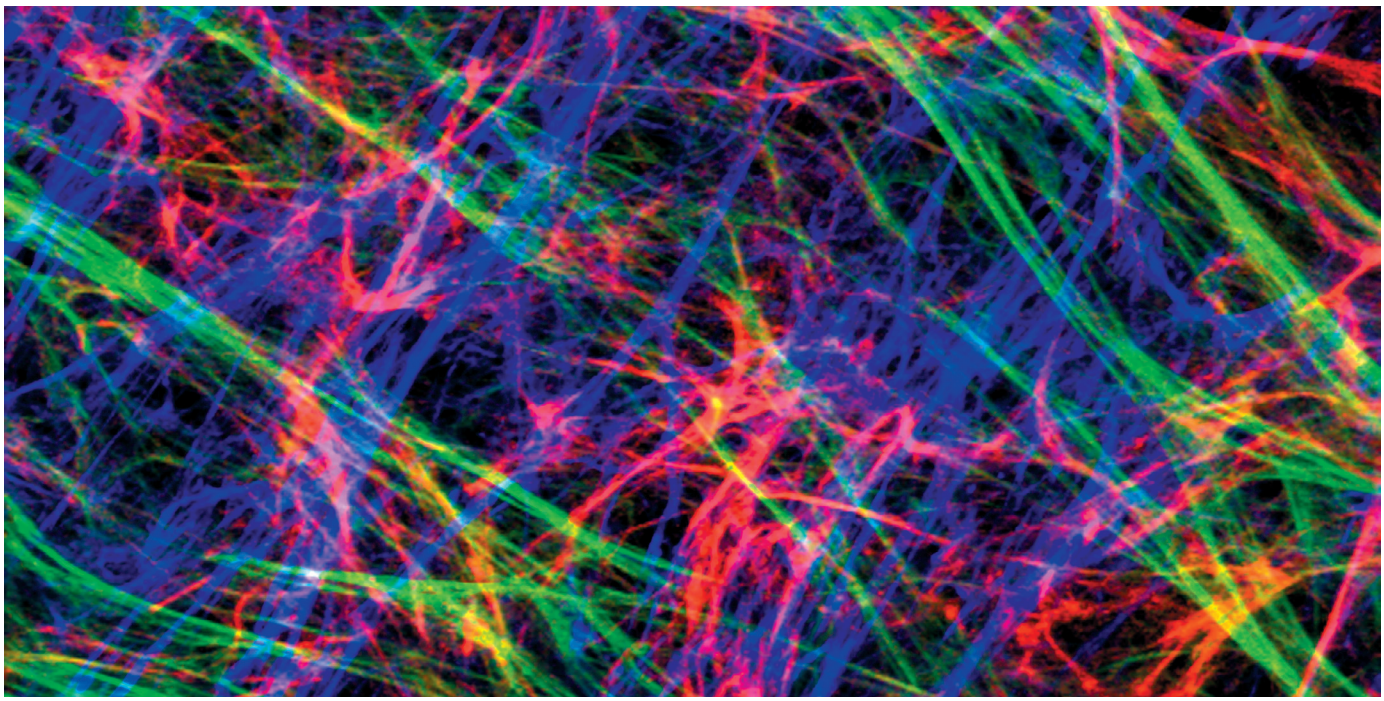
TECHNOLOGY FEATURE

MATRIX MIMICS SHAPE CELL STUDIES

The extracellular matrix governs a surprising number of cellular functions. New techniques are revealing how cells and matrix communicate — and why this cross-talk matters.

EDNA CUKIERMAN/GETTY



The extracellular matrix is a mixture of materials secreted by cells that provides structural and signalling support.

BY JYOTI MADHUSOODANAN

Muscle stem cells are constantly poised to proliferate and repair injuries. But when cultured in the laboratory, these cells retain their self-renewal abilities only on soft gels — not hard plastic plates. How do they know the difference?

