. The researchers also employ a experimental strategy, "yeast display" which expresses designed