Biomaterials researchers develop increasingly dynamic materials to mimic tissue-based and cellular events and cues. Credit: E. Dewalt/Springer Nature

# How some labs put more bio into biomaterials

The materials–biology interface is challenging to characterize, but new assays are on the way.

Vivien Marx

Biomaterials researchers straddle disparate worlds as they develop materials to mimic dynamic tissue-based and cellular events and cues. They draw on such fields as materials science, chemistry, physics, nanofabrication, informatics, physiology, cell biology, genomics and immunology[1–3]. Some of this research, and the commercial ventures it inspires, can lead to, for example, enhanced ways to mimic cells and tissue-based processes, improved approaches to tissue regeneration or better modes of delivering drugs into the body. Such applications draw on basic insight about the complex materials–biology interface that is challenging to characterize and for which new assay types are needed. Some biomaterials labs draw those disparate worlds closer by using 'omics approaches to 'read out' a material's impact on cells and fine-tune the traits of their materials.

Some scientists see a dearth of in vitro–based and computational ways to assess the biological effect of materials in high throughput[4]. They and others see opportunities in the convergence of 'omics technologies and materiomics, as the study of material properties and functions is sometimes called. The idea is to accelerate biomaterials development and to better the predictions about a biomaterial's performance in vivo.

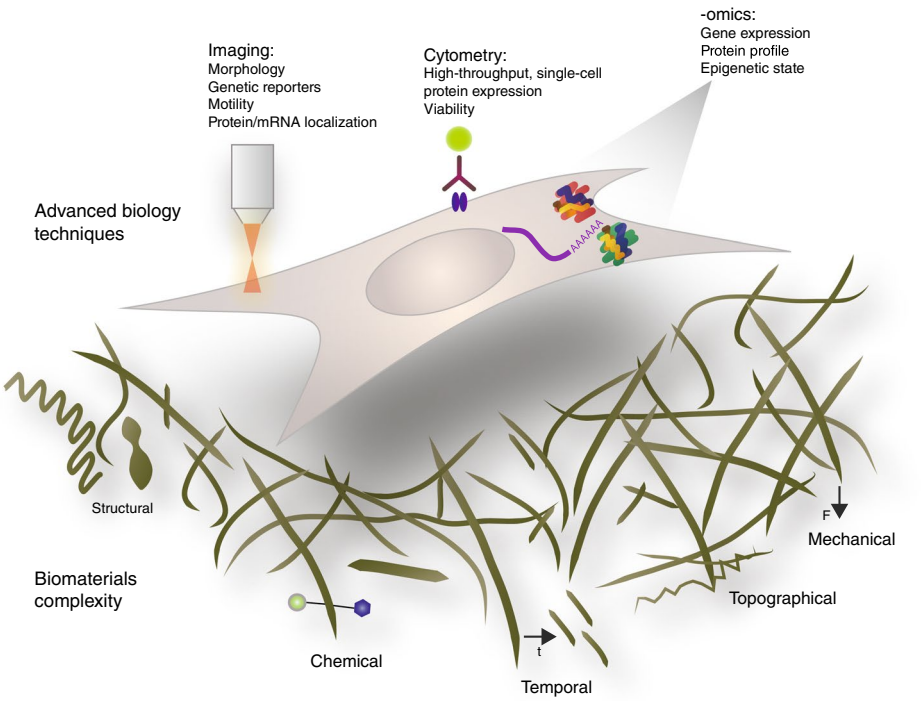
At the University of Michigan, researcher Carlos Aguilar calls his lab the Nano-Omic-Bio-Engineering lab. As a graduate student at the University of Texas, he trained in micro- and nanofabrication, then worked with stem cells and on bioinformatics at the Lincoln Laboratory, which is affiliated with MIT. "Now, in our lab, I'd say most of the work we do is biology and we use and develop devices and tools to understand specific biological functions," he says. His team works regularly with engineers, physiologists, clinicians, bioinformaticians and biologists. "I think it's fun to be at that interface," he says.