In 2017, cell biologist Penney Gilbert at the University of Toronto in Canada and her colleagues discovered a clue[1]. Certain receptor proteins on muscle stem cells respond differently to their binding partners depending on whether the underlying growth substrate is soft or stiff. Cells, it seems, can tune their responses to stimuli according to the physical properties of their environment.

Cells are surrounded by the extracellular matrix (ECM), a cocktail of proteins, signalling molecules and chemicals that cells exude as they grow. Gilbert had previously reported that the stiffness of a cell's ECM influenced the ability of muscle stem cells to self-renew; the 2017 work suggested a mechanism by which the ECM directed this process.

Cells use the matrix to impart strength and shape to tissues such as bone or brain. As a result, scientists had long dismissed the ECM as just a scaffold — like a garden trellis — that cells use for support. They now know that the matrix plays an active part in cellular behaviour. Cues from the ECM can guide stem cells to repair damaged tissues, re-form blood vessels damaged by a stroke and alter cellular responses to chemotherapy.

"If you asked anybody 25 years ago about the function of the extracellular matrix, they would've said it was structural," says bioengineer Stephen Badylak of the University of Pittsburgh in Pennsylvania. "Now it's the opposite: it's recognized as a reservoir of signalling molecules that serves as a sort of information highway between cells."

The ECM is inspiring developments in cell culture, bioengineering and more, resulting in materials that better reflect how cells live and behave in tissues. Many of the materials are being used in the clinic for regenerative medicine. In the laboratory, researchers use them to understand how the matrix can influence cells, and how to improve engineered ECMs. But using them can be tricky. Working out the best matrix for an experiment is one of the biggest hurdles to someone starting out, Gilbert says. "Each synthetic or naturally derived biomaterial has different pros and cons, and homing in on the system that best meets your needs is a current challenge."

TAKEN FROM TISSUES

Until the 1980s, cells were thought to control their surrounding matrix. But to Mina Bissell, a cell biologist now at the Lawrence Berkeley National Laboratory in California, the conversation between cells and matrix seemed more bidirectional. In 1982, she proposed the ▶

then-controversial idea that the matrix communicated with a cell's nucleus to direct its functions[2]. The right ECM, she and others found, could drive mouse mammary cells to make milk and rat liver cells to make enzymes. By tweaking the matrix, even mutation-carrying tumour cells could be made to act like healthy cells.

By the mid-1980s, Badylak had begun to explore whether healthy matrices stripped of their cells could be used to stimulate tissue regeneration in animals. This stripping process, dubbed decellularization, involves treating tissues with a mix of chemicals, including detergents and enzymes, to remove cells while leaving the matrix intact.

"It's a balancing act," says bioengineer Karen Christman of the University of California, San Diego. Researchers must remove enough matrix proteins and molecules called proteoglycans to avoid an inflammatory response when the matrix is implanted into the recipient, but retain enough to provide cells with the cues they need to grow.

Certain tissues are trickier than others. The brain is extremely soft and falls apart easily, Christman says, whereas the enzyme-rich pancreas must be treated with inhibitors of protease enzymes, which digest proteins, to preserve the matrix.

Decellularized materials can be powdered and reconstituted into hydrogels to form potent therapeutics. Once injected into the body, such a hydrogel "reassembles into a structure that's very similar to the original in terms of pore size, fibre diameter and biochemical cues", Christman says. Such materials have been used to heal tendon tears, rotator-cuff injuries and burned skin, for example.

Although ECMs vary from tissue to tissue, therapeutic materials can originate from different organs or even different species. For some tissues, it almost doesn't matter what ECM is used, Badylak says; for others, "it definitely makes a difference".

Matrices from the pig small intestine, bladder or dermis, for example, can all repair human skeletal muscle. But for the oesophagus, only an ECM from the same tissue will suffice. And in the central nervous system, a foreign matrix works better than a nervous system one. Badylak's team found that urinary bladder ECM stimulates neuronal stem cells to proliferate better than an ECM derived from the central nervous system[3].

