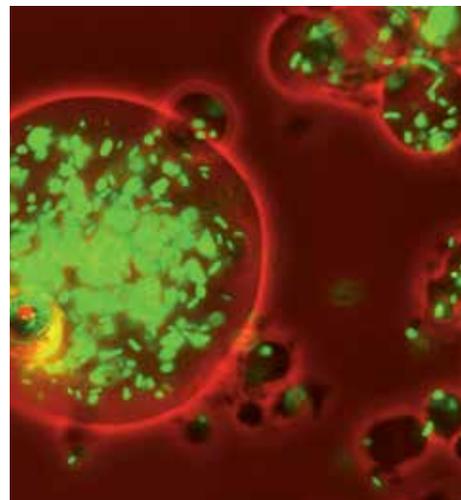
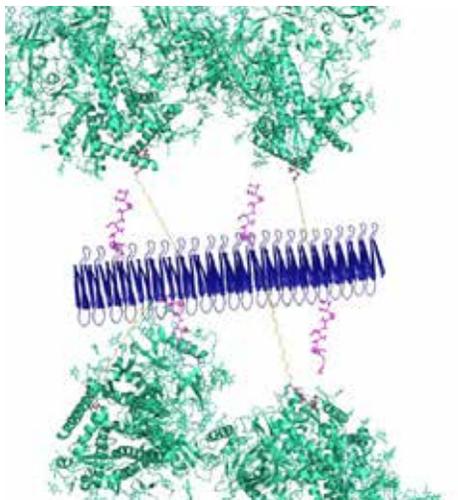
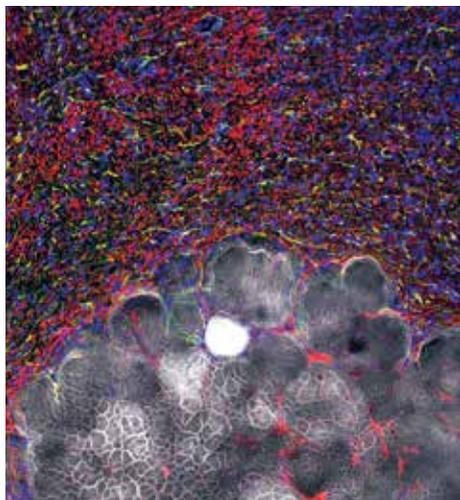
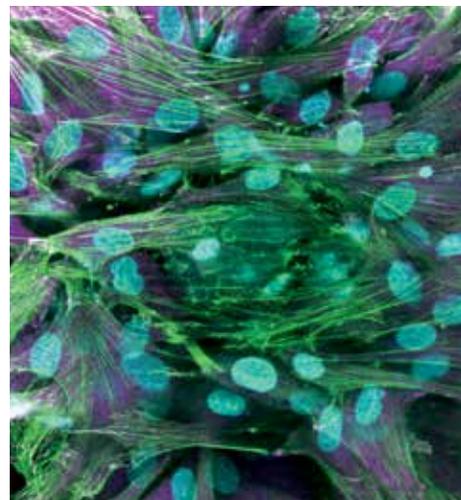
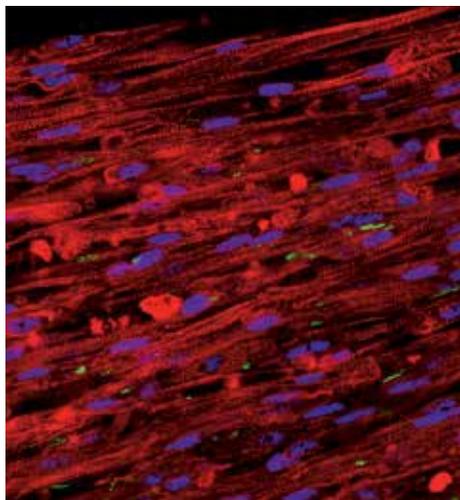
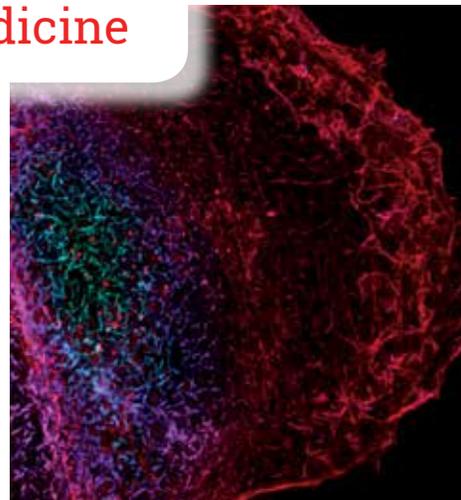
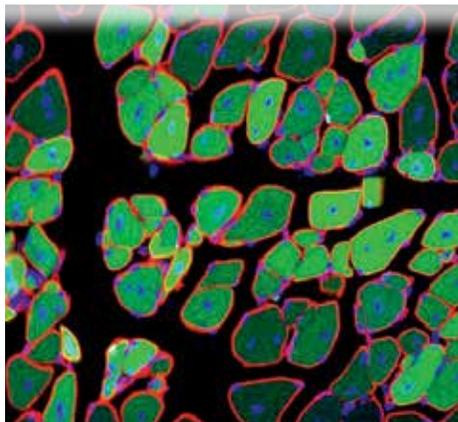
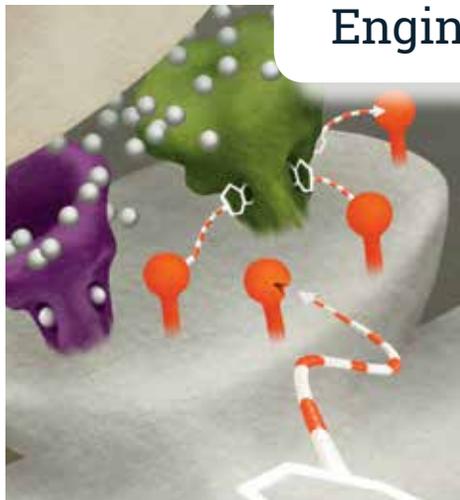


DukeBME

MAGAZINE

BIOMEDICAL ENGINEERING AT DUKE UNIVERSITY

Engineering **Biology** for Medicine





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LEFT: *The interior of the Fitzpatrick Center for Interdisciplinary Engineering, Medicine and Applied Sciences, home to Duke BME.*



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A CONFERENCE TO REMEMBER



Dear Colleagues and Friends,

Good work is rarely accomplished alone. For me, the 2019 Engineering Biology for Medicine conference served as an energizing reminder that breakthrough discoveries often emerge due to collaborations at the intersection of engineering, biology and medicine.

It was wonderful to welcome colleagues from schools as far-flung as Berkeley, Chicago, Columbia, Cornell, Georgia Tech, Harvard, Hopkins, MIT, UCSF, Yale, and ETH Zurich to Duke BME for the May 2019 conference, which was co-sponsored by Nature Medicine and Nature Biomedical Engineering.

With sessions covering topics that spanned mechanobiology, synthetic and systems biology and immune engineering, this event was a wonderful opportunity for leading researchers to highlight successful research, and discuss how their work could solve long-standing problems within the biomedical field.

I was thrilled to have several Duke BME faculty among the speakers presenting their own work and introducing sessions. In our newest issue of the *Duke BME Magazine*, we take a deeper look at their impressive research, with stories about researchers using lab-on-a-chip technologies to study diverse diseases, work in the emerging field

of mechanobiology, novel approaches to mapping the human brain, and features about work at the intersection of tissue engineering and regenerative medicine.

Our faculty are just one of the reasons Duke BME is consistently ranked among the best biomedical engineering programs in the United States, and I'm thrilled with the opportunity to highlight what is just a small fraction of their excellent research. Duke BME has blazed an exciting trail in the last fifty years, and I know there is more innovative work on the horizon.

Ashutosh Chilkoti
 Chair
 Duke Biomedical Engineering

The Numbers: DukeBME

HIGHLY RANKED:

TOP 10 Rank among US universities—*U.S. News & World Report*

Duke BME is the:
2nd largest graduate program at Duke,
7th largest undergraduate major, and
#1 undergraduate major in Duke's Pratt School of Engineering

#3 Graduate biomedical engineering program

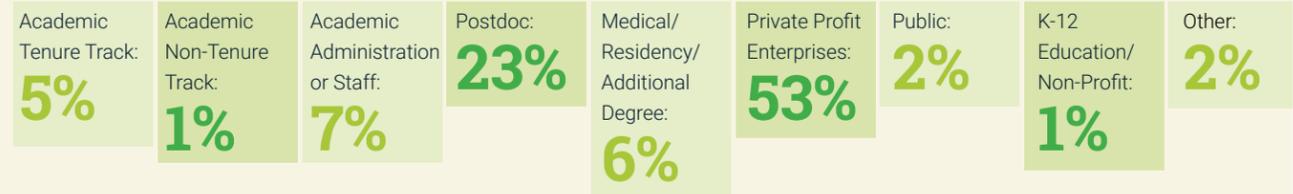
#3 Undergraduate biomedical engineering program

FACULTY:

40 Tenured or tenure-track faculty

2 NIH pre-doctoral training grants:
 Medical Imaging (Nightingale, PI)
 Biomolecular and Tissue Engineering (Gersbach, PI)

