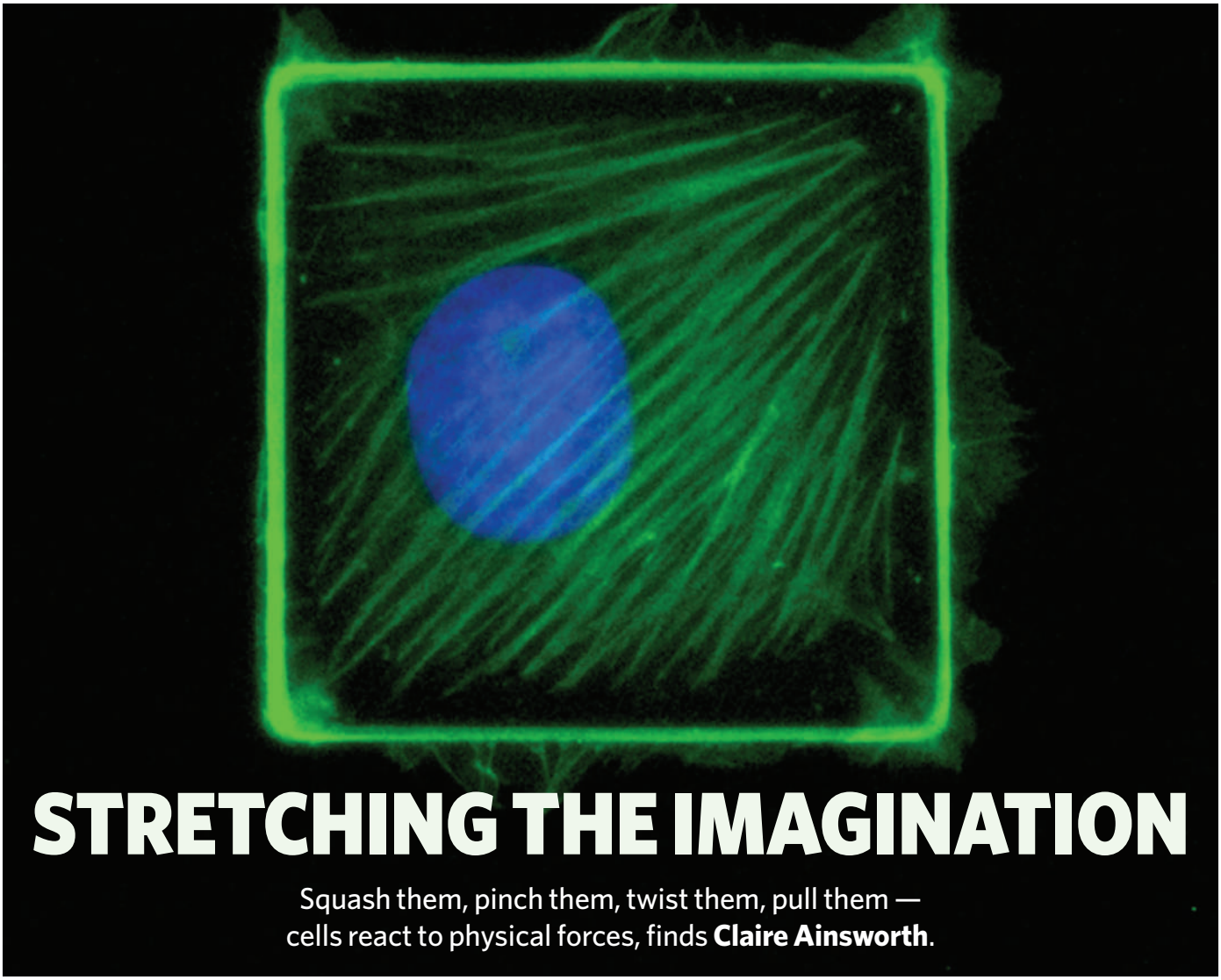


C. CHEN & D. INGBER

STRETCHING THE IMAGINATION

Squash them, pinch them, twist them, pull them — cells react to physical forces, finds **Claire Ainsworth**.