Whatever its source, each batch of decellularized material is unique, and must be tested to ensure all cells have been removed, Christman says, as well as for its mechanical and signalling properties. But that's sometimes easier said than done: researchers aren't always sure which properties of decellularized materials — tensile strength, polymer chemistry or ligand composition — actually trigger specific cellular functions.

Decellularized materials "can be very powerful in their ability to trigger behaviour of cells", says bioengineer David Mooney at Harvard University in Cambridge, Massachusetts. "But they also suffer because they are undefined."

STARTING FROM SCRATCH

To isolate, mimic and understand the specific properties of the ECM, some researchers are exploring synthetic matrix replacements. These matrices are built using polymers and a few specific ligands for cells to attach to, and have well-defined chemical and physical characteristics.

At the Max Planck Institute for Molecular Biomedicine in Münster, Germany, bioengineer Britta Trappmann has found that matrix stiffness, degradability and 'stickiness' can all spur cells that form blood vessels to switch between multicellular and single-cell modes of migration[4]. Single cells quickly invade new regions, but the multicellular mode is needed so cells can collectively form a blood vessel. Ideally, bioengineers would be able to design tissue implants that direct which mode cells use.

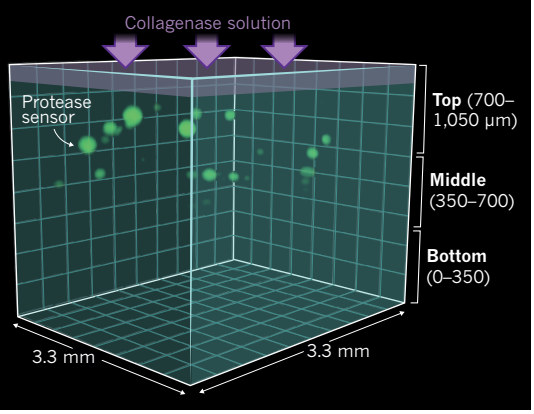
The chemical palette for such matrices typically includes the natural polymers collagen and hyaluronan, as well as synthetic versions such as polyethylene glycol or polyvinylidenefluoride–trifluoroethylene; the choice depends on the biological question, Gilbert says. When studying whether a matrix made of hyaluronan could improve survival rates for muscle stem cells injected into tissues, Gilbert's team found that hyaluronan confounded the data because, rather than helping cells adhere to other proteins, the polymer itself bound to cell-surface receptors[5].

How these polymers are turned into scaffolds also varies. Treena Arinzeh, a biomedical engineer at the New Jersey Institute of Technology in Newark, studies how mechanical forces can trigger electric currents that influence stem-cell differentiation. Arinzeh uses electrospinning, in which a voltage is applied to a jet of polymer ejected from a syringe, to create sheets of fibres in which spacing and size can be precisely controlled at the nanoscale level[6]. The sheets are stacked to form 3D structures, which Arinzeh has used to study how certain stem cells differentiate in a defined matrix.

Artificial matrices are also being tested for clinical use. Bioengineer Tatiana Segura at Duke University in Durham, North Carolina, developed a material based on hyaluronan that is studded with nanoparticles bearing vascular endothelial growth factor. When injected into mouse brains that had been damaged to replicate a stroke, the gel polymerized into a hydrogel that filled the cavity left by the stroke damage[7]. Creating an implant that precisely fits the shape of the cavity is tricky, but injecting a liquid that solidifies *in situ* could solve the problem. Importantly, the gel promoted blood-vessel formation, which is "really important in the context of brain repair", Segura says.

ENZYME ACTIVITY IN THE MATRIX

To study the enzymes that model the extracellular matrix, fluorescent sensors of protease activity are embedded in a matrix-mimicking hydrogel. A purified enzyme, collagenase, is added, and the sensors fluoresce as the enzyme diffuses down.



KRISTI S. ANSETH

STUDYING CELLULAR FUNCTIONS

Mooney says that when developing an ECM, whether naturally derived or synthetic, researchers need to consider first its composition — which protein ligands should be present, their density and their affinity to cellular receptors — and then its mechanical properties, such as elasticity, stiffness, shape and whether these physical attributes change over time.

