a solid foundation:

BIOMATERIALS GUIDE STEM CELL THERAPY

by Janelle Weaver

Kevin Healy is enchanted by biomaterials, right down to the props in his office. There's the vascular stent: a Teflon-covered, man-made tube that keeps arteries open. Then there's the Teflon-based vascular graft: a long tube that channels blood in patients with weakened vessels. These items are made of polymers, or large molecules consisting of repeating units, that are relatively harmless inside the body. Healy hopes to infuse them with stem cells, inject them, and repair damaged tissue in a wide range of organs, from hearts to brains.

A professor in the bioengineering and materials science departments at UC Berkeley, Healy is developing networks of polymers, called hydrogels, which act as a type of jungle gym for stem cells to grow on. Once injected into the body, they promise to restore damaged tissue without causing an immune reaction. Made of similar building blocks as cells and tissues, they're naturally broken down by the body, leaving little trace.

"Think of a hydrogel as a contact lens. It's dry—it's just a large molecule—but when you add water to it, it actually holds the water like a sponge," Healy says. A lab demo by graduate student Kimberly Kam makes that clear. When she injects a clear gel from a syringe into a flask of water, it looks stringy, like a piece of spaghetti. But when she pumps the same gel into a warmer body of water, it becomes stiff and opaque. The hydrogels are thermo-responsive "smart materials" that sense changes in their environment. If surgically injected as a liquid, they become stiffer when they warm to the body's temperature. Then they're ready to act as a scaffold for cells. By making the gel harder or softer, scientists can coax stem cells to become different types of cells.

Much past research has focused on identifying the chemicals that to determine a stem cell's fate. But the importance of mechanical forces is just starting to garner attention. Healy is one of a handful of scientists around the world who is developing materials to guide what stem cells become. Healy's lab is teaming up with chemical engineer David Schaffer and bioengineer Sanjay Kumar at UC Berkeley to pinpoint the molecules that sense mechanical properties, such as scaffold hardness, and translate these signals into a cell's fate. Together, the three labs hope to set the stage for safer, more effective stem cell therapies for a range of diseases, including heart disease, Parkinson's, Alzheimer's, retinal diseases, and spinal cord injuries.

Healy's hydrogels

The idea behind hydrogels is to mimic the normal environment found outside cells. Cells are arranged into tissues and organs by a sort of glue called the extracellular matrix (ECM), a network of proteins that anchor cells in place. Proteins hanging off of cells connect with the ECM, like kids' arms on monkey bars. The hydrogel has little snippets of ECM glued onto it; these bind to the proteins hanging off of cells and hold the cells in place.

Once injected into the body, the hydrogel changes shape and lets cells grow into it. The hydrogel also releases chemicals that help cells recover. Eventually the artificial

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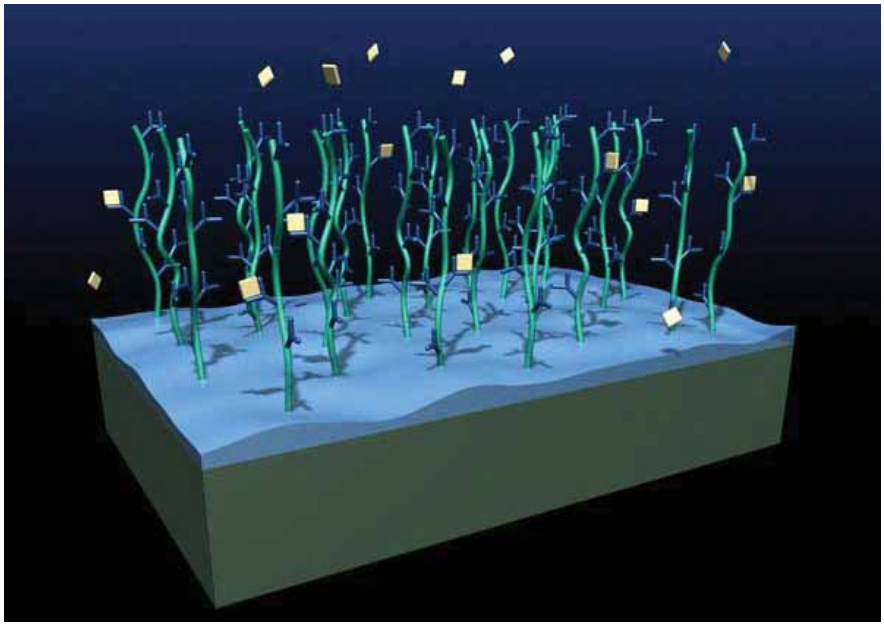
hydrogel is replaced by natural cells in the body. Hydrogels can stabilize damaged tissue and attract healthy cells.

