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# Healing touch: the key to regenerating bodies

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YOU started life as a single cell. Now you are made of many trillions. There are more cells in your body than there are stars in the galaxy. Every day billions of these cells are replaced. And if you hurt yourself, billions more cells spring up to repair broken blood vessels and make new skin, muscle or even bone.

Even more amazing than the staggering number of cells, though, is the fact that, by and large, they all know what to do - whether to become skin or bone and so on. The question is, how?

"Cells don't have eyes or ears," says Dennis Discher, a biophysical engineer at the University of Pennsylvania in Philadelphia. "If you were blind and deaf, you'd get around by touch and smell. You'd feel a soft chair to sit on, a hard wall to avoid, or whether you're walking on carpet or concrete."

Until recently, the focus was all on "smell": that is, on how cells respond to chemical signals such as growth factors. Biologists thought of cells as automatons that blindly followed the orders they were given. In recent years, however, it has started to become clear that the sense of touch is vital as well, allowing cells to work out for themselves where they are and what they should be doing. Expose stem cells to flowing fluid, for instance, and they turn into blood vessels.

What is emerging is a far more dynamic picture of growth and development, with a great deal of interplay between cells, genes and our body's internal environment. This may explain why exercise and physical therapy are so important to health and healing - if cells don't get the right physical cues when you are recovering from an injury, for instance, they won't know what to do. It also helps explain how organisms evolve new shapes - the better cells become at sensing what they should do, the fewer genetic instructions they need to be given.

The latest findings are also good news for people who need replacement tissues and organs. If tissue engineers can just provide the right physical environment, it should make it easier to transform stem cells into specific tissues and create complex, three-dimensional organs that are as good as the real thing. And doctors are already experimenting with ways of using tactile cues to improve wound healing and regeneration.

Biologists have long suspected that mechanical forces may help shape development. "A hundred years ago, people looked at embryos and saw that it was an incredibly physical process," says Donald Ingber, head of Harvard University's Wyss Institute for Biologically Inspired Engineering. "Then when biochemistry and molecular biology came in, the baby was thrown out with the bath water and everybody just focused on chemicals and genes."

While it was clear that physical forces do play a role - for example, astronauts living in zero gravity suffer bone loss - until recently there was no way to measure and experiment with the tiny forces experienced by individual cells. Only in the past few years, as equipment like atomic force

microscopes has become more common, have biologists, physicists and tissue engineers begun to get to grips with how forces shape cells' behaviour.

One of the clearest examples comes from Discher and his colleagues, who used atomic force microscopy to measure the stiffness of a variety of tissues and gel pads. Then they grew human mesenchymal stem cells - the precursors of bone, muscle and many other tissue types - on the gels. In each case, the cells turned into the tissue that most closely matched the stiffness of the gel.

The softest gels, which were as flabby as brain tissue, gave rise to nerve cells. In contrast, gels that were 10 times stiffer - like muscle tissue - generated muscle cells, and yet stiffer gels gave rise to bone (*Cell*, vol 126, p 677). "What's surprising is not that there are tactile differences between one tissue and another," says Discher. After all, doctors rely on such differences every time they palpate your abdomen. "What's surprising is that cells feel that difference."

The details of how they do this are now emerging. Most cells other than blood cells live within a fibrous extracellular matrix. Each cell is linked to this matrix by proteins in its membrane called integrins, and the cell's internal protein skeleton is constantly tugging on these integrins to create a taut, tuned whole. "There's isometric tension that you don't see," says Ingber. In practice, this means changes in external tension - such as differences in the stiffness of the matrix, or the everyday stresses and strains of normal muscle movement - can be transmitted into the cell and ultimately to the nucleus, where they can direct the cell's eventual fate.