One way to grow cells in a matrix for 2D studies is to crosslink the polymer so it forms a semi-solid gel and then add cells on top. Another is to mix the matrix material and the cells, and then solidify the scaffold to create 3D structures.

Because cells typically grow more slowly in 3D cultures than in 2D ones, Mooney's team often grows the cells in 2D before moving them to 3D. But Mooney suggests asking yourself whether a 3D culture is even necessary. Cultures grown in 3D are difficult and time-consuming, and from a biological standpoint, certain aspects of cell behaviour "can be very readily and appropriately modelled in a 2D culture", he says.

Cells in 2D cultures can be collected from the surface and used in standard protocols for techniques such as gene-expression analyses and enzyme assays; with 3D cultures, "you need to get rid of the matrix to access cells", Mooney says. Adding a chelating chemical to bind calcium can dissolve some gels, and enzymes can be used to digest matrix materials.

To image the ECM, "researchers often treat a matrix like a piece of tissue", Mooney says. Light-microscopy techniques can help researchers peer beneath the matrix surface. To look deeper, they can 'cryosection', fix and stain samples, just as they do with animal tissues.

For biomedical engineer Jennifer Leight at Ohio State University in Columbus, matrices are tools for studying the enzymes that cells use

to digest and rebuild the ECM. "There are not a lot of ways to study things that cells secrete into the matrix," says Leight, who works on matrix metalloproteinases (MMP), enzymes that cells secrete to degrade collagen during growth and tissue turnover.

Leight designed a peptide sensor, based on a collagen sequence cleaved by an MMP, that emits a fluorescent signal when the enzyme cuts it, and incorporated it into a hydrogel[8]. This allowed her to track the activity of the enzyme (see 'Enzyme activity in the matrix').

Similar sensors can be designed to study other secreted proteins, she says, and the reagents needed to make them are now available commercially. "It greatly reduces the barrier to more general use."

UNDERSTANDING THE NUANCES

But other obstacles remain. Although both synthetic and artificial materials are, in principle, easy to access, no common protocols exist to create these materials in a standardized way. Each lab has its own methods, so comparing data, even relating to the same tissue, is tricky.

Questions about how implanted materials assemble and degrade *in vivo* also linger. Segura, for example, can measure the polymer properties of the hydrogel injected into a mouse brain affected by a stroke. But because the dead tissue left behind after a stroke contains cell debris and various fluids, the hydrogel in the lab is "not at all what actually gets polymerized *in vivo*", Segura says. And it's impossible to visualize what happens in the depths of the brain. "We can only make sure that what we inject is the same every time."

When speaking to researchers starting out with ECMs, Gilbert says their most frequent question is 'what's the best biomaterial for my experiments?' There's no easy answer. "You don't typically see side-by-side comparisons to be able to say, this is the advantage of this material over that one," she says, "That makes it hard to really home in on the best choice."

Nonetheless, says Christman, the pay-off is worth the effort. Any material, old or new, requires similar safety and standardization studies, she says, "I don't think people should feel discouraged or scared to design brand new materials and push them towards the clinic." ■

Jyoti Madhusoodanan *is a science writer in Portland, Oregon.*

1. Safaee, H. *et al. Cell. Mol. Bioeng.* **10**, 501–513 (2017).
2. Bissell, M. J., Hall, H. G. & Parry, G. *J. Theor. Biol.* **99**, 31–68 (1982).
3. Crapo, P. M., Tottey, S., Slivka, P. F. & Badylak, S. F. *Tissue Eng. A* **20**, 313–323 (2014).
4. Trappmann, B. *et al. Nature Commun.* **8**, 371 (2017).
5. Davoudi, S. *et al. Biomaterials* **173**, 34–46 (2018).
6. Damaraju, S. M. *et al. Biomaterials* **149**, 51–62 (2017).
7. Nih, L. R., Gojgini, S., Carmichael, S. T. & Segura, T. *Nature Mater.* **17**, 642–651 (2018).
8. Shin, D. S. *et al. ACS Biomater. Sci. Eng.* **4**, 378–387 (2018).