Harvard University bioengineer David Mooney, who is also at the Wyss Institute for Biologically Inspired Engineering, and his colleague Max Darnell mention that the design of more complex biomaterials has been enabled by, for example, advances in polymerization and the ability to use click chemistry to integrate different types of chemical and molecular functionalities into materials[5]. Combining techniques in materials and 'omics is appealing. When scaled up, this can mean combinatorial materials design coupled with high-throughput gene expression profiling. It's promising, says Johns Hopkins University bioengineer Jennifer Elisseeff, but, she says, one always has to "remember that things may be different in vivo." "We're really excited about it; I think it's absolutely the direction to go," says Mooney, but it also deserves a note of caution. "We're not going to be able to analyze everything with every technique." The sheer amount of data associated with such approaches "would be staggering," he says, making it hard and costly to extract meaning from it. What is needed is a framework through which labs can decide which types of analyses to use in order to obtain the right amount of usable data and not in a way that is prohibitively expensive. "This is something we need to think about as a community," he says—how to bring together this "explosion" in materials science with the "explosion" in analysis techniques happening in the life sciences to "marry these things in a rational way and that allows us to make better materials."

Mooney and his team avoid data explosion by integrating high-throughput biology assays in a more stepwise fashion in biomaterials design and development. He and his group have been using gene expression analysis to characterize how factors such as viscoelasticity, stiffness and adhesion ligands can regulate broad patterns of gene expression, and they use these

findings to design materials for particular applications. His group has experience in picking variables to tweak for a desired result, and it continues to matter, he says, "but we think that increasingly we'll be using these 'omics approaches in concert with that to help us better understand the landscape and figure out the right path."

His team has, for example, developed and tuned alginate hydrogels to control stem cell differentiation. As they made materials, they studied the impact of varying viscoelastic properties on stem cell fate decisions. In the area of immunology, the team is developing biomaterials to bridge the gap between in vitro assays and mouse models of immunotherapy, and to bridge the in vitro–in vivo gap in other domains, too. "What we'd all like is to be able to do cell culture studies and use those to really predict what happens in the body." It could simplify research and reduce the use of experimental animals. It's a goal both in biomaterials and in the organs-on-chip field, a space that's growing. "I think we're still at a very early stage, but it clearly is going to be really important."




Biomaterials can be made to be dynamic, and labs can fine-tune them for particular applications. Assays deliver detailed readouts. Labs explore combining materials science and 'omics techniques. Credit: Mooney lab, Harvard Univ./Wyss Inst. at Harvard Univ. Adapted with permission from ref. [5], Springer Nature

## Mechanomodulation

Over ten years ago, by culturing mesenchymal stem cells on materials with varying levels of stiffness, labs showed how materials can alter cell fate decisions, says Aguilar. The extracellular matrix (ECM) is more than an architectural scaffold for cells and tissues: the biomolecules in this fibrillary network affect cellular development, growth and migration. Labs have worked with decellularized ECMs but have moved to engineering these materials synthetically and building in many types of tunability. For example, light can be used to cleave certain elements in a hydrogel used to mimic the ECM. When engineering ECM mimics, labs might design for potential external uses, such as skin wounds. For less accessible locations, injectable materials can be designed, also to change after injection, says Jacqueline Larouche, a graduate student in the Aguilar lab. A change in pH or antibody presence might trigger drug release from a biomaterial, or a biomaterial can be glucose-sensitive as part of an insulin-delivery system. Labs get two types of readouts with their ability to make materials that are dynamically tunable and with the use of sequencing, says Aguilar, "so we have two great handles to understand how extrinsic cues from the environment couple into molecular networks that determine or alter cell fate." Stem cells behave according to their genetic makeup and also microenvironmental signals, which might be mechanomodulation: biomaterials can be tuned to induce epigenetic signatures on stem cells and encourage a certain type of cellular reprogramming. Scientists at the University of California, Berkeley, Ecole Polytechnique in Palaiseau, France, and Shanghai Jiao Tong University cultured mouse fibroblast cells on substrates with etched microgrooves, and found that certain grooves made reprogramming more efficient, which can possibly obviate the need to use small-molecule epigenetic modifiers in reprogramming[6]. The substrates were made of poly(dimethylsiloxane) etched with parallel microgrooves 3 µm deep and spaced, on separate substrates, at 10 µm, 20 µm and 40 µm. When exposed to transcription factors Oct4, Sox2, Klf4 and c-Myc—the OSKM cocktail—the microgrooves enhanced fibroblast reprogramming, with the substrate with 10 µm showing the most pronounced effect.