Sometimes the hydrogel carries stem cells, which encourage growth of new tissue, and other times it releases drugs, which have a targeted effect at the site of injection. "We're putting the drug right at the site where the drug is needed," Healy says. One example is atropine, which is used to treat myopia, or nearsightedness. The hydrogel's structure helps hold the drug or drug delivery system in place. So far, the treatment has not been carried out in humans.

Healy's lab is fine-tuning the three-dimensional design of the hydrogel to increase the odds that it will stimulate new growth in damaged tissue. He hopes one day to translate his research to clinical applications.



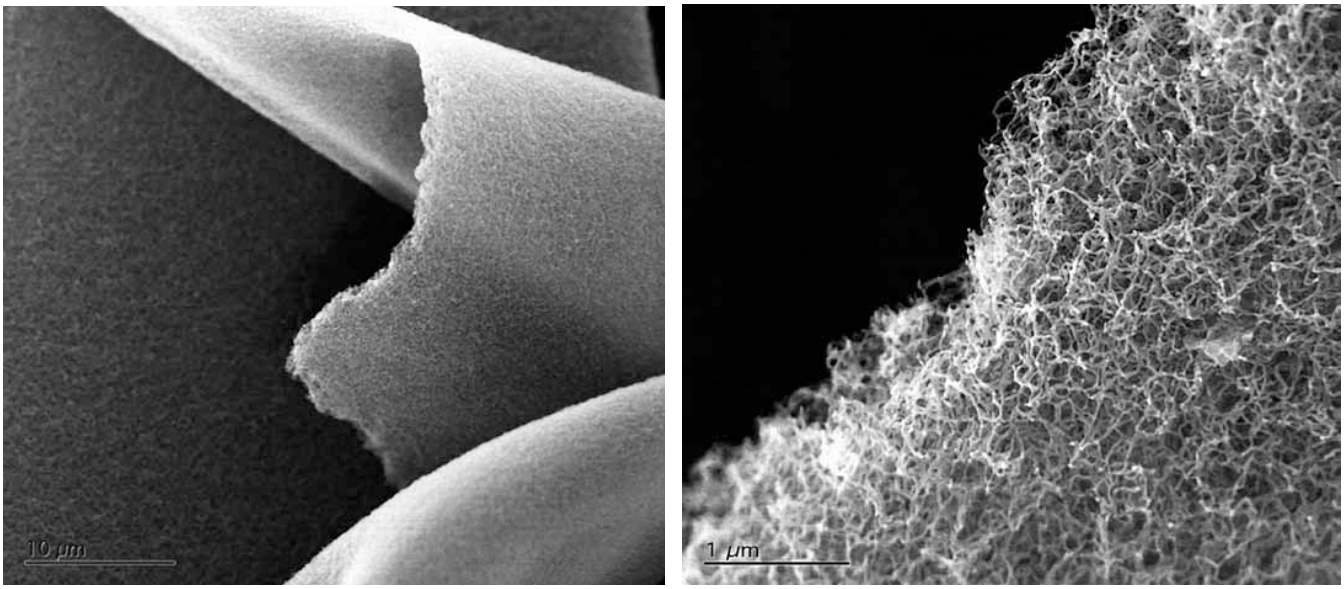
At the molecular scale, hydrogels are composed of long, brush-like polymers that absorb water molecules (yellow) and swell, softening the material into a gel (hence the name). Certain hydrogels absorb water at cold temperatures and expel it at high temperatures, so when they are warmed (after being implanted in the body, for example), they harden and form rigid structures ideal for tissue engineering.

Fickle stem cells

Human embryonic stem (hES) cells are derived from embryos and have the potential to become any cell in the body. They multiply until they are told to "differentiate" by extracellular signals and become tissue-specific cells. But this amazing ability poses a great risk: if the cells continue to divide uncontrollably after transplantation, they can lead to a tumor. Growing stem cells that are safe and effective for regenerative medicine is a huge challenge. In February 2009, scientists reported the first known case of a stem cell transplant that caused a tumor in a patient.

Hydrogels could help overcome the problems with stem cells in two ways. First, they could be used alone, without stem cells, to help repair damaged tissue. This would bypass all the ethical, medical, and technological challenges associated with stem cell research. Indeed, Healy is investigating whether hydrogels can help strengthen eye tissue in animal models of myopia. Second, hydrogels might help guide stem cells to the correct fate in a Petri dish, thereby ensuring a pure colony of cells that would not later form a tumor inside a patient. Then the hydrogels and accompanying stem cells would be injected into damaged tissue. For the latter option to work, it's essential to understand the chemical and mechanical cues that guide stem cell differentiation.