The race for enzymatic DNA synthesis heats up

An alternative to chemical oligonucleotide synthesis inches closer to reality.

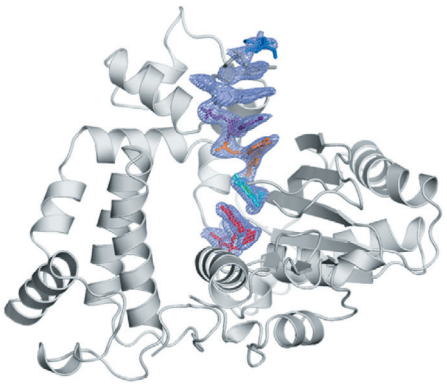
BY JEFFREY M. PERKEL

For decades, biologists have built custom DNA sequences chemically, from phosphoramidite building blocks that replicate natural bases. But the method is impractical beyond 200 bases, and environmentally hazardous. New enzymatic strategies could circumvent those limitations.

In June 2018, George Church, a geneticist based at Harvard University in Cambridge, Massachusetts, and his colleagues reported encoding and decoding short messages in enzymatically synthesized DNA (H. H. Lee *et al.* Preprint at bioRxiv https://doi.org/c2cs; 2018); two months later, Molecular Assemblies, a biotechnology company in San Diego, California, announced a similar achievement.

In July, Sebastian Palluk and Daniel Arlow, in Jay Keasling's synthetic-biology laboratory at the University of California, Berkeley, published a strategy that they used to build ten-base oligonucleotides (S. Paluk *et al. Nature Biotechnol.* http://doi.org/gdqkff; 2018), and founded Ansa Biotechnologies to commercialize the approach.

And in October, DNA Script, based in Paris, announced that it had synthesized a 150-base DNA strand of defined sequence — an achievement that William Efcavitch, Molecular Assemblies' chief scientific officer, calls a "milestone". (At least two other companies also are pursuing enzymatic strategies: Nuclera Nucleics and Evonetix, both based near Cambridge, UK.)



MARC DELARUE, ADAPTED FROM J. GOUGE *ET AL. J. MOL. BIOL.* **425**, 4334–4352 (2013).

TdT, a template-independent DNA polymerase.

Key to enzymatic synthesis is terminal deoxynucleotidyl transferase (TdT), a DNA polymerase that requires no template. "It can add nucleotides without taking instructions," explains Marc Delarue, a structural biologist at the Pasteur Institute in Paris who collaborates with DNA Script. In theory, the approach can generate longer molecules than can chemical synthesis. It's also environmentally friendlier.

To control the sequence, developers must stop the enzyme after each step. Ansa tethers the nucleotide to the enzyme, thus physically blocking the DNA; others are developing TdT variants and modified DNA bases that act as reversible terminators. For DNA-based information storage, in which data are encoded in the transitions between bases rather than in their precise arrangement, the native enzyme and nucleotides can be used.

Enzyme-written DNAs are not yet commercially available. Nor can any published strategy rival chemical synthesis in length or efficiency. Palluk and Arlow reported 97.7% average coupling efficiency in their paper; Integrated DNA Technologies (IDT), a DNA-synthesis firm in Coralville, Iowa, touts 99.5%. Yet less than 40% of molecules are correct at 200 bases; longer molecules would require higher efficiencies.

Still, says Emily Leproust, chief executive of the synthetic-DNA firm Twist Bioscience in San Francisco, California, "someone will crack it, and it's going to be great for the field". Adam Clore, technical director of synthetic biology at IDT, reckons that a "commercially viable product" is "probably several years off".

Those products could fill niches that chemistry cannot: long, complex sequences — synthetic gene libraries, for instance — for which assembly from shorter segments can add significant delays. "Any technology that can make that faster is going to be very valuable," says Christopher Voigt, a synthetic biologist at the Massachusetts Institute of Technology in Cambridge. "There is no Nobel prize that needs to happen," Leproust says. "It's just hard engineering." ■