The lecturer flattened a tangle of sticks and elastic on to the desk. He let go, and the structure pinged back into shape. It was a demonstration of 'tensegrity', a term constructed by the engineer Buckminster Fuller for situations in which push and pull have a 'win–win relationship' with each other. Fuller mashed the word together — from the components tensional integrity — to describe the way sculptor Kenneth Snelson used taut wires and stiff poles to make strong yet flexible monuments.

Among the students, Donald Ingber could see the ingenious engineering in the sculptures — but he also saw biology. Ingber was an undergraduate in molecular biophysics at Yale University; the course in three-dimensional design had seemed apt. But what he saw there changed the course of his professional life. At that time, in the late 1970s, researchers were publishing the first scientific papers describing how cells are propped up by an internal scaffolding, or cytoskeleton. "I immediately thought: 'Oh, so cells must be tensegrity structures'," Ingber says. On returning to the lab, he eagerly explained this idea to one of the postdocs, who was less than impressed. "He told me: 'Just never mention that again'," Ingber recalls.

Then, as now, most cell biologists had little time for architecture and engineering. When they want to understand why a cell behaves the way that it does, they try to identify the genes, proteins and signalling molecules that are thought to exert control. But to Ingber there was an obvious gap between the dramatic events that mould a developing embryo and the molecular explanations that were given for them in his developmental-biology class. "What I saw before my eyes was something that was incredibly physical, mechanical in nature: twisting, bending, folding," he says, "and then I got into cell biology, and it was all chemical."

Thirty years on, work from Ingber's group and many others has started to convince cell biologists to embrace the missing physics. Their findings are remarkable. Pull a stem cell in one way and it starts developing as a brain cell; stretch it in another, and a bone cell is its more likely fate. Change the mechanical stresses on cancer cells and they can start to behave more like healthy ones. Among this work's implications, few are more important than the consequences for cell therapy and tissue engineering, in which researchers hope to use new cells to repair damaged organs. If these cells encounter the 'wrong' kinds of mechanical stresses, they could conceivably end up doing more harm than good.

The discoveries are giving biologists a fresh appreciation of the body's physical nature. Hearts pump, muscles stretch, blood surges, feet pound. And on the microscopic scale, fluids flow and cells jostle with their neighbours. When Ingber, now at Harvard University, talks about his ideas today, he doesn't get quite the same frosty reception that he once did. "There's no doubt," he says, "in the past five years it has exploded."

Popular mechanics

Even in the 1970s, the ideas were not entirely new. The importance of mechanical forces was appreciated by embryologists in the 1800s and early 1900s, long before they had signalling proteins and chemical gradients to play with. Swiss biologist Wilhelm His, for example, experimented with metal, clay and rubber to try to mimic events in development, such as

how the future brain starts forming as a roll of tissue on the back of a mammalian embryo. "To think that heredity will build organic beings without mechanical means is a piece of unscientific mysticism," he wrote in 1888 (ref. 1).

The molecular biology revolution of the 1960s onwards pushed much of this aside, as researchers focused on genes and proteins. But the mechanical ideas never fell completely out of favour. Physiologists know that astronauts' bones get thinner when they escape gravity, and that hefting weights inflicts physical damage on muscle cells that stimulates them to grow. But it was widely felt that the role of mechanical stress would be limited to these and other cell types that needed it in order to function normally.

Then, in 1978, Judah Folkman and Anne Moscona at Harvard Medical School published one of the first studies to experimentally stretch mammalian cells — in this case, cells extracted from cow's blood vessels and other tissues. They coated plastic culture dishes with various concentrations of a sticky polymer and grew the cells on top. The stickier the substrate, the flatter the cells stretched out, and the more the cells stretched out, the more they divided. It was considered a landmark paper — one of the first to show that cell shape influences growth[2]. And it had a "profound effect" on Ingber when he read it that year. "It resonated with my sense that physicality was critical to developmental control," he says. Ingber later contacted Folkman and eventually went on to complete a postdoc in his lab.

Researchers now know that almost all human cells test the mechanical properties of their microenvironment in the body, and use it to adjust their growth. This is determined by the extracellular matrix, a lattice of proteins and other molecules to which cells in solid tissues anchor themselves like a horde of old canvas tents staked out at a rock festival. Instead of poles and ropes, cells have their internal cytoskeleton. This includes a mesh of fibres made up of actin protein that lines the cell's membrane, plus tough 'actomyosin bundles' in which actin combines with the protein myosin II. The tent pegs in this case are proteins called integrins that span the cell membrane, gripping the actomyosin filaments inside the cell and the extracellular matrix on the outside.