10-Year PhD Career Paths

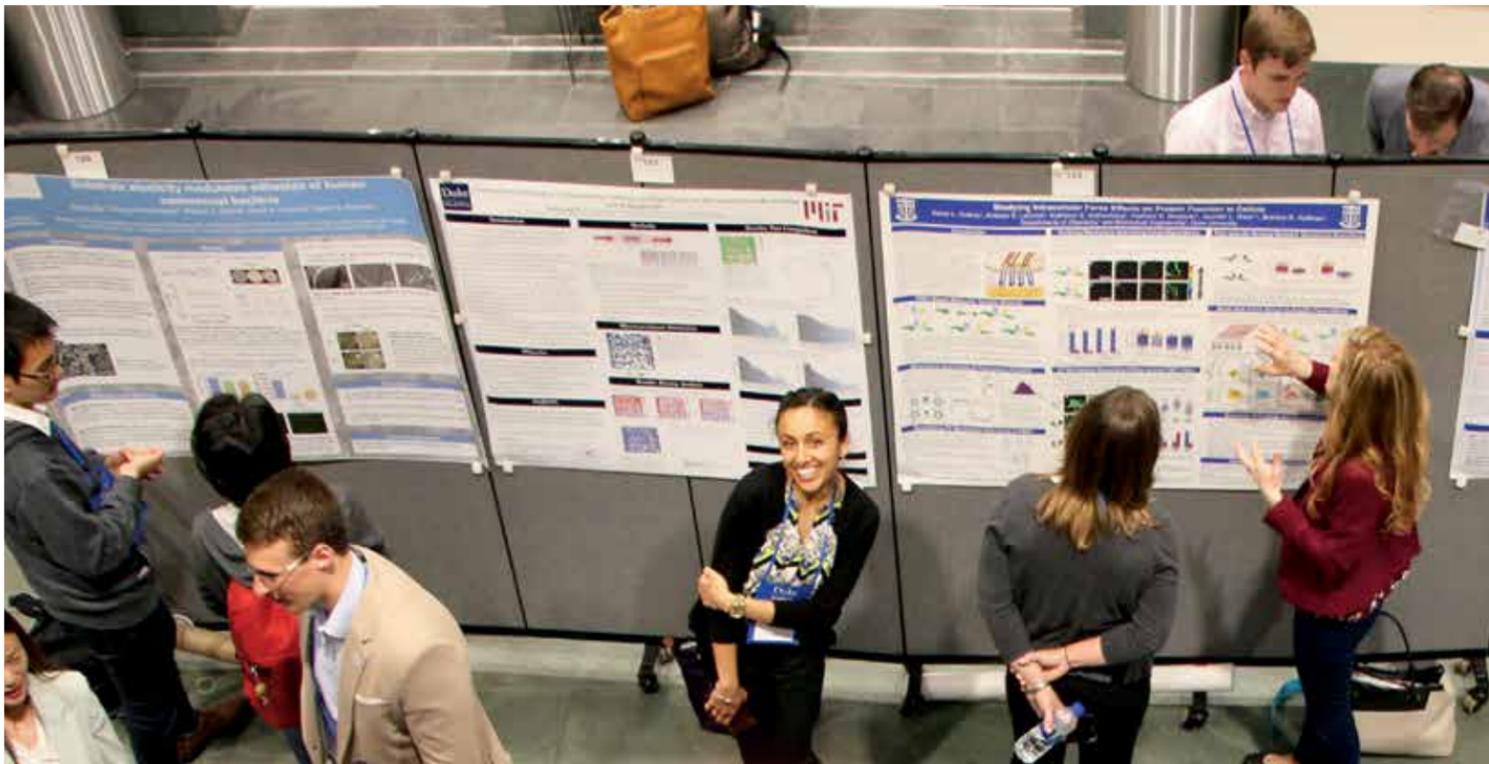


BME faculty are involved in the following centers and institutes:

Center for Biomolecular and Tissue Engineering (CBTE) | Fitzpatrick Institute for Photonics (FIP) | Duke MEDx | Duke Cancer Institute (DCI) | Duke Center for Genomic and Computational Biology | Duke Global Health Institute (DGHI) | Duke Institute for Brain Sciences (DIBS) | Center for Entrepreneurship and Research Commercialization (CERC) | Center for Global Women's Health Technologies | Center for the Study of Neurodevelopment and Improving Children's Health Following Environmental Tobacco Smoke Exposure (NiCHES) | Woo Center for Data and Precision Medicine | Center for Advanced Genomic Technologies (CAGT)

RESEARCH FY 2019:





Engineering Biology for Medicine

Inaugural Conference Held at Duke

The new conference hosted by Duke Biomedical Engineering, Nature Biomedical Engineering and Nature Medicine created a collaborative environment to share innovative research

”
The conference provided a snapshot of the most exciting work going on across the different areas of bioengineering and conveyed the promise of the field for improving health.

ENGINEERING THE IMMUNE SYSTEM **THE BRAIN** ENGINEERING MECHANICS FOR MEDICINE
TISSUE ENGINEERING NEUROENGINEERING **GENOME AND EPIGENOME EDITING**
 MICROBES FOR MEDICINE **ENGINEERED MICROENVIRONMENTS** AND MORE...



LEFT:
 Researchers present their work during the poster sessions at the Engineering Biology for Medicine conference.

When Ashutosh Chilkoti took over as chair of Duke University's Department of Biomedical Engineering, one of his goals was to host a specialized conference for biomedical engineers in partnership with a high-impact research journal.

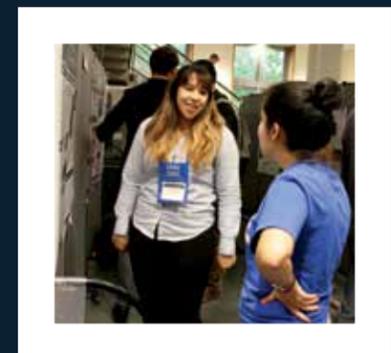
On May 19, 2019, that idea came to fruition in the form of "Engineering Biology for Medicine", a conference hosted by Duke BME, *Nature Biomedical Engineering* and *Nature Medicine*. Spanning four days and held on Duke's campus, the event brought together more than 200 biologists and engineers — including faculty, post-doctoral fellows and graduate students from universities and research institutions across the United States and from overseas.

"The Engineering Biology for Medicine conference was developed to promote engaging discussions about how to harness human biology to devise strategies for probing, diagnosing and combating human disease. Biomedical engineering is highly collaborative, and we wanted to create a conference that embodied that aspect of the field," says Chilkoti. "We were highly pleased with the breadth of speakers that we were able to attract, and the conference was a great opportunity for researchers to highlight innovative research."

"In my view, the conference was packed with high-quality talks, and there were lots of questions from the audience and lively discussion with the speakers," says Michael Basson, a senior editor at *Nature Medicine* and one of the hosts of the conference. "I feel that the conference provided a snapshot of the most exciting work going on across the different ar

reas of bioengineering and conveyed the promise of the field for improving health."

Running from May 19-22, "Engineering Biology for Medicine" featured talks that spanned immune engineering, tissue engineering, synthetic and systems biology, genome engineering, and neuroengineering. In addition to speakers from Duke University, presenters



also included researchers from a variety of universities across the United States, including Harvard University, Massachusetts Institute of Technology, Johns Hopkins University, Georgia Tech, Columbia University, and the University of California, San Francisco.



“I left Duke with lots of notes to take home and ponder on.”

ABOVE, CENTER: Yiyang Gong presents his research during a New Innovator Mini-Session at the conference.

“This is a different crowd than I normally see at conferences. Often meetings are somewhat siloed, so you’ll have meetings that are just on genome engineering, or developmental biology,” says Luke Gilbert, an assistant professor from UCSF and a conference speaker. “This meeting was delightful because you’re bringing together different communities of engineers and biologists. And I’ve been able to learn a lot, especially about mechanosensing and organ-on-a-chip models. It was a nice mix of people who are in the same field as me, but more than anything I’ve really enjoyed meeting new people and hearing about their work.”

In addition to notable faculty from prestigious universities, the conference featured New Innovator Mini-Sessions, where researchers who had received the NIH New Innovator Award could discuss their work.

“It was important for us to include these sessions so we could highlight the exciting work from these young researchers,” says Chilkoti. “The NIH New Innovator Award is an impressive accolade, as it celebrates exceptionally creative early career investigators as they pursue high-impact projects.”

Graduate students and post-doctoral fellows could also share their work during the nightly poster sessions, giving students and faculty a chance to network and check in on new research in various

labs. At the end of the conference, Gabriel Butterfield (University of Washington), Daniel Dewran Kocak (Duke) and Imran Ozer (Duke) received the poster awards, which included a cash prize and the opportunity to present their work in front of all attendees.

Beyond various research presentations, the conference also included a Meet the Editors session, where attendees asked editors from *Nature Biomedical Engineering*, *Nature Medicine* and *Science Translational Medicine* for advice and feedback about crafting compelling research stories and the processes of peer review and publication.

“The quality of the speakers, their talks and the interest of the audience was evident,” says Pep



Chilkoti and others within the Duke and Nature teams hope to arrange follow-up conferences every few years that highlight exciting areas of interest in biomedical engineering and bring together high-profile researchers and promising younger investigators with complementary expertise and interests.

“I think people who attended Engineering Biology for Medicine left our conference with lots of new ideas and new connections,” says Chilkoti. “Our goal was to create an event that would bring together some of the most exciting minds in the field, and I think it was a resounding success.” ■

ABOVE: Ravi Bellamkonda addresses the crowd at the Engineering Biology for Medicine conference.

LEFT: Conference attendees attend a reception held at the Nasher Museum on Duke's campus.

THE BODY On Defense

In his lab at Duke, Joel Collier uses synthetic nanomaterials to boost the body's natural defenses

When a person gets sick, the invading virus or bacteria often triggers an immune response, sending a wave of white blood cells to attack the source of the illness. While the body's natural defenses can often dismantle the infection, the immune reaction can sometimes cause more harm than intended.

That's where Joel Collier's lab comes in.

Collier, an associate professor in Duke University's Department of Biomedical Engineering, creates biomaterials that can help understand and control adaptive immune responses. Most of the lab's projects involve using biomaterials, peptides and proteins to devise immune therapies for a variety of diseases, ranging from cancer to Crohn's disease.

"Figuring out how we can design these materials to self-assemble and appropriately interact with the immune system is a big challenge," says Collier. "Every type of immunological cell that interacts with these materials does so in a different

manner, so we have to build our materials in a way that stimulates the right combination of cells to behave the right way."

One of the main foci within immune engineering is devising how to induce the immune system to combat diseases that lack highly protective vaccines, like HIV, malaria and influenza. To address this issue, Collier and his research group collab-

orate with the Duke Human Vaccine Institute to explore how they can use engineered nanomaterials to improve vaccines.

"Broadly neutralizing antibodies are one of the holy grails of vaccination, and the folks at the Duke Human Vaccine Institute have spent years refining their ideas and technologies for raising the best antibodies," says Collier. "In collaboration with Genevieve Fouda, Sallie Permar, and Kevin Saunders, we are arraying these antigens on nanomaterials. HIV, for example, is very diverse, so you need antibodies that will neutralize as many strains as possible."

"We were able to explore this topic using seed fundign from MEDx and Duke's Center for AIDS Research (CFAR)," says Chelsea Fries, one of the PhD students in the Collier lab leading this work. "Our group brings new nanomaterials to the table, providing technology that allows us to control how immune cells interact with HIV antigens. We hope that the unique architecture of our materials will promote stronger responses from B cells, which can make antibodies to fight diverse strains of HIV virus."