## Niche engineering

Meet the satellite cell. It's a type of muscle cell and rather "magnificent," says University of Michigan's Aguilar. As a resident stem cell in our muscles, this satellite cell is sort of a doctor on call: "when you get injured, this is the stem cell that regulates injury repair," he says. In response to injury, cells undergo all sorts of transcriptional and epigenetic changes, which he and his lab assess with high-throughput sequencing assays. As we age, he says, the chromatin in these cells becomes dysregulated and the doctor on call has some issues. The muscle cell's genes are not turned on or expressed at the needed levels. Single-cell profiling has further revealed how heterogeneous these stem cells are. And their niche affects their diversity and behavior. "It's neat because what we have found says the niche, or place the stem cell lives, dictates what the stem cell does," says Aguilar.

One big challenge in this research, he says, is that removing these stem cells from their niche to study them in the lab is like forcibly removing long-time residents from their home. The cells' behavior changes, their gene expression is altered and they miss out on sending and receiving their niche's biochemical cues. Such changes make it harder to characterize the cells' in vivo behavior. Aguilar has high hopes for two emerging research angles, one of which is the integration of high-resolution molecular readouts from single cells, which could help scientists tease out precisely the facets of engineered systems that best replicate in vivo conditions. The other involves reprogramming the cells, shifting them from an identity as fibroblasts to muscle cells in order to experimentally test whether they can replicate more accurately the cell states they observe in vivo. Like people, stem cells are sensitive and heterogeneous, says Aguilar. To understand their actions

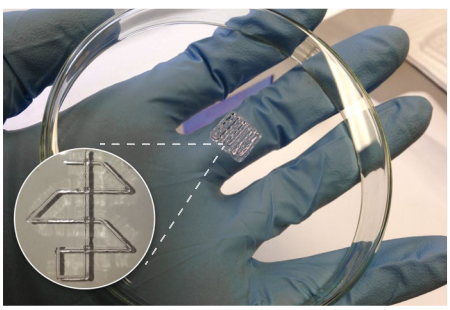
or functions, such as what triggers their emission of cytokines or growth factors involved in building tissue, labs have to learn how to "read" the molecular networks that determine these traits, aspects such as chromatin state and gene expression.

When building a niche, tunable materials need varying degrees of elasticity, stiffness and porosity, to name just a few traits. Open research questions remain about the impact of each trait, says Aguilar. The elasticity of the surrounding matrix is the most complex to study, he says. Labs look at how cells pull through integrins, how they alter their cytoskeletal tension and focus forces into and through the nucleus into chromatin. In his view, sensitive genomic assays and engineered systems "can start to help unravel some of these questions."

## Hydrogels

Hydrogels exist in a dizzying array of varieties and are fundamentally a class of flexible materials made of crosslinked polymers and water. Labs make their own hydrogels from ingredients they buy, or they can buy hydrogels they need.

AmacaThera, a University of Toronto spin-out, commercializes the work of Molly Shoichet, a biomaterials researcher at the university. The team develops hydrogels for different applications. To determine material traits for a given application, the team works backward from the biology, says co-founder Michael Cooke. For example, the company's scientists look at the optimal duration of the release of a drug to then engineer a hydrogel that delivers drugs for that time. Their first product is an injectable, drug-releasing hydrogel to treat postoperative pain and is part of an overarching goal to help decrease the need for opioids where possible. In developing this hydrogel, they checked in with over 100 surgeons and anesthesiologists to understand what they sought in a material and product. "We are aiming not to change medical practice but more to develop a delivery material by which we can improve the drugs that are already used by surgeons," says Cooke. The company has as its focus sustained drug release applications. It got its start in Shoichet's lab; received support, for example, from the university, the non-profit MaRS Innovation that supports university spin-outs in Toronto, and Ontario Bioscience Innovation Organization; and has received government grants and venture capital seed funding. Its current home is in JLABS, an incubator funded by the pharmaceutical company Johnson & Johnson.



Hydrogel-based inks can help to protect cells from the shearing stresses of bioprinting. Credit: Biogelx; E. Dewalt/Springer Nature

Labs might use hydrogels to make synthetic ECM, which is not identical but can be similar to native ECM. Native ECM contains many biomolecules, such as collagen and fibronectin. Biogelx, spun out of the University of Strathclyde in 2013, sells biomaterials for 3D cell culture such as tunable peptide-based synthetic hydrogels. These hydrogels can be used as synthetic ECMs. The company has also designed an ink for room-temperature 3D bioprinting applications. The ink helps to protect cells from the shearing stresses of bioprinting, with which labs can, for example, 'print' a microenvironment to study cells. (All Biogelx comments were relayed through a spokesperson.)