Despite some progress, stem cell research tends to advance in fits and starts. It works one day, it works for two weeks, it works for two months and then one day it stops. "You consider it a success when you can actually make differentiated stem cells in your lab, but it doesn't always mean that someone else will be able to repeat it," Healy says. Hydrogels, on the other hand, aren't as touchy because they are completely syn-



Scanning electron microscope images of hydrogels created at UC Berkeley. The porous structure of hyrogels forms a scaffold to which cells can attach.

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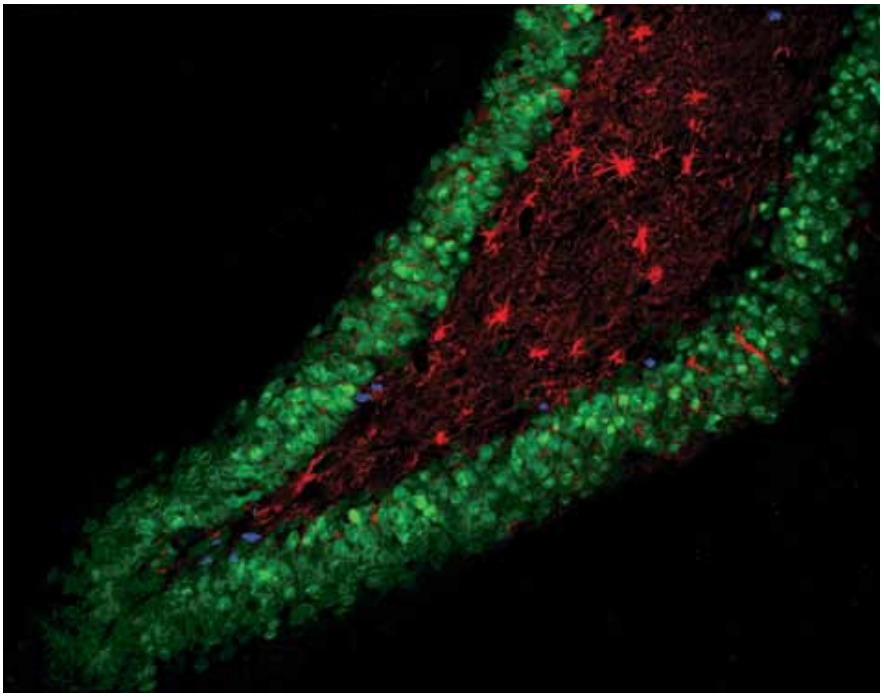


The adult brain contains neural stem cells that continually divide to generate new neurons throughout life. This image shows neurons (green), astrocytes (red), and stem cells (blue). If we learn enough about the signals that control the behavior of these cells, they could potentially be harnessed to regenerate neural tissue.

thetic. "Now, even though there's this push for using stem cells, if you could get the job done without stem cells, it's much easier from a practical and regulatory standpoint," Healy says.

Another problem with stem cells is that they typically don't survive and integrate with existing tissue once transplanted. In the case of cardiac therapies, "they sit there isolated by themselves," Healy says. "They're not electrically or mechanically coupled to the heart. They're not doing anything. They actually could be doing more harm if they're beating off cycle." He hopes hydrogels could help stem cells integrate with surrounding tissue by providing a scaffold that holds cells in place. Research in this area might make it possible to transplant stem cells into the heart so that they beat in synchrony with other heart cells.