Collier's lab has also broadened their efforts to focus on non-infectious diseases like cancer. Collier and Yaoying Wu, a post-doctoral fellow in his lab, are working with John Sampson, MD, the Robert and Gloria Wilkins Professor of Neurosurgery in the Duke University School of Medicine, who studies glioblastoma, a deadly form of brain cancer. Using specific antigens identified by the Sampson lab, Collier and Wu are exploring how they can use a synthetic version of their platform to boost the activity of both B cells, which make antibodies, and T cells, which recognize and kill infected cells directly, to combat cancer.

In addition to these projects, the Collier lab has zeroed in on a relatively unexplored research topic: how to use the immune system to fight inflammatory diseases.



"We hope that the unique architectures of our materials will promote stronger responses from B cells, which can make antibodies to fight diverse strains of HIV virus."

One of the hallmarks of inflammatory diseases is the overproduction of inflammatory signaling proteins known as cytokines. One of the most important inflammatory cytokines is a protein called TNF. Currently, deploying manufactured antibodies that target TNF is the best way to lower its levels. But this method requires patients to inject themselves regularly with the antibody, which is expensive, does not work for many patients, and can eventually stop working.

Instead, Collier and his team are investigating ways to use nanomaterials to induce the body's immune system to become an anti-TNF antibody factory itself. But that is easier said

than done, as patients would also need a way to regulate the amount of antibodies in their system.

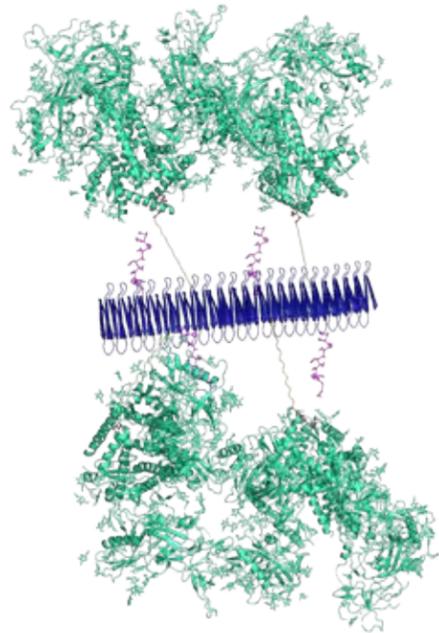
"If our idea is successful, you would get an initial dose and then you'd be able to get a booster far less frequently," says Collier. "The big challenge now is learning how to tune the antibody level up, down, or off. But if we can develop these strategies, we've got a whole new way of treating inflammatory dis-

ease, and that's very exciting. We can also think about targeting other harmful proteins in the body, so the approach could ultimately have very broad applicability."

According to Collier, these neglected frontiers in immunotherapy and immune engineering are among the most exciting aspect of his group's research in Duke BME.

"Most immunotherapy research is focused on cancer and infectious diseases, but I also like to steer our group to places where few people are looking. I find that you can have a bigger impact if you can go places where others aren't," says Collier.

"We're active in the more traditional and competitive areas of vaccine development, but often I think our biggest long-term potential involves our least traditional projects. And we're putting ideas out there, right? We might get all the way there, we may get part of the way, but putting ideas out there allows the rest of the scientific community to also run with them." ■



A self-assembling peptide nanofiber decorated with engineered HIV antigens and T cell epitopes. (Credit: Chelsea Fries)



LEFT: The Collier Lab

ABOVE: Peptides with specific sequences form higher-order structures like the nanofibers shown above. These peptide sequences can be extended to incorporate epitopes from cancer, infectious disease, or autologous proteins. (Credit: Chelsea Fries)



UNDERSTANDING Cells Under Pressure

Brenton Hoffman's lab explores how mechanical forces can shape and control our cells

Most researchers today understand biology through the principles of chemistry. Cells can communicate through chemical signals, and traditional medicine has long focused on how to treat disease by modifying those signals. But according to Brenton Hoffman, an assistant professor of biomedical engineering at Duke University, this approach is incomplete, as it ignores a major factor in cell biology: physics.

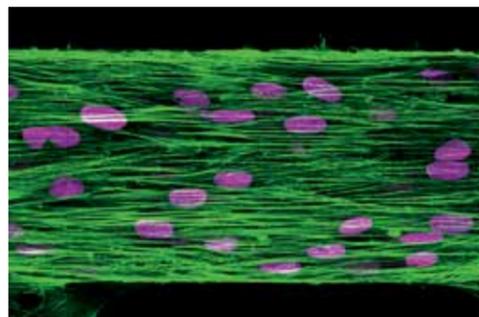
When you apply a force on a cell, that pressure can alter and control its structure and behavior. For example, the force generated by blood flow pushes on the cells in blood vessels, helping them grow into the correct shape and behave normally. The same principle, Hoffman says, can be applied to a disease like breast cancer.

“A breast tumor is normally discovered when someone feels a lump, and we know that a lump is a local increase in stiffness, which means it’s a mechanical input to cells,” says Hoffman. “As we learn more about these mechanical inputs, we’re examining whether the physical changes in cells and their local environments that make cancerous tissue stiff may actually be a trigger for cancer to spread, just like if you have a perturbed growth factor or a mutated receptor.”

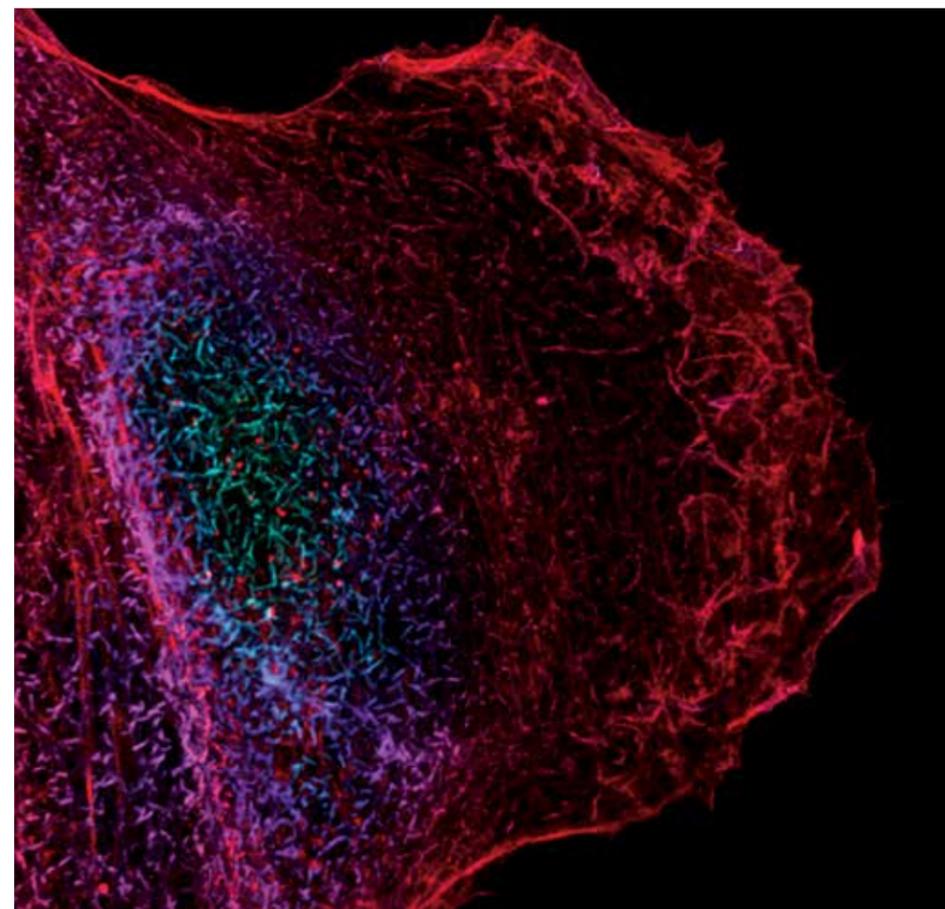
Until recently, researchers had no way to explore how these processes worked, but the Hoffman lab has circumvented this problem by creating tunable protein sensors capable of measuring forces inside living cells. These tension sensors contain a specially engineered module that emits a fluorescent glow under normal conditions, but dims if it experiences a change in force. The sensor is placed inside specific proteins that physically connect the cell to its surroundings. When these proteins feel a physical force, they stretch, causing the sensor to dim.

“You could argue that we’re making the tools to do basic studies, but that’s necessary, especially in a newer field,” says Hoffman. “If we can understand the pathological mechanics, we can control, or maybe eliminate, the pathological signaling that drives mechano-sensitive disease.” ■

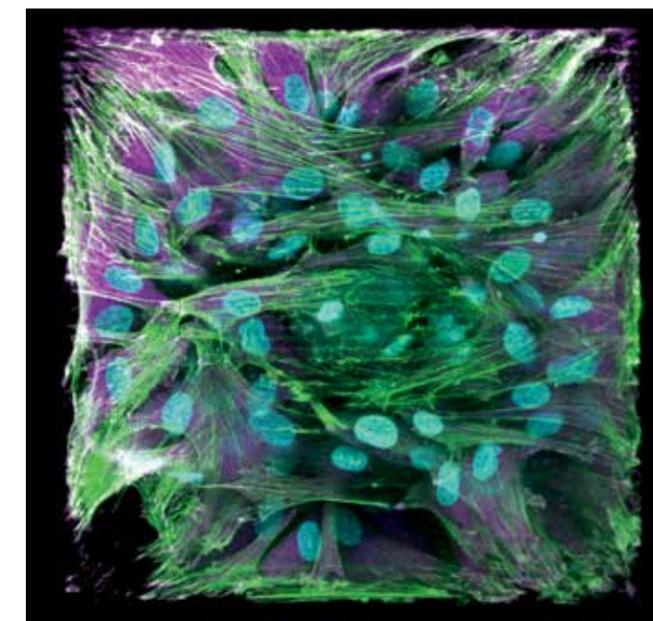
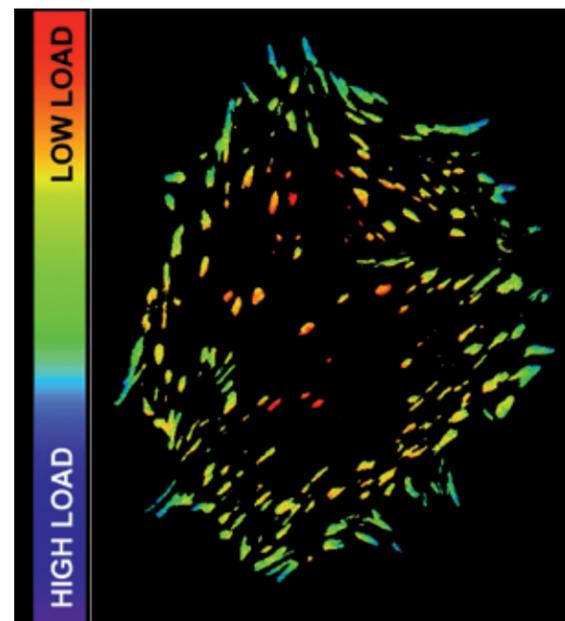
“If we can understand the pathological mechanics, we can control, or maybe eliminate, the pathological signaling that drives mechano-sensitive disease.”