According to the company's business development manager Elia Lopez-Bernardo, Biogelx works with labs to learn of their experimental requirements, such as whether they are working in 3D or 2D, the scaffold thickness they seek and any experimental challenges they have faced. In some cases, labs are looking to switch from a material they know that might not, for example, be stiff enough. In her observation, researchers "tend to overestimate the time and effort a switch can take." By varying peptide concentration, Biogelx materials can be tuned to stiffnesses ranging from 0.5 kPA to 100 kPA, she says. The company offers gels with several peptides and is currently working on a newer hydrogel combination with additional short peptide sequences. Fully synthetic and characterized hydrogels make experiments more comparable, and the hydrogel's biocompatibility is provided by the integration of short "mimetic sequences" from ECM proteins, says Mhairi Rodgers, the company's head of operations.

When materials scientists characterize the physical and chemical properties of their materials, they sometimes lack "real-world" applications, observes Laura Goldie, head of technical services, while cell biologists hunting for functional biomaterials can lack the skills to tailor materials to their needs. To bridge this gap, the company launched an academic collaboration program, which gives labs deals on company products. Ongoing projects include gel-based

He calls his lab the Nano-Omic-Bio-Engineering lab, says Carlos Aguilar at the University of Michigan. Here, with graduate student Jacqueline Larouche. Credit: B. Baier

biosensors, liver organoid models, stem cell media platforms, 3D cancer models and bioinks for tissue engineering. Biogelx is also involved in two research consortia.

On a larger scale, the German pharmaceuticals company Merck KGaA, which acquired Sigma-Aldrich in 2015, offers many types of hydrogels for researchers in academia and drug discovery seeking to mimic the natural environments of cells or tissues of interest. Culturing cells in 3D such as by encapsulating them in water-swollen hydrogels is thought to be a more physiological approach than growing cells in 2D planar cultures, and labs can track changes in cell shape, differentiation, gene expression and drug sensitivity, says Nick Asbrock, global product manager for stem cell and 3D cell culture at Merck Life Science. (Asbrock's comments were relayed through a company spokesperson.)

The company sells hydrogels that are ready to use and ingredients for labs designing their own. Its synthetic hydrogel system, TrueGel3D, gives labs a high degree of control over a given 3D hydrogel environment, says Asbrock. The hydrogel is a blend of dextran, polyvinyl alcohol and polyethylene glycol (PEG). When labs fine-tune their chemically defined environment, they can adjust gelation speed, gel stiffness, migration, cell binding motifs and cell recovery. Users might, for example, add more or fewer crosslinkers. They can control gelation speed by choosing fast or slow thiol-reactive gel crosslinkers. And they can choose degradable or non-degradable crosslinker types as they, for example, control the functions of cells encapsulated in hydrogel. The non-degradable crosslinker carries thiol groups on the ends of the PEG

molecule and is well suited for spheroid cultures, says Asbrock.

The hydrogel with the cell-degradable crosslinker might be applied, for example, for experiments about cell migration. These hydrogels include peptide sequences that can be cleaved by enzymes in the family of matrix metalloproteases, which provide homeostasis to the cell's ECM. But some of these enzymes are active in cancer and help a tumor to spread by easing the way cancer cells burrow through the ECM. Many labs use animal-derived hydrogels, which Merck KG also sells. "However, there are limitations to these types of products, including lot-to-lot inconsistencies, animal cross-contamination, temperature-sensitive gelation methods and the lack of the control to customize the hydrogel composition," says Asbrock. "There is not a universal hydrogel for every cell type." Nor is there a shortcut for labs optimizing the conditions for their cell types, but TrueGel3D might offer a lab greater consistency from one experiment to the next as a team fine-tunes microenvironments around cells and tissues of interest.

Controlling the gelation speed, he says, has proven valuable for labs working on drug delivery methods, since they can avoid issues with temperature-sensitive animal-derived hydrogels and the need to pre-chill all lab equipment for that work. Labs can stagger multiple experiments at a time using TrueGel3D and avoid worrying about the hydrogel forming until the crosslinkers are added in the final step, says Asbrock. This hydrogel is transparent, which makes imaging easier. Animal-derived hydrogels can be opaque. In Asbrock's view, cell biologists without much material chemistry expertise can use these materials and kits. "We cannot test every cell application but can provide guidance on which hydrogel chemistry to use," he says.