In 2006, Ingber's team used a femtosecond laser to cut the actomyosin filaments and found that they immediately retract, revealing that they are under stress just like the tensed wires in Snelson's sculptures[3]. The role of the poles seems to fall to another part of the cytoskeleton, the microtubules, which buckle under severe stress.

Bioengineers, meanwhile, have attempted to measure the elasticity of various tissues in the body. The simplest way involves hanging a weight from a hunk of tissue and measuring how much it stretches. More sophisticated methods include atomic force microscopy, which uses a cantilevered tip to lightly prod cells and measure their springiness.

Last year, bioengineer Paul Janmey and his colleagues at the University of Pennsylvania, Philadelphia, used atomic force microscopy to show that connective tissue cells called fibroblasts can tune their internal stiffness to match that of the substrate on which they are growing[4]. "That was actually quite a surprise," says Janmey. He thinks that this type of surveillance could help cells respond to change in the tissue around them. Imagine a cell that is normally pegged down on all sides in the skin — but whose moorings are weakened when the skin is cut. The physical change could be felt just as fast, if not faster, than chemical signals released by the wound. "The cell already knows that mechanically, something has gone haywire and can respond to that," says Janmey.

Ingber says that this idea is already being used in hospitals to promote healing, an idea he is working on in collaboration with Dennis Orgill, a plastic surgeon at Harvard's Brigham and Women's Hospital. Orgill seals a sponge-like device over hard-to-heal wounds such as those left by deep surgery, or diabetic foot ulcers. He then attaches this to an oscillating suction pump. The suction stretches the cells so that they divide, form new blood vessels and regenerate skin tissue, thus healing the wound[5] — the same type of behaviour that Folkman observed in his cultured cells 30 years ago. Ingber says the system works better (and is far cheaper) than artificial skin or synthetic growth factors.

K. JOHNSON

"What I saw before my eyes was something that was incredibly physical, mechanical in nature."
— Donald Ingber