can use mechanics to study what he calls mechano-sensitive diseases and address problems in biol-



Super-resolution microscopy reveals the complex architecture of the actin cytoskeleton in a migrating epithelial cell (left). Cells utilize this contractile actin network to probe the mechanical stiffness of their environment, stretching proteins at the interface with their surroundings. Hoffman uses fluorescent protein sensors inserted within these proteins (bottom left) to measure forces across them and understand how these mechanical signals are converted into downstream biochemical ones, both in the context of single cells and in multi-cellular constructs (opposite page and bottom right) that more closely mimic behaviors at the tissue scale. (Credit: Evan Gates, Julie Malavade and Christopher Gilchrist)



BIG IDEAS ON A Small Surface

George Truskey and Shyni Varghese use custom lab-on-a-chip technologies to create novel models of tissues, organs and diseases

Several small slides sit in an incubator in Shyni Varghese’s lab at Duke University, where they are connected to an assortment of machines via a network of delicate clear tubes. Roughly the size of viewing slides used in microscopes, these slides are actually microfluidic chips that house living samples of cells and tissues.

Varghese, a professor of biomedical engineering at Duke University, uses this lab-on-a-chip technology to create miniature models of tissues, organs and even tumors.

One of the major challenges in studying disease and developing therapies involves the different responses each provoke in different organs and tissues in the body. Cells in the liver will react differently to disease or various pharmaceuticals than cells in the heart, and drug reactions and disease symptoms can also be inconsistent from person to person. Using animal models or

petri-dish studies with human cells to understand these responses has proven less than perfect.

The Varghese lab’s chips help address this shortcoming by allowing the researchers to mimic the disease conditions similar to that in the human body.

“Our work tries to capture the architecture of the microenvironment of the organ within the device, so the cells are arranged the same way they’d be arranged inside the organ, and they’re subject to the same mechanical forces that are in the body,” says Vardhman Kumar, a PhD student in the lab who specializes in the technolo

gy. “You can make individual organ-on-a-chip models using cells from the gut, the skin, or even cells from the eye. Anything you can think of in the body can be engineered and assembled on a chip.”

A Full-Body Model

Previously, the Varghese lab developed models for the heart and liver, but they’ve since turned their attention to other systems, including musculoskeletal tissues. To model this system, they created a knee joint which, Kumar says, will allow them to examine how biomechanics and inflammation affect joint function and health.

The team has also expanded their platform to study the lungs, creating a tissue model that can pulsate to mimic how the lungs expand and contract as humans inhale and exhale. In addition to enabling them to study various physiochemical cues in normal lung development, this model allows the researchers to recapitulate different aspects of lung diseases.

“With the lung chips, for example, we’ll be able to mimic coughing and other behavior that’s associated with idiopathic pulmonary fibrosis, a lung disease that causes scarring and difficulty breathing,” says Varghese, a Duke MEDx investigator who also holds joint appointments in mechanical engineering and materials science and in orthopaedic surgery at the Duke University School of Medicine.

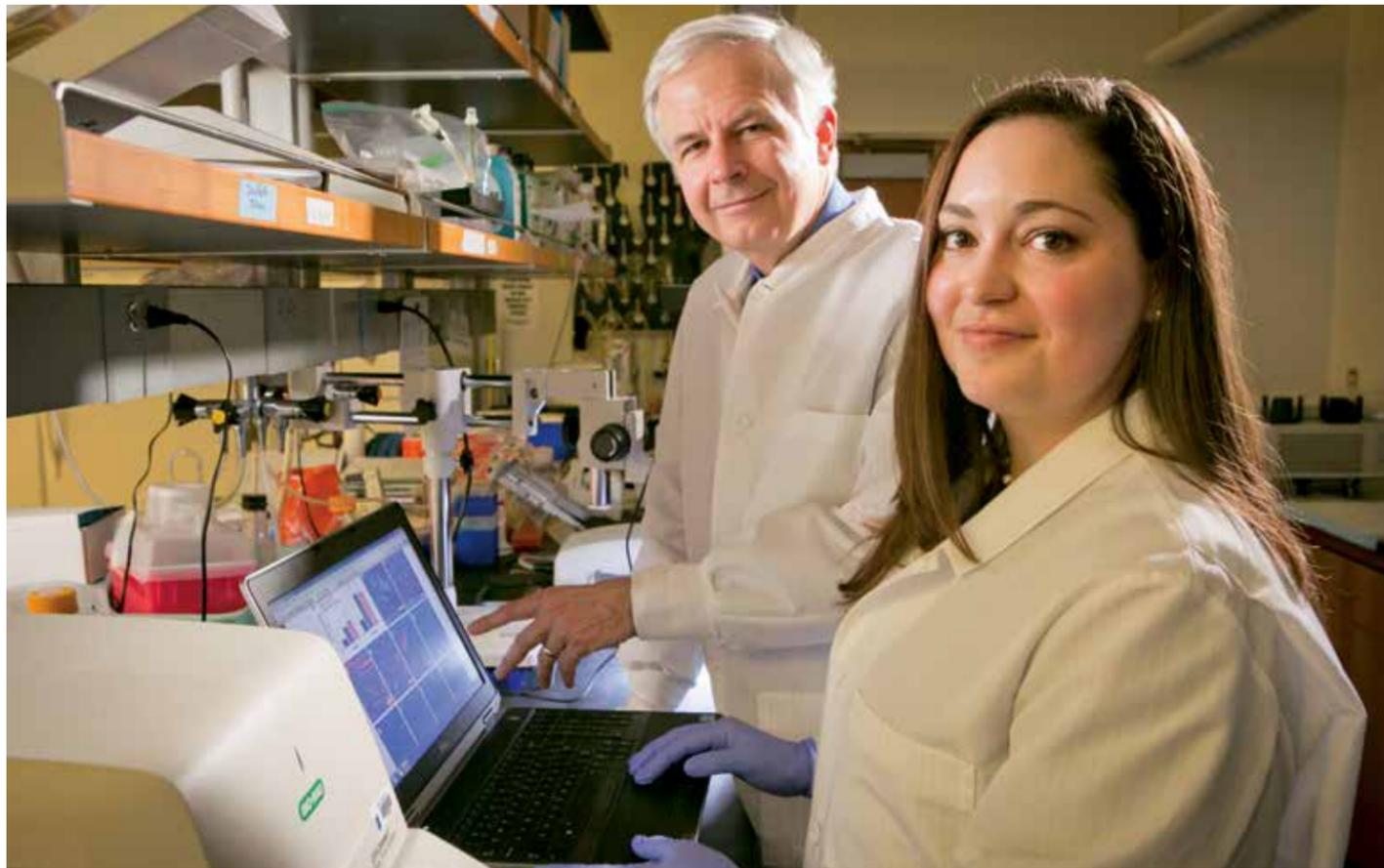
A larger focus for the lab, she adds, is figuring out how to integrate these individual systems for a body-on-a-chip platform. “We want to use this platform to test for cross-talk between different organs at the same time, which is more realistic than studying these organs in isolation,” Varghese says.

For example, her lab is developing “tumor on-a-chip systems” to examine the role of the cancer microenvironment on disease progression and diagnosis. Not only can the researchers test the efficacy of various drugs and immunotherapies on the tumor itself, they can also study how the drugs affect the entire body by connecting the tumor chips to the body-on-a-chip network.

“We’re trying to integrate these small-scale but very robust human-specific models to look at how immune cells can be recruited to a tumor site and to understand the role of the cellular environment on cancer spread



ABOVE: Shyni Varghese
LEFT: Vardhman Kumar examines a microfluidic chip.



George Truskey and Christina Fernandez examine slides from their microfluidic chips

and growth,” says Varghese. “Our goal is to use this system to address large biological questions and ultimately identify new therapeutic targets.”

Studying Cardiovascular Disease

A couple of buildings down from the Varghese lab, George Truskey, the R. Eugene and Susie E. Goodson Professor of Biomedical Engineering at Duke, is using his own version of a lab-on-a-chip platform to study how physical forces affect the function of blood vessels and skeletal muscle.

has zeroed in on how they can use these models to study the connection between cardiovascular and autoimmune diseases.

Earlier this year, the World Health Organization reported that cardiovascular disease is the leading cause of death worldwide, with nearly half of all Americans suffering from the disorder.

Typically researchers use animal models, particularly mice, to study the disease and perform initial tests for drugs, and humans are involved in the later stages of the drug approval process. However, due to the difference between animal and human physiology, animals don’t accurately replicate the disease

and drug response in humans, leading to failure of the drug to be approved. By making vessels with human cells, the Truskey lab can more closely approximate the disease and drug response in the body.

“When some individuals have a disease that causes inflammation, like lupus, rheumatoid arthritis or Crohn’s disease, they also have a higher risk of developing cardiovascular disease,” says Truskey. “Everyone knows that high levels of cholesterol can put you at risk for developing cardiovascular disease, but we want to explore the role of inflammation.”

“The goal would be to identify some novel biomarkers that may be helpful in assessing how inflammation influences cardiovascular disease.”

These sophisticated chips contain a central well for tissue samples, and channels that can pump nutrients and other materials into the tissue and create blood flow, mimicking its natural environment.

Truskey and his lab initially developed the chips to model human blood vessels so they could study cellular response to various drugs. Now, the lab

Toward that end, Truskey and his lab have created blood vessel models that mimic the early stages of atherosclerosis, a cardiovascular condition in which fats and other substances build up along the walls of blood vessels, making them thick and stiff. The team plans to combine these models with their skeletal muscle models of rheumatoid arthritis.