Translating firmness to fate

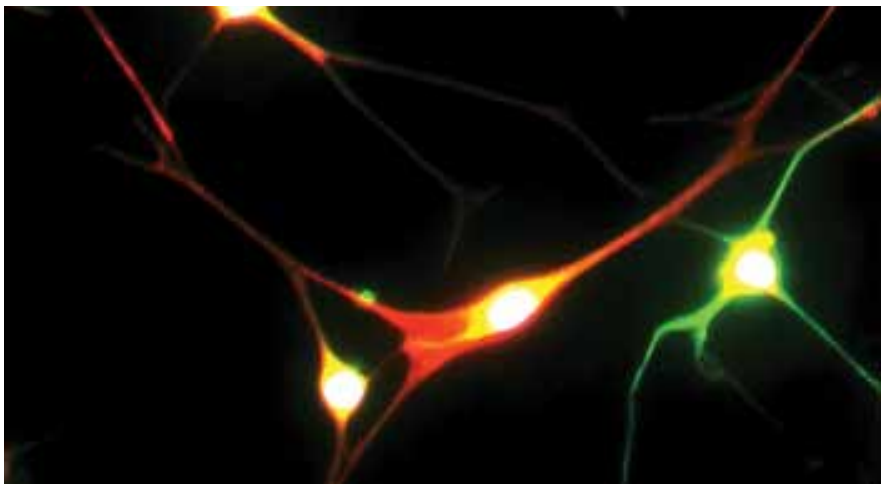
Healy, Schaffer, and Kumar are doing everything they can to get stem cells to behave. They hope one day to inject hydrogels filled with stem cells into patients with neurodegenerative diseases and spinal cord injuries. But first, they must figure out how stem cells choose their fates in a dish.

The first report that gel stiffness influences the fate of stem cells came in 2006 from Dennis Discher's lab at the University of Pennsylvania. Then, in 2008, Schaffer and Healy were the first to report that neural stem cells respond to stiffness. Harder hydrogels are more likely to produce glial cells—cells in the brain that form tough scar tissue. Soft hydrogels more often lead to neurons. How the physical environment in a dish influences the fates of stem cells sheds new light on past experiments, which typically ignored the role of mechanical forces.

The fact that stiffness affects stem cell fate may seem surprising to those outside the field, but there were previous clues in biology. You may think that cells are mechanically insulated from the environment, but even cells inside the brain pulsate as an aftereffect of a beating heart. Bones remodel in response to forces placed on muscles and ligaments. Scar tissue and tumors are harder than surrounding brain tissue. Even different cell types within a brain region have different levels of stiffness. Many cells are constantly pushing and pulling on their environment. "We think that this process of mechanical loading has something to do with what those cells will eventually become," Kumar says.

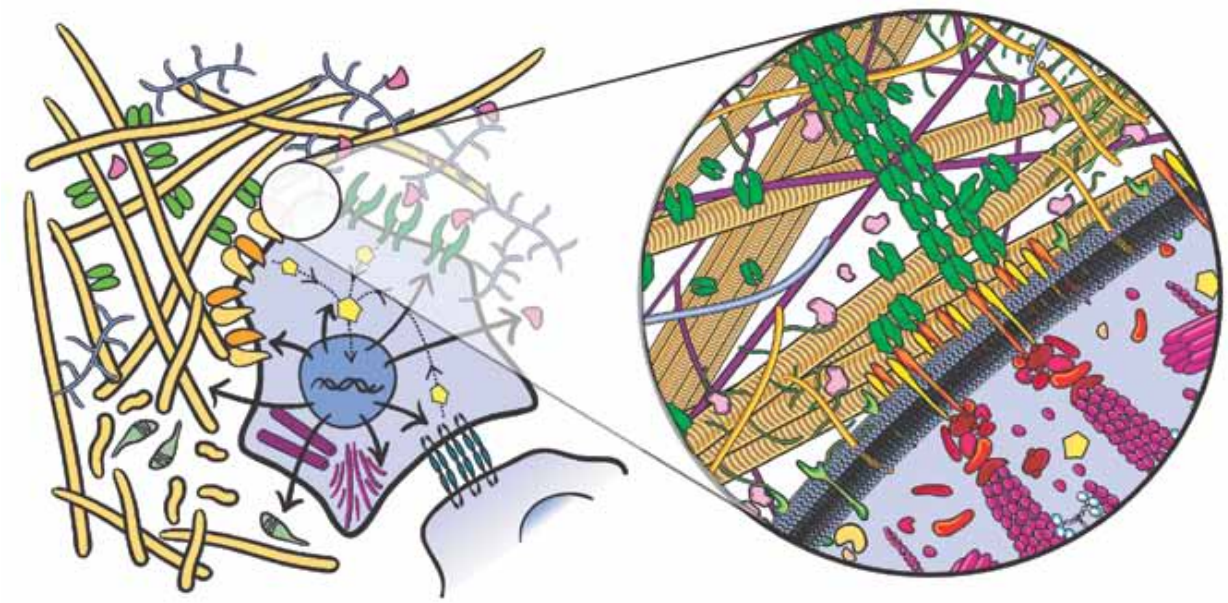
Both chemical cues and mechanical stiffness affect a stem cell's choices. If you add a chemical that tells the stem cell to become a specific type of cell, making a stiffer matrix will help tip the balance, making the differentiation more reliable. If you add another chemical that tells cells to replicate themselves, an intermediate stiffness will work best. "Just figuring out how to control cell behavior by providing the appropriate chemical cues is something that the field is working out," Kumar says. "To take the next step, to do this mechanically, is quite challenging but could potentially be very rewarding."