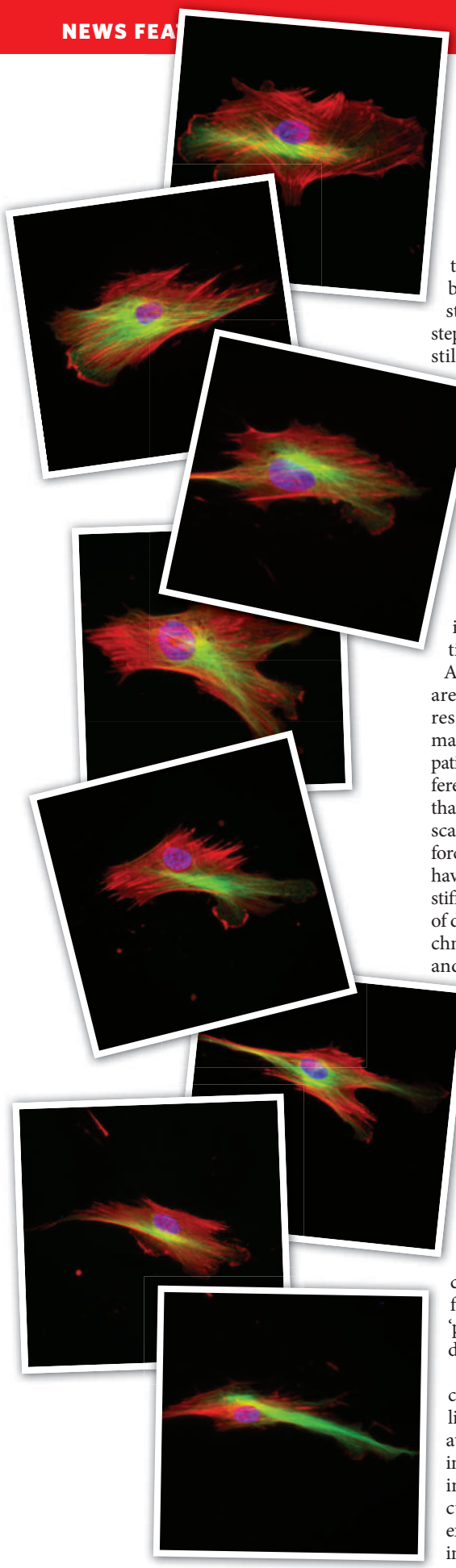
Eschewing growth factors

Monitoring the physical environment could also serve a purpose during development, by helping cells to detect where they are, and to migrate, divide or differentiate appropriately. This has required a particular change in thinking for many biologists. The field has long been dominated by the idea that developmental decision-making is directed by the chemical signals inherited from a cell's parent, or received from its neighbours and the environment. That way of thinking leads researchers to encourage stem cells to form heart muscle cells, neurons or other cell types simply by adding cocktails of proteins known as growth factors.

In 2006, cell and molecular biophysicist Dennis Discher at the University of Pennsylvania reported he had done away with growth factors and allowed force to trigger a change in cell fate[6]. His team studied mesenchymal stem cells, a kind of cell that normally grows in the soft, fatty bone marrow where it generates bone progenitors, but that is also thought to move to other locations and give rise to a range of cell types including nerve and muscle. They grew the cells on gels made from polyacrylamide and collagen that mimicked the softness of bone marrow. By changing the degree of chemical crosslinking in the polyacrylamide, the team was able to alter the stiffness of the gel to be more like that of different body tissues.

F. LEMMENS/ALAMY

Cells may share the quality of 'tensegrity' with the constructions of sculptor Kenneth Snelson.



Day in the life: a stiff substrate converts stem cells into bone cells over 24 hours.

A. ZAJAC

On a relatively soft base that resembled the sponginess of brain tissue, the stem cells began to form the precursors of neurons; on stiffer, muscle-like substrates the cells took steps towards forming muscle stem cells; and on still stiffer substrates resembling developing bone they started to become bone-forming cells. The team still had to add growth factors to get the cells to differentiate fully, but by the time the stem cells had become comfortable for three weeks on their soft, medium or hard mattresses, growth factors that would normally get stem cells to switch between one developmental pathway and another had little effect.

Findings such as these have important implications for regenerative medicine, Discher says. A number of clinical trials are now under way in which researchers inject mesenchymal stem cells into the hearts of patients who have previously suffered a heart attack, in the hope that the cells will help repair the scarring caused by the attack. By using atomic force microscopy, Discher and his collaborators have found that in rats this kind of scar tissue is stiffer than normal heart muscle, resembling that of developing bone[7]. And last year, Bernd Fleischmann of the University of Bonn, Germany, and his colleagues found that mesenchymal stem cells formed bony spurs after they were injected into the scar tissue of damaged mouse hearts[8]. Although there are no published reports of human patients developing these problems, "You have to think about the microenvironment that you put cells in," Discher says.

The new appreciation of cells' mechanical environment is also a complication in the lab. A number of studies have revealed how poorly cell-culture conditions mimic those that a cell would encounter in real tissue. Mammalian cells grown on a typical, flat, glass or plastic tissue culture dish, for example, do not develop the directional 'polarity' that they do in real tissue, but they do when grown in three-dimensional gels[9].

Many groups are now developing sophisticated cell-culture methods that have more lifelike mechanics. Melody Swartz, a bioengineer at the Swiss Federal Institute of Technology in Lausanne (EPFL), and her team are studying the effects of 'interstitial flow': the gentle current to which almost all human cells are exposed as the fluid that bathes them seeps into the lymphatic system, the body's drainage network. The team has found that fibroblasts align themselves perpendicular to this flow during wound healing and inflammation. Swartz thinks that interstitial flow distributes chemical signals through tissue, and that cells may be so accustomed to its mechanical presence that they are lost without it. "They need the functional aspects — the mechanical environment — to act normally," she says. Her team has recently shown that the lymphatic cells themselves, which are notorious for losing many of their characteristic cell functions in culture, regain their character if she subjects them to flow.