“This linkage would resemble what occurs physiologically in the bloodstream,” says Truskey. “Our rheumatoid arthritis model would essentially serve as a reservoir for inflammation, and we’re curious if linking it with our blood vessel model would exacerbate the symptoms of cardiovascular disease. The goal would be to identify some novel biomarkers that may be helpful in assessing how inflammation influences cardiovascular disease.”

Hope for Rare Diseases

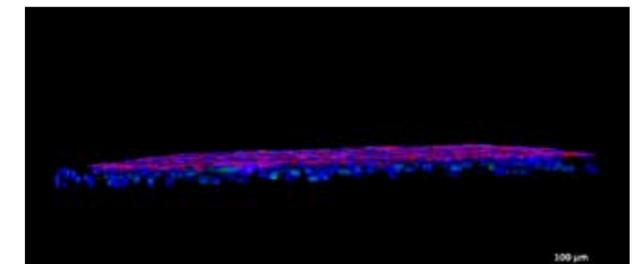
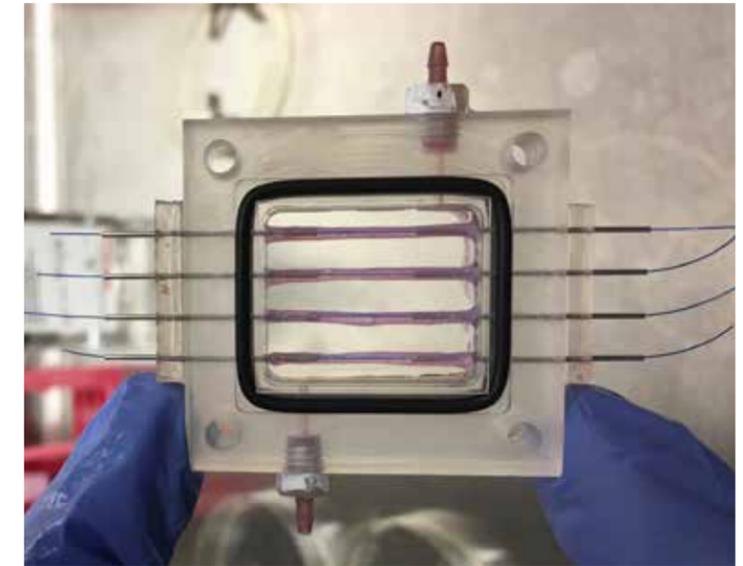
The lab has also employed tissue chips to study rare diseases, like Hutchinson-Gilford Progeria Syndrome. Known as progeria, the disease causes symptoms that resemble accelerated aging in children; patients typically die of heart disease or stroke, caused by weakened blood vessels, by the age of 14. There are less than 250 known cases worldwide.

“This disease is so rare and deadly that it’s challenging to run clinical trials with compounds that may help alleviate symptoms associated with the disease or provide some sort of treatment,” says Truskey. “But that’s where our blood vessel models prove to be very helpful.”

Using induced pluripotent stem cells from patients with progeria, the team was able to grow engineered blood vessels that displayed many of the symptoms seen in people with the disease. This new model allowed them to test a variety of pharmaceuticals, revealing more about the efficacy of various therapies.

After finding success with disease models, the Truskey lab has also started using their tissue chips to study the human placental barrier in collaboration with Liping Feng, MD, an assistant professor of obstetrics and gynecology in the Duke University School of Medicine.

The placenta is created in the uterus during pregnancy, where it helps provide oxygen and nu-



TOP: A chip containing four engineered blood vessels

BOTTOM: A 3D view of the engineered blood vessel with the endothelial layer shown in pink. (Credit: Dr. Xu Zhang)

trients to the fetus. Many of the complications that occur during pregnancy, such as pre-eclampsia, are linked with irregular placenta development. But researchers have long struggled to study these complications and address larger questions about pregnancy because they’ve lacked an effective research model for the human placenta.

Using a synthetic membrane interspersed with cells from human placenta cells, the team was able to create a model that could mimic the blood flow through a placenta. Although the work is still in its early stages, Truskey and his collaborators hope to use their organ-on-a-chip to better understand how viruses, drugs and other molecules travel from the mother to the fetus.

“This technology has advanced to the point where you can create many of the key features of tissues and organs in the lab, and that opens a lot of possibilities—not just to understand normal function, but to model diseases,” says Truskey. “In the end we hope that it will help speed up and improve the drug development process if you show that these can be used as efficient models.” ■

“Our hope is to use DART to better understand how we can alleviate pain without introducing the negative side effects [of opioids].”

ZOOMING IN ON Neuronal Behavior

There are more than 86 billion neurons in the brain. These cells communicate by sending electrical and chemical signals across pathways and are responsible for controlling everything from simple movement to the processing and storing of complex thoughts and memories. The sheer enormity of this neural network makes the brain a difficult frontier for exploration, but Michael Tadross and Yiyang Gong, two faculty in Duke University's Department of Biomedical Engineering and recipients of the NIH Young Innovator Award, are using distinct approaches to decipher the body's most complex organ.

A Molecular GPS to Target Neurons

Michael Tadross, MD, is an assistant professor in Duke BME, where his lab uses a novel technology dubbed DART, or Drugs Acutely Restricted by Tethering, to deliver pharmaceuticals to specific cells within the brain. By harnessing DART, Tadross and his lab hope to develop a better understanding of how drugs work in the brain.

“Drugs have been really important in the history of neuroscience and neuropsychiatric disease, and I think we take it for granted that brain diseases are biological diseases. There was a time when that wasn't widely believed to be true,” says Tadross. “The turning point came in the 1950s, after drugs were accidentally discovered to have an effect on disorders like depression.”

This discovery set off a race to discover exactly what these drugs are binding to in the brain, with the goal of better understanding the fundamental basis of brain disease. More than 60 years later, researchers still lack this deeper understanding.

But DART could address these long standing questions by enabling Tadross and his team to target drugs to one type of neuron at a time.

Drugs today are unable to discriminate between different groups of cells. For example, when someone is treated for a mood-relat-

ed disorder like depression, they are prescribed a drug called an SSRI. These drugs target a receptor that reabsorbs the chemical messenger serotonin, a neurotransmitter that plays a role in mood. SSRIs block these receptors, boosting the amount of serotonin in the brain.

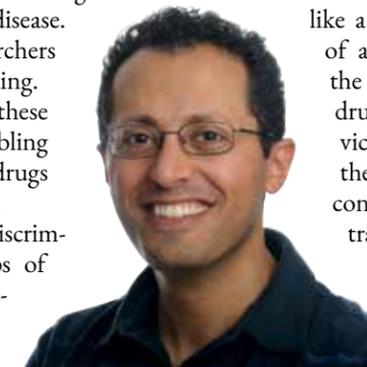
The problem with this approach is that there can be thousands to millions of cells that express the same receptor, so when you take a drug, it affects all of those cells at the same time, often leading to a complex set of effects. While SSRIs, for example, may effectively treat depression, they can also have a negative effect on anxiety.

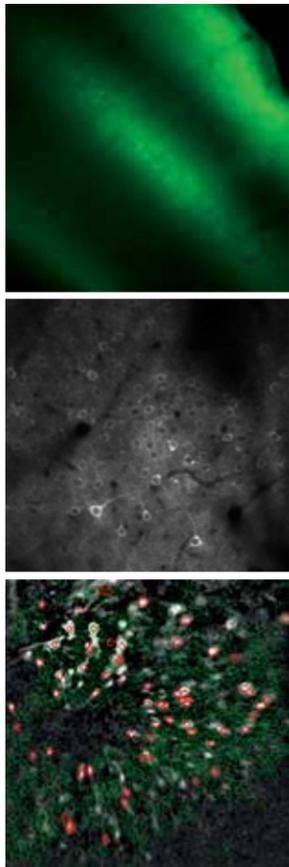
“There has already been a lot of work tailored toward locally infusing drugs into specific parts of the brain, like the amygdala or the prefrontal cortex,” says Tadross. “But DART allows you to do that to a far greater degree of specificity.”

DART works by genetically programming specific cell types to express a virus that acts like a GPS beacon. This beacon, made of a protein found in bacteria, coats the surface of target cells, and attracts drugs loaded with a special homing device. When a drug is introduced into the system, it's captured by this beacon and creates a locally high concentration of the drug.

“There is a universal on-off switch for every drug, and that's the dose,” says Tadross.

OPPOSITE: DART genetically programs cells to express a virus (in red) that attracts drugs loaded with a homing device, creating a locally high concentration of the drug. These drugs can then manipulate specific molecules (in green) for a desired outcome, like blocking the absorption of a neurotransmitter like serotonin. (Credit: Julia Kuhl)





Fluorescent sensors label and report activity from the brain. TOP: Fluorescent sensors label the excitatory neurons in the upper layers of the cortex. MIDDLE: High resolution two-photon image of neural activity details the structure of neurons labeled with fluorescent calcium sensors. BOTTOM: Deep learning algorithms rapidly find firing neurons (red outlines) from the complete set of active neurons (green highlights) in one-photon movies. (Credit: Gong Lab)

“The homing device and GPS beacon are so efficient that the concentration of the drug on the cells of interest accumulates to become 1,000 times higher than anywhere else within a few minutes, so it’s only acting where you want it to. The simplicity of that idea allows it to work for virtually any drug.”

Tadross and his lab are using DART to untangle the mysteries of the human brain, starting with movement disorders like Parkinson’s disease. The team has focused their efforts on the striatum, a structure in the basal ganglia, which helps control speech, movement and posture. Among the different cell types in the striatum are D1 and D2 cells, which are tightly intermingled, but communicate to different brain regions.

In Parkinson’s disease models, cells that produce the neurotransmitter dopamine begin to die off, which results in less dopamine being sent into the basal ganglia. To compensate for this drop in dopamine, the brain tries to rewire itself by altering signals carried by the neurotransmitter glutamate, creating an imbalance.

To explore the role of glutamate in Parkinson’s disease, Tadross and his lab worked with a Parkinson’s mouse model. These animals have difficulty moving, develop a curved posture, and can only turn in one direction. Rather than treat the disorder by increasing dopamine (the standard treatment), they used DART to stop the D2 type of neuron from compensating by delivering a glutamate antagonist specifically to these cells in the striatum. The animal’s movement dramatically improved. Remarkably, this improvement was only seen when the drug was targeted exclusively to D2 cells; delivering the drug to both D1 and D2 cells had no benefit.