Since stem cells have yet to turn into specific cell types, biologists expected them to be extra sensitive to the environment, and this does indeed seem to be the case. Ning Wang, a bioengineer at the University of Illinois at Urbana-Champaign, found that the embryonic stem cells of mice are much softer than other, more specialised cells. This softness means that tiny external forces can deform the cells and influence their development (*Nature Materials*, vol 9, p 82).

For instance, if stem cells are exposed to flowing fluid, they turn into the endothelial cells that line the inner surface of blood vessels. In fact, fluid flow - particularly pulses that mimic the effect of a beating heart - is proving crucial for growing replacement arteries in the laboratory. The rhythmic stress helps align the fibres of the developing artery, making them twice as strong, says Laura Niklason, a tissue engineer at Yale University. A biotech company Niklason founded, called Humacyte, has begun animal testing on arteries grown this way.

Surprisingly, pulsatile motion can help heal injuries in situ too. At Harvard, Ingber and his colleague Dennis Orgill are treating patients with difficult-to-heal wounds by implanting a small sponge in the wound and connecting this to a pump. The pump sucks the cells surrounding the wound in and out of the sponge's pores, distorting them by about 15 to 20 per cent - an almost ideal stimulus for inducing the cells to grow and form blood vessels and thus boost the healing process, says Ingber.

Meanwhile, tissue engineers are finding that they can grow far better bone and cartilage by mimicking the stresses that the tissues normally experience in the body. For instance, human cartilage grown in the lab is usually nowhere near as strong as the real thing. Recently, however, Clark Hung, a biomedical engineer at Columbia University in New York City, has grown cartilage that matches its natural counterpart strength for strength. The secret, he has found, is rhythmically squeezing the cartilage as it grows to mimic the stress of walking.

The secret of growing cartilage that is as strong as the real thing is to mimic the effects of walking

Hung says this is partly because the pressure helps to pump nutrients into cartilage, which has no blood vessels. But his experiments suggest that the loading alone also plays an important role. His team hopes the engineered cartilage will eventually be used to resurface arthritic human joints.

Even relatively mild stresses make a big difference. Attempts to grow replacement bone by placing stem cells in a culture chamber of the desired shape have not been very successful, with the cells often dying or producing only weak bone. But Gordana Vunjak-Novakovic, a biomedical engineer also at Columbia, has found that mimicking the internal flow of fluid that growing bones normally experience helps maximise strength. Last year, her team used this approach to successfully grow a replica of part of the temporomandibular joint in the jaw from human stem cells, producing a naturally shaped, fully viable bone after just five weeks.

"If you don't stimulate bone cells, they don't do much," says Vunjak-Novakovic. "But if you do, they wake up and start making bone at a higher rate."

There is still a long way to go, however. The replica bone lacks the thin layer of cartilage that lines the real bone, and it also lacks a blood supply, so it begins to starve as soon as it is removed from the culture chamber.

Again, though, the answer could be to provide the cells with the right physical cues. For example, Vunjak-Novakovic has used lasers to drill channels in the scaffolds used to grow heart muscle in the lab. When fluid begins flowing through these channels, endothelial cells move in to line the channels while muscle cells move away. "Each of the cells will find its own niche," she says. Her team is now testing to see whether stem cells will turn into endothelial cells in the channels and into muscle cells elsewhere. Early results suggest that they will.

Even small differences in forces can influence development. Christopher Chen of the University of Pennsylvania grew flat sheets of mesenchymal stem cells and exposed them to a mixture of growth factors for bone and marrow development. The cells on the edges of the sheets, which were exposed to the greatest stresses, turned into bone cells, while those in the middle turned into the fat cells found in marrow, as in real bone (*Stem Cells*, vol 26, p 2921).

If this kind of sorting-out according to physical forces is widespread in development, it could be very good news for tissue engineers. Instead of having to micromanage the process of producing a replacement organ, they need only to provide the right cues and let the cells do the rest.