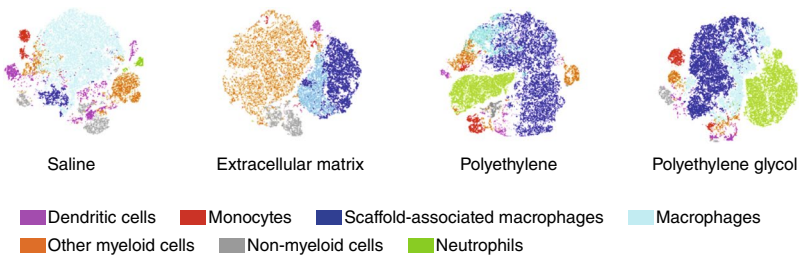
## Clear and strong

Biomaterials labs push to make complex materials that emulate complex biology. Not only can materials have varying degrees of stiffness, porosity or elasticity, says Aguilar, but also there are ways to turn soft materials into stiff ones, such as to emulate the fibrosis and tissue hardening that follow a heart attack.

Other researchers, such as Elisseeff, who also directs the Translational Tissue Engineering Center at Johns Hopkins, have tuned for a different set of material properties—for example, strength and transparency. She and her team seek ways to assist with repairing injuries to the cornea of the eye. Although transplantations with donor corneas are common, people needing transplants outnumber donors. For recipients of donor corneas, immunological rejection over time can leave people visually impaired, even blinded. A synthetic cornea replacement has to be strong and transparent. Elisseeff and others have experimented with collagen vitrigel, which is a collagen mimic vitrified in different ways. It's tricky to get the vitrification conditions, the timing and temperature, just right to achieve the desired ultrastructure of the collagen fibers. The process she and her team developed made the materials strong for a corneal mimic, but they lacked the right ultrastructure and transparency levels.

The team chose to work with cyclodextrins, which are cyclic oligosaccharides that can mimic cyclic proteoglycans. "It was pretty cool to see how these circular cyclodextrin molecules could modulate the assembly of collagen, and by controlling temperature and evaporation rates, we could produce materials that were strong and elastic like the cornea," she says. Plenty of optimization steps lie ahead, but she hopes such research can lead the way to a viable replacement for cornea transplants.

To Elisseeff, biomaterials research can potentially lead to ways of regenerating damaged or lost tissue. The interaction between biology and material is intricate given how the tissue's microenvironment and its cues shape cellular behavior. That's why collaboration is a key ingredient in such projects, she says, whether one comes to the field as a biologist or a materials scientist.




Different biomaterials used on muscle wounds in mice: saline solution as a control, particulate extracellular matrix (ECM) derived from bladder tissue, polyethylene and polyethylene glycol (PEG) led to different immune responses. Here, *t*-distributed stochastic neighbor embedding analysis of flow cytometry data three weeks after injury. Credit: Adapted with permission from ref. [7], Elsevier

Collaboration is a key ingredient in biomaterials projects, Jennifer Elisseeff says, whether one is a biologist or a materials scientist.

Credit: Johns Hopkins University

As a bioengineer, she is keen on making materials for pressing needs. But at the moment, she and her team are not making new materials. Instead, the researchers are taking on a more fundamental issue central to all of biomaterials research and applications: a better characterization of the immune response to and interaction with materials. "Because the immune system is so complicated, we really have to do it in vivo," says Elisseeff. "Nothing we did in vitro really predicted much of the in vivo."

Materials long thought to be inert, such as the synthetic polymer PEG, have been shown in some studies to generate immune responses. This can be an issue with drugs made as PEGylated conjugates.

When Elisseeff and her team studied the microenvironment around biological and synthetic scaffolds placed onto tissue wounds in mice, they found diverging immune profiles, also when PEG is present. "It definitely does something," she says. Separately, she is advancing the emerging field of immunoengineering in which biomaterials target the immune system and trigger a desired response. That might be to support wound healing or to stimulate the immune system to combat cancer in a person's body.

Adding 'omics to materials science is a way to mechanistically dissect the interplay between materials and a biological system. It's a tall order, but as biomaterials labs put more bio into their biomaterials, they see promise. ❐

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