“At first we were surprised to see such a dramatic effect, since we knew that glutamate antagonists have historically never worked to treat Parkinson’s. In retrospect, it made perfect sense. You can think of it like a seesaw where there is an imbalance in the circuit; targeting a drug to just one of the cells is a great way to rebalance the seesaw,” says Tadross. “There are still many questions left to answer, and we’re now using newer DARTs to study how different neurotransmitters contribute to this disorder.”

Beyond investigating movement disorders, Tad-

ross and his team are interested in using DART to study issues like addiction, especially as it pertains to opioids. In 2018 alone, the Centers for Disease Control and Prevention reported that more than 68,000 people died from drug overdoses in the United States, and opioids were responsible for a majority of these deaths. The Tadross lab hopes to use their technology to examine which cells in the brain are the most helpful for pain relief and which cells are driving addiction.

“Every drug has some good things and bad things associated with it, but while there hasn’t been another drug that’s been able to match the pain-relief benefits of opioids, these drugs are so dangerous and addictive that their costs have outweighed their benefits,” says Tadross. “Our hope is to use DART to better understand how we can alleviate pain without introducing those negative side effects.”

“Wouldn’t it be great to understand how they work and finally develop a therapy that was designed on purpose rather than accidentally?” asks Tadross. “We don’t know the answers to those big questions, but we now have the tools that can point us in the right direction.”

Lighting Up the Brain’s Activity

When Yiyang Gong describes his lab as interdisciplinary, he’s not exaggerating. His team creates tools at the intersection of neurobiology, optics and engineering. Gong is an assistant professor in Duke University’s Department of Biomedical Engineering, and his lab studies brain circuitry by recording, perturbing and controlling brain activity in various situations.

“Our goal is to record neural activity in novel ways,” says Gong. “Our lab uses interdisciplinary techniques to understand how neurons communicate with each other. We’re achieving this using sensors that we developed that flash in response to neural signals, and pairing that with microscopy tools that help us record those flashes.”

Neurons generate and communicate messages partly by changing their voltage. These electrical signals can be measured as single pulses from a neuron called an action potential, or as oscillating waves of activity from a group of neurons working together.

Gong and his lab have engineered voltage sensors made from pieces of DNA that can be delivered to target neurons using a virus. Once the virus

is in place, the DNA encodes a protein that sits on the cell membrane and flashes in response to the neuron’s changing voltage. By genetically targeting different types of neurons with his sensor, Gong hopes to understand how these cell types oscillate together and contribute to different types of brainwaves.

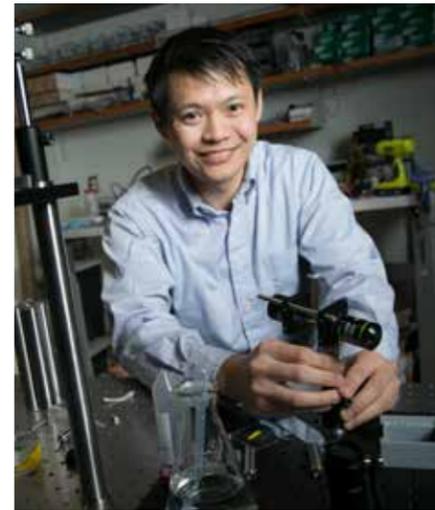
Since their inception, Gong has been able to use these sensors in cell cultures, intact tissues and even live animal models. Now, the lab aims to improve these sensors by both increasing their speed and differentiating them by introducing multiple colors.

“That second point is especially important, because we’d like to see how neuron 1 communicates with neuron 2, or how population 1 com-

real-time behavioral studies.

Currently, identifying neurons in recordings is a painstaking process. The most accurate method involves a human analyst circling every ‘spark’ they see in a recording, often requiring them to stop and rewind the video until every targeted neuron has been identified. This process, called segmentation, is challenging and time-consuming, and a researcher can spend anywhere from four to 24 hours working on a 30-minute recording.

But the new algorithm developed by Gong and Farsiu can accurately identify and segment neurons in mere minutes.



Yiyang Gong

“We’re trying to get a detailed view of how neurons communicate with each other, and we’re accomplishing this by developing sensors that can fluoresce in response to neural signals, and then microscopy tools that can help us record those flashes.”

municates with population 2,” says Gong. “These insights could help reveal the neural origin of complex actions like learning, remembering and decision-making.”

In addition to developing these genetic sensors, Gong and his team are exploring how they can advance microscopy tools to record their flashes. Previously, researchers have developed tools that can measure a few action potentials a second. According to Gong, there hasn’t been a push to speed up the imaging tools because that’s as fast as traditional optical sensors for neural activity have responded.

“With the advent of the genetically encoded voltage sensors, we also needed to develop really fast microscopy on the same timescales,” says Gong. “Our goal is to develop faster versions of fluorescence microscopes that can still image large portions of the brain.”

The Gong lab is also working on new methods to help process their incoming data. In collaboration with Sina Farsiu, the Paul Ruffin Scarborough Associate Professor of Engineering at Duke, Gong has developed an automated process that can rapidly gather and process neuronal signals for

“Data analysts have spent hours and hours processing minutes of data, and that’s created a bottleneck in neuroscience that’s been a problem for a long time. But our algorithm can process a 30-minute video in 20 to 30 minutes,” says Gong. “We were also able to generalize its performance, so it can operate equally well if we need to segment neurons from another layer of the brain with different neuron size or densities.”

Gong is already using this new technique to study the relationship between neural activity and different behaviors in mice. By understanding which neurons fire during specific activities, his lab could learn how researchers can manipulate brain activity to modify behavior.

“The overall idea is that we can combine the technologies to not only do the overall data processing applications in the near future, but to use this tech in the far future to understand what the brain is doing in real time,” says Gong. “Neuroscience is a very cool discipline, and my lab allows us to take a lot of engineering concepts and apply them to the great unknown of brain activity.” ■

PROGRAMMING

Cell Behavior

With work spanning gene circuits, antibiotic resistance and the mathematical modeling of cellular networks, Lingchong You explores how to best to harness microbes for medical purposes | **By Mary-Russell Roberson**

When Lingchong You earned his PhD in chemical engineering in 2002, synthetic biology was in its infancy. Yet he was drawn to the field right away.

“Synthetic biology to me is just like programming,” You says. “Every cell is like a computer. In this case the code is in the form of DNA.”

You had spent years building mathematical models of viral infection in graduate school and was ready to do some experiments with living cells. As a postdoctoral researcher in the Caltech lab of Frances Arnold (who won a Nobel Prize last year), he dove into gene circuit design, which involves manipulating the genes of microbes so they will perform a desired function, like producing pharmaceuticals or biofuel.

“I just fell in love with this type of research, integrating both mathematical modeling and experiments,” he says. “As an engineer, you cannot get more ‘engineering’ than this! You introduce stuff into cells and look at how they behave and even predict how they will behave.”

Today, as a professor in Duke’s Department of Biomedical Engineering, You studies and manipulates the dynamics of microbial communities with the goal of developing practical applications in medicine and other fields.

One of his areas of research involves the growing problem of antibiotic resistance.

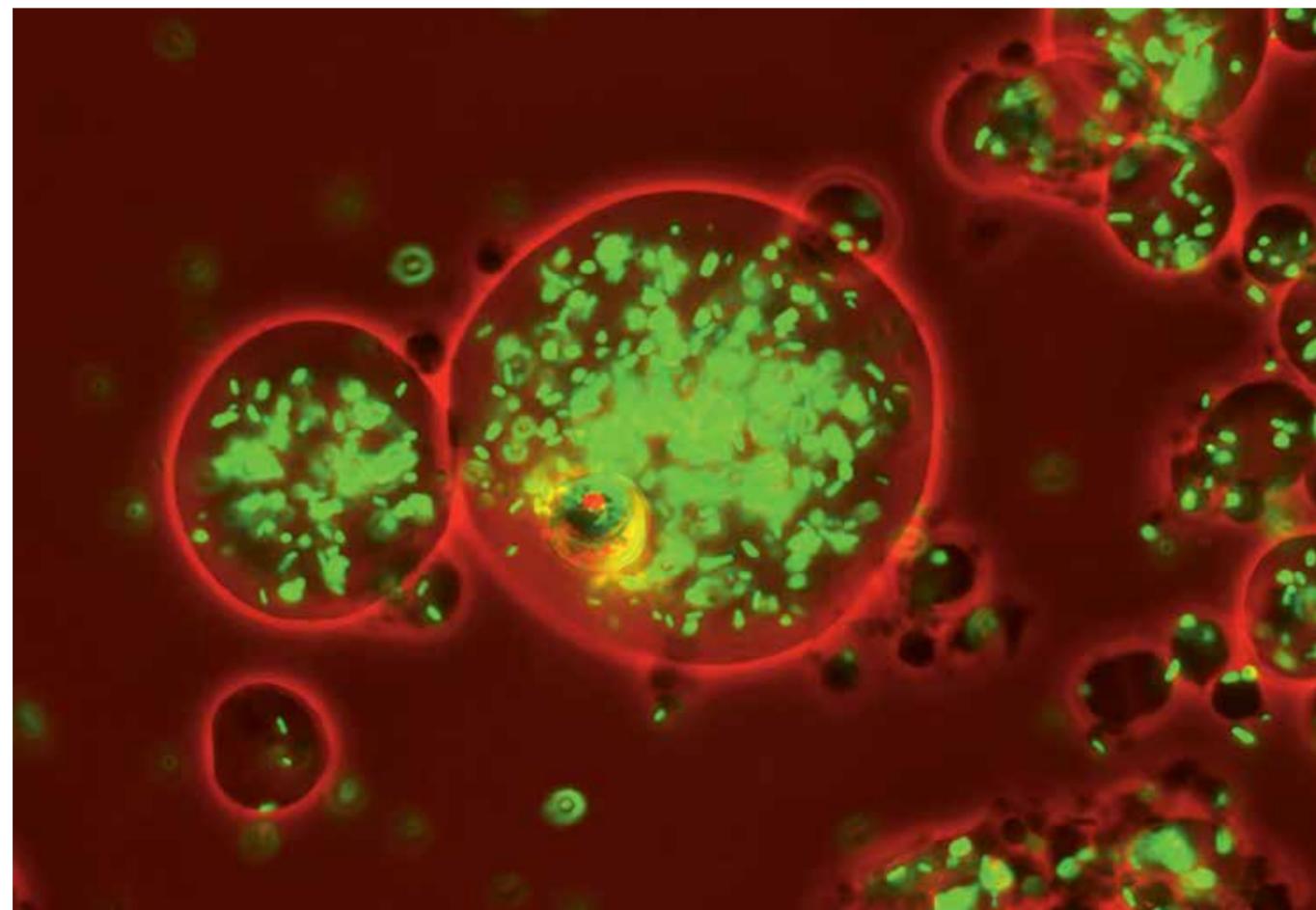
“We’re facing this antibiotic resistance crisis and there is a huge innovation gap in terms of lack of development of new antibiotics,” You says. “In my view, in the foreseeable future we have to deal with the situation by using the existing antibiotics in a more clever way.”