"I don't think mechanobiology is a separate field from biology," says Swartz. "It's something you can either choose to consider or not, but it is always present." Not all labs are considering it — those that have switched to three-dimensional culture systems are still a minority, partly because cells grown that way are harder to study with standard microscopy and other techniques. But if mechanical stress has yet to be fully appreciated by cell biologists, it is becoming hard to ignore among developmental ones. As Ingber observed, nowhere are physical forces more apparent than in the developing embryo where tissues twist, fold and writhe into the beginnings of adult tissues and organs.

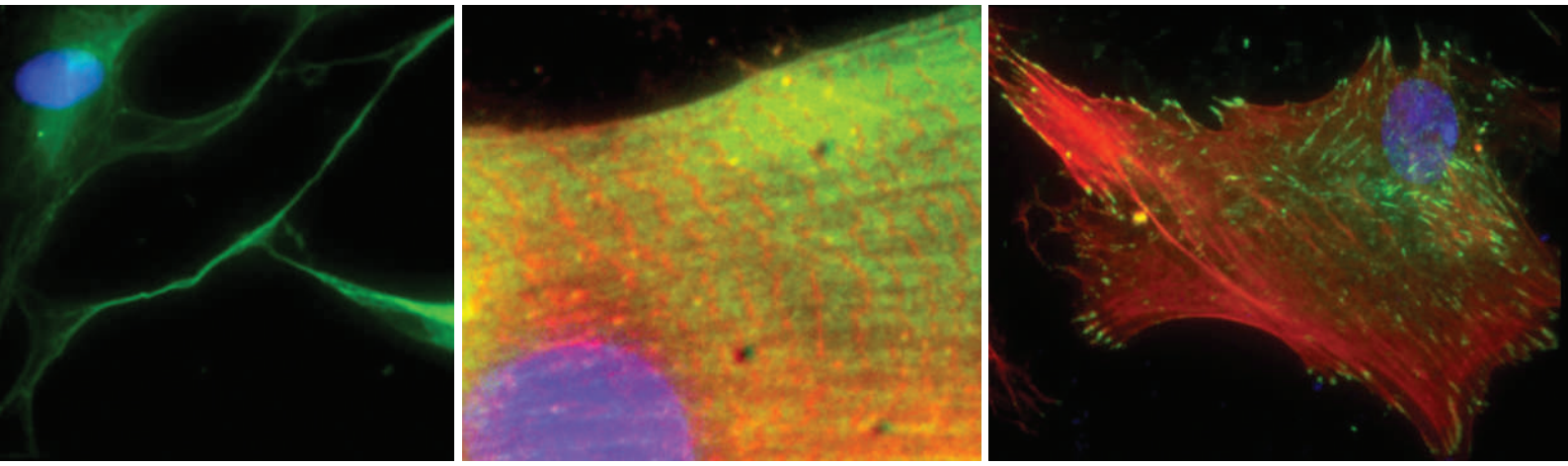
> **"The genome must be aware of the shape it is in charge of developing."**
> — Emmanuel Farge

Let's twist again

In 2003, biophysicist and developmental biologist Emmanuel Farge at the Curie Institute in Paris gently squashed entire fruitfly embryos under a tiny sheet of glass, and found that they switched on a gene called *twist* in nearly all their cells, instead of the particular subsets where it should be expressed[10].

Farge has since shown that the normal compression of tissue that occurs as the embryo changes shape is required to switch on this gene in the nascent gut[11]. First he removed the cells that normally exert pressure on the gut as it grows, and showed that *twist* expression dropped. Then he devised a way to artificially exert a tiny force inside these embryos by injecting magnetic nanoparticles and tugging on them with an electromagnet. The expression of *twist* was restored.

Such studies challenge the idea that an embryo's shape and patterning is driven only by a genetic program, suggesting instead that shape and patterning can also drive gene expression. This mechanical control could provide cells with feedback on their changing position in the growing embryo. "The genome must be aware at key stages of the shape it is in charge of developing," says Farge. It also means that genes acting in two physically separate tissues — such as those that control tissue compression, and those that control *twist* — can still interact. This



Mesenchymal stem cells grown on soft, medium or rigid matrices start developing into (left to right) neurons, muscle cells and bone cells.

A. ENGLER

kind of action at a distance, Farge speculates, might be involved in an embryo-wide, "global" system that coordinates development.