If tissue engineers provide the right physical cues when growing organs, cells will do the rest

Indeed, it makes a lot of sense for some developmental decisions to be "devolved" to cells. The growth of tissues like muscles, bone, skin and blood vessels has to be coordinated as our bodies develop and adapt to different activities and injuries. A rigid genetic programme could easily be derailed, whereas using tactile cues as guides allows tissues to adapt quickly as conditions change - for instance, carrying heavy loads will make our bones grow stronger.

This kind of plasticity may play a vital role in evolution as well as during the lifetime of individuals. When the ancestors of giraffes acquired mutations that made their necks longer, for instance, they did not have to evolve a whole new blueprint for making necks. Instead, the nerves, muscles and skin would have grown proportionately without needing further changes in instructions. The result of

this plasticity is a developmental programme that is better able to cope with evolutionary changes, says Ingber.

There is, however, a drawback. When disease or injury changes the stiffness of a tissue, things can go awry. Some researchers suspect that tissue stiffening plays a role in multiple sclerosis, in which nerves lose their protective myelin sheath (*Journal of Biology*, vol 8, p 78). It may also play a role in some cancers (see "Lumps and bumps").

It could also explain why many tissues fail to heal perfectly after an injury. To prevent infection, the body needs to patch up wounds as quickly as possible. So it uses a form of collagen that is easier to assemble than the normal one. "It's a quick patch, things are sealed off and you go on - but it's not perfect regeneration," says Discher. The quick-fix collagen is stiffer than normal tissue, as anyone with a large scar will tell you.

After a heart attack, for example, the dead portion of the heart muscle scars over. Why, Discher wondered, don't heart muscle cells then replace the scar tissue? To find out, he and his colleagues grew embryonic heart cells on matrixes of differing stiffness. When the matrix was the same stiffness as healthy heart muscle, the cells grew normally and beat happily. But if the matrix was as stiff as scar tissue, the cells gradually stopped beating (*Journal of Cell Science*, vol 121, p 3794).

The constant work of trying to flex the stiffer matrix wears the cells out, Discher thinks. "It's like pushing on a brick wall. Finally, they give up."

Discher believes the solution may lie in finding a way to soften the scar tissue so that heart cells can repopulate it. Several enzymes, such as matrix metalloproteinases and collagenases, might do the job, but overdoing it could be risky. "If you degrade the matrix too much, you lose the patch," he warns.

The stiffness of scar tissue may also prevent regeneration in nerve injury, because nerve cells prefer the softest of surroundings. "It might just be that the growing tip of the axon senses that there's a stiff wall ahead of it and doesn't grow through because of that," speculates Jochen Guck, a biophysicist at the University of Cambridge in the UK.

There is still a long way to go before we fully understand how cells sense and respond to the forces on them. But it is becoming clear that the touchy-feely approach could be the key to regenerating the body.

### Lumps and bumps

Many tumours are stiffer than the tissues in which they form - after all, doctors often first detect many cancers of organs such as the breast and prostate by feeling a hard lump. Some researchers now suspect that this stiffness is not always just a consequence of the cancer. It may be a cause as well.

A team led by Paul Janmey, a biophysicist at the University of Pennsylvania in Philadelphia, has found that the cycle of cell division in breast cells stops when they are grown on a soft gel, keeping them in a quiescent state (*Current Biology*, vol 19, p 1511). Anything that signals stiffness - even just touching a cell with a rigid probe - can be enough to start it dividing again.

Similarly, when Valerie Weaver, a cancer biologist at the University of California at San Francisco, and her team used chemicals to soften the extracellular matrix in which breast cells were growing in the lab they found the cells were less likely to become malignant (*Cell*, vol 139, p 891). If her findings are confirmed, they could explain why women with denser breast tissue are more likely to develop breast cancer.

Some researchers, too, have reported seeing tumours form around the scars from breast-implant surgery. "This needs to be looked at again," says Weaver. If the link is confirmed, it might be possible to block tumour growth by interfering with the way cells detect stiffness.