He and members of his lab have shown that pathogens can pass small bits of DNA that confer antibiotic resistance back and forth among themselves. These DNA bits are called plasmids, and they get shared in a process called horizontal gene transfer.

In a recent project, You analyzed hundreds of bacterial pathogens that show resistance to beta-lactam antibiotics such as penicillin and its cousins. He found that at least 25 percent of the pathogens can transfer resistance among themselves via horizontal gene transfer. In other words, a cell that has never been exposed to an antibiotic can still contain the plasmid that confers resistance to it. Furthermore, horizontal gene transfer happens rapidly enough to keep the plasmid in circulation in a microbial community, even though its presence is costly to an individual cell in the absence of an antibiotic. “That’s why it’s so difficult to eliminate this antibiotic resistance,” You says. “We’re trying to understand how you can control the plasmid dynamics.”

You and his students have engineered a synthetic plasmid that can replace the antibiotic-resistant plasmids in bacteria. The idea is that the synthetic plasmid, or something like it, could one day be given as a supplemental medicine along with a beta-lactam antibiotic. If antibiotic-resistant plasmids are present, the synthetic plasmid will spring into action to replace and eliminate them, leaving

Graduate student Yangxiaolu (Will) Cao speaks with Lingchong You in the You lab space.



The image shows fluorescent bacteria encapsulated in alginate beads. This process has been used by the You lab to generate living functional materials for versatile bio-manufacturing. (Credit: You Lab)

the door open for the antibiotic to kill the pathogenic cells. Once the antibiotic-resistant plasmids are gone, the synthetic ones become inactive.

“This is one example of synthetic biology where we designed a genetic program to control cell behavior,” You says.

He is working on several other aspects of antibiotic resistance as well. In collaboration with Deverick Anderson, an infectious disease physician in the Department of Medicine, he described the dynamics of a microbial community that is resistant in the face of antibiotic treatment, and compared that to the dynamics in a “resilient” community—one that appears to be resistant but is not. The researchers showed in the lab how a resilient community can be knocked out with first-line antibiotics by using a customized strategy of timing and dosing.

You is also active in another branch of synthetic biology—programming microbes to build patterns or structures. “Look at nature,” he says. “Nature gives us all these living functional materials. I grew into this shape from a single cell. If you think about that, it’s quite amazing. As an engineer, I

wonder how can we do something like this?”

In one project, You and colleagues engineered bacteria such that they spontaneously grew into dome shapes. When provided with gold nanoparticles, the bacteria incorporated them into the structures. The researchers then used a pair of these conductive domes to create a pressure sensor in an electrical circuit.

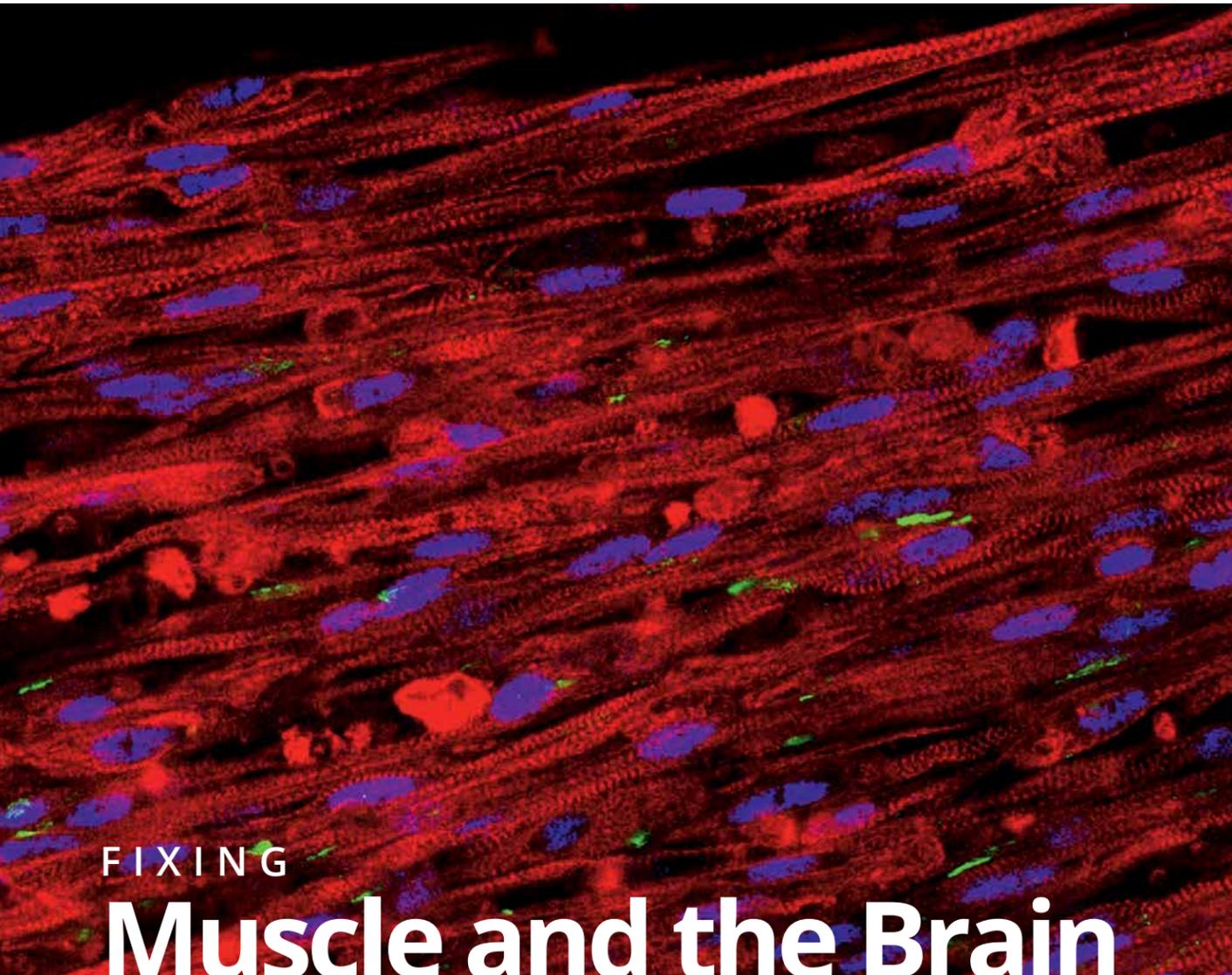
“This is the first time anyone has built a structure in a predictable manner using living cells that carry out a specific function,” You says.

You’s research projects may seem wide-ranging,

“Nature gives us all these living functional materials. I grew into this shape from a single cell. If you think about that, it’s quite amazing. As an engineer, I wonder how can we do something like this?”

but they are all driven by the same questions: how do microbial communities behave in space and time, and how can those behaviors be modified?

“Deep down I’m very curious,” You says. “It’s incredibly, incredibly fascinating to me—all of this.” ■



FIXING Muscle and the Brain

Nenad Bursac and Tatiana Segura are engineering hydrogel microenvironments to physically and chemically support the growth of skeletal muscle and brain tissue | By Ken Kingery

ABOVE: A stained cross section of the new muscle fibers. The red cells are striated muscle fibers, the green areas are receptors for neuronal input, and the blue patches are cell nuclei. (Credit: Bursac Lab)

With the number of times the word “gel” comes up in the research of Nenad Bursac and Tatiana Segura, one would be forgiven for thinking they might work in the haircare or running shoe industries. Their gels, however, are aimed at much more difficult tasks than holding hair styles or cushioning feet. Bursac and Segura, both professors of biomedical engineering at Duke University, are two of the leading researchers working to grow functional tissues both inside and outside of the human body. While the gels being engineered in their laboratories are initially more liquid than solid, they

promptly solidify to provide a crucial three-dimensional scaffolding for cells to organize and grow. And by adding the perfect combination of growth factors, enzymes, nutrients and supporting cells, their laboratories are successfully “engineering” some of most challenging human tissues, including skeletal muscle and brain matter. In 2014, Bursac and his team were the first to grow human skeletal muscle that contracts and responds just like native tissue to electrical pulses, biochemical signals and pharmaceuticals. While this initial success required a muscle biopsy to isolate and grow human “almost muscle cells” called myoblasts, they soon demonstrated the abil-



“...muscle cells derived from induced pluripotent stem cells form muscle fibers that contract and react to electrical and biochemical stimuli mimicking neuronal inputs, just like native muscle tissue.”

ity to achieve similar results starting from cellular scratch—human induced pluripotent stem cells.

The method took years to develop, with the researchers making educated guesses and taking baby steps toward their goal. The difference-maker was their unique cell culture conditions and 3-D matrix, which allows the cells to grow and develop much faster and longer than the 2-D approaches that are more typically used.

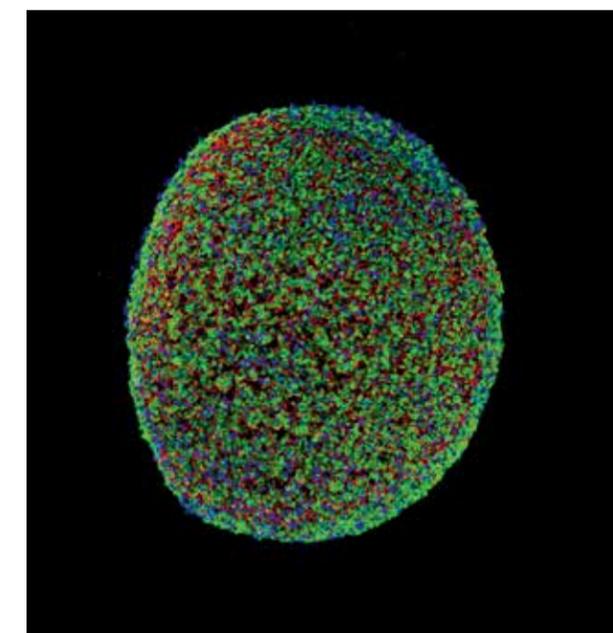
In the 2018 study, Bursac and his group showed that after two to four weeks of 3-D culture, muscle cells derived from induced pluripotent stem cells form muscle fibers that contract and react to electrical and biochemical stimuli mimicking neuronal inputs, just like native muscle tissue.