Given its importance in influencing cells in embryonic and adult tissues, it should come as no surprise that mechanical environments are now thought to contribute to disease. Even five years ago, says cell biologist Valerie Weaver, at the University of Pennsylvania, "there was a lot of scepticism" when she presented her work investigating links between tissue stiffness and cancer. "I know there was a little bit of snicker." But now a whole body of work has shown that manipulating the extracellular matrix in the cancer's microenvironment can switch normally dividing cells into excessively dividing, cancerous ones and vice versa[12].

Weaver and her colleagues investigated how mechanical forces on the outside of the cell could be converted into cancer-promoting signals on the inside — one of the key questions in the field[13]. Her team used a machine known as an electromechanical indentor, which presses on tissue in culture, to show that cancerous mammary tissue is stiffer than healthy tissue. Then they grew normal mammary cells in gels stiffened to different extents with collagen, so that they resembled either healthy tissue or tumours. Mammary cells grown in soft gels organized themselves into structures characteristic of normal breast tissue, whereas cells grown in stiff 'cancerous' gels did not. They found that the stiff gels pulled more on the membrane-spanning integrins, and this boosted the activity of an integrin-controlled signalling pathway that regulates tension in the cytoskeleton.

Crushing cancer

Weaver suggests that many oncogenes — genes which, when mutated, predispose a cell to becoming cancerous — can also activate biochemical pathways that increase internal tension. And in unpublished work, Weaver and her colleagues have shown that these changes may be some of the earliest events in cancer. They found that the extracellular matrix surrounding cells that harbour mutated oncogenes becomes stiffer even before those cells form invasive tumours, because the constitution of the extracellular matrix is changing. "That microenvironment is changing dramatically long before you get a tumour," says Weaver.

If her ideas are correct, then interfering with the mechanics of a cancer cell might override aberrations in its genes. And that's what Weaver found in her 2005 study: when she added chemicals that block integrin signalling to the cells grown in stiff gels, they grew into more normal-looking mammary-gland tissue. These signalling pathways might make a possible cancer drug target. And Ingber is pursuing the idea that cancer might be reversed by physically manipulating a tumour's microenvironment by, for example, implanting artificial materials that mimic the structural, mechanical and chemical properties of healthy tissue.

> **"Cells need the mechanical environment to act normally."**
> — Melody Swartz

Ingber is also becoming more and more convinced that cells behave like Snelson's deformable sculptures. When he used a laser to break a single actin fibre, the shape of the entire cell altered to accommodate the change[3]. So, just like other tensegrity structures, the cytoskeleton can transmit a force from one point through the entire assembly. These mechanical signals are transmitted faster than a chemical signal can diffuse across a cell[14], and Ingber thinks that such mechanical changes may physically deform other molecules attached to the cytoskeleton, including many associated with the cell's metabolism. This cascade of events could eventually cause a dramatic change in cell behaviour[15].

Ingber goes even further, suggesting that living creatures are a Russian doll of tensegrities. On the largest scale, muscles tense against bones. Inside the body, the forces in these and other tissues are picked up by integrins and relayed to the cytoskeleton. The cytoskeleton itself is made up of macromolecular structures that are themselves tensegrities at the molecular level. Such a hierarchy of systems, he says, could explain how mechanical signals such as gravity or movement are transmitted from the macro to the micro and nano scales.

In the past two years, Ingber may have found the means to test his ideas. In 2007 he received seed money to establish a Harvard Institute for Biologically Inspired Engineering. And in October 2008, philanthropist Hansjörg Wyss gave US$125 million towards it. Looking back, Ingber says, "I was lucky to have my first 'Aha moment' as an undergraduate at a liberal arts college. In the United States, undergraduates can explore any field, and hence know no bounds between biology, chemistry, physics, architecture or art."

It is a boundlessness he tries to convey to the students he teaches today. Those who visit his office find that it is filled with tensegrity models. He even has a small Snelson sculpture on the window sill, one that the artist gave him. And in the classroom, some Harvard undergraduates are already exploring how to develop expandable lightweight structures based on tensegrity and cell architecture. But these are not designs for beautiful sculptures — they are water carriers for the developing world. ■

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