When it came to pinpointing which molecular pathway translates mechanical cues to fate choices, the answer was easy. Schaffer's team dug through previous studies and zoomed in on Rho GTPases because of their role in changing the mechanics and shape of the cell. "We've begun to march down the pathway on the inside of the cell and ask how mechanical information is conveyed, molecule by molecule, from the



Neurons (green) and astrocytes (red) that were differentiated from adult rat neural stem cells grown on a peptide-grafted hydrogel surface. Neural stem cells respond to the stiffness of the medium, with harder gels generating astrocyte differentiation, and softer gels generating neurons.

FROM TOP: DAVID SCHAFFER; LAUREN LITTLE



By incorporating proteins for cell adhesion (green and pink) directly into the hydrogel matrix (yellow), cells can more readily attach to the hydrogel matrix via complementary proteins on their membranes (yellow and orange). These adhesion proteins also act as signals for stem cells, telling them about the tissue environment and what kind of cell to become. This causes stem cells to produce more tissue-specific proteins which are secreted into the hydrogel and help to further define the new tissue.

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cell surface to the nucleus, where ultimately the decisions about cell fate can be made," Schaffer says.

While most past research has focused on how freely moving chemical cues can affect stem cell fate, Schaffer and Healy look at the role of molecules, such as ECM snippets, which are fixed onto the hydrogel matrix. The advantage of such unmoving cues is that they can present the cell with spatially organized patterns. These patterns cause receptors on the cell surface to cluster differently, which changes how cells respond to signals in their environment, as well as to mechanical forces inside the cell. Schaffer and Healy are now examining how fixed patterns may help turn hES cells into neural cells. By manipulating the physical environment of the stem cells, scientists may gain more control over determining their fate and reducing the threat of tumor formation.

The future of hydrogels

Hydrogels show great promise because they are safe—most do not trigger immune reactions—and they can easily be mass produced. But this is not the case with stem cells. Up to a billion cells might be needed for a single patient. "That's an enormous number of cells compared to what we typically deal with in the lab," Healy says. Before stem cell–laced biomaterials can reach the clinic, scientists must overcome several hurdles. "There's no real clinical output yet," Healy says. "I think we're still far away from that."

To date, most work examining the effects of stiffness on stem cell differentiation has been done in cell culture dishes, but it remains to be seen how this will translate to tissues in the body. "As scientists, we forget that cells live in the body, not in a Petri dish," says Theo Palmer, a neurologist at Stanford University. "It's really difficult to say that all the work we do in the very constrained environment of the Petri dish really represents the range of cellular responses that occur in living, behaving organisms." Clinical adoption will also require the development of new procedures that direct stem cell–laden hydrogels to the damaged tissue. "It's one thing to make a hydrogel in the lab," says Kumar, "but how do you get it to the site where it's needed? It would be nice to inject a liquid that hardens once it reaches the site of action."

Early therapies may involve the injection of hydrogels, either alone or with a drug, into diseased tissues to slow degeneration, Palmer says. Next would be to incorporate adult stem cells into hydrogels. "This therapy could be applied right away to treat stroke or spinal cord injuries," Palmer says. "The lowest hanging fruit is to deliver cells, not to restore the damaged circuitry, but to have them be healthier and happier after transplant and protect the local environment a little better."

Ideally, scientists would like to incorporate hES cells into these hydrogels because they have the potential to become myriad cell types that could restore damaged neural circuitry or tissue. That's harder, because scientists will need to make sure all the stem cells become the right cell type; undifferentiated cells can lead to a tumor. What's more, the cells would have to integrate properly with the existing circuitry. "If you pattern the stem cells first and mix them with the right kind of scaffold, that would promote neurons to survive after transplantation," Palmer says. "Then that becomes really exciting."

The idea of using hES cells to treat diseases never would have occurred to Healy when he first began his scientific endeavors. As an undergraduate, he was captivated by artificial hearts, and his project involved fabricating vessels using plastic. He pointed out to his adviser that the blood kept clotting in the plastic replica; then he asked him why. The response: "Nobody knows how to make something that will work in the human body."

By developing an innovative gel that mimics the natural cellular environment, guides the fate of stem cells, and may repair damaged hearts, Healy and his collaborators sure have brought biomaterials a long way.

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