The pluripotent stem cell-derived muscle fibers also develop reservoirs of “satellite-like cells” that are necessary for normal adult muscles to repair damage and regenerate, while the muscles made using muscle biopsy had much fewer of these cells. The stem cell method is also capable of growing many more cells from a smaller starting batch than the biopsy method.

Both of the advantages point toward a possibility of using this new method for regenerative therapies and for creating models of rare diseases

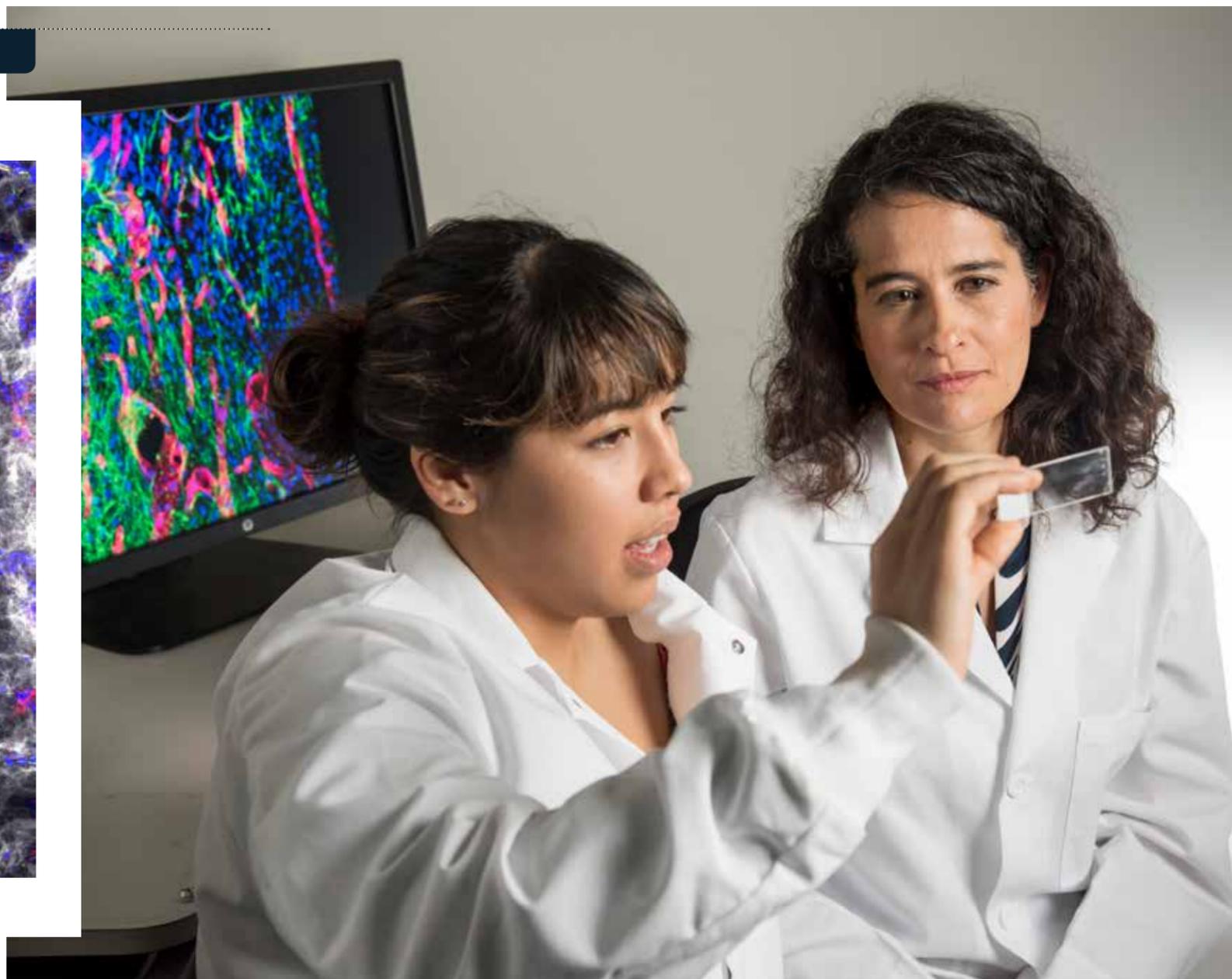
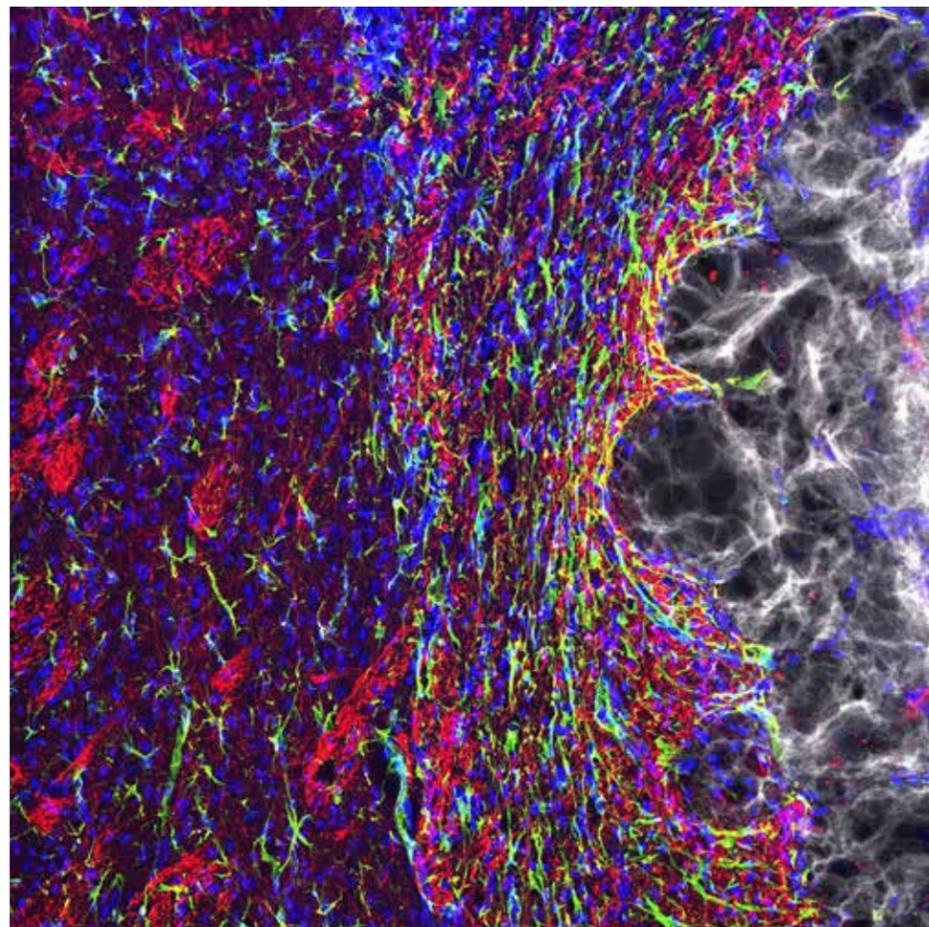
for future studies and individualized health care.

“The prospect of studying rare diseases is especially exciting for us,” says Bursac. “When a child’s muscles are already withering away from something like Duchenne muscular dystrophy, it would not be ethical to take muscle samples from them and do further damage. But with this technique, we can just take a small sample of non-muscle tissue, like skin or blood, revert the obtained cells to



ABOVE: Nenad Bursac

LEFT: A cross section of a muscle fiber grown from induced pluripotent stem cells. The green indicates muscle cells, the blue is cell nuclei, and the red is the surrounding support matrix for the cells. (Credit: Bursac Lab)



FAR LEFT: The image shows hydrogel that was injected into the stroke core. Axonal filaments are shown in red, astrocytes are dyed green, nuclei appear blue and the hydrogel is white. (Credit: Elias Sideris)

LEFT: Tatiana Segura with graduate student Katrina Wilson.

“We have something that is working, and hopefully we can now figure out the mechanisms for that improvement, because that is what leads to new engineering.”

a pluripotent state, and eventually grow an endless amount of functioning muscle fibers to test.”

The technique also holds promise for being combined with genetic therapies. Researchers could, in theory, fix genetic malfunctions in the induced pluripotent stem cells derived from a patient and then grow small patches of completely healthy muscle. While this could not heal or re-

ing perhaps the most stubborn organ in the human body—the brain.

The brain has a limited capacity for recovery after stroke and other diseases. Unlike some other organs in the body, such as the liver or skin, the brain does not regenerate new connections, blood vessels or new tissue structures. Tissue that dies in the brain from stroke is absorbed, leaving a cavity, devoid of

place an entire body’s worth of diseased muscle, it could be used in tandem with more widely targeted genetic therapies or to target more localized muscle repair.

In Segura’s laboratory, researchers are focusing on repair-

blood vessels, neurons or axons, the thin nerve fibers that project from neurons.

In a recent study conducted with mice, Segura sought to coax the healthy tissue surrounding the cavity into healing the stroke injury. She engineered a gel to inject into the stroke cavity that thickens to mimic the properties of brain tissue, creating a scaffolding for new growth.

This artificial material creates an environment that helps local, native stem cells do their best work to promote healing—providing the physical structure as well as the biological cues that encourage cells to grow. For example, the gel is infused with molecules that stimulate blood vessel growth and suppress inflammation, since inflammation results

in scars and impedes regrowth of functional tissue.

After 16 weeks, the stroke cavities contained regenerated brain tissue, including new neural networks—a result that had not been seen before. The mice with new neurons also showed improved motor behavior, though the exact mechanism wasn’t clear.

“We have something that is working, and hopefully we can now figure out the mechanisms for that improvement, because that is what leads to new engineering,” says Segura. “If you can discover which pathways are causing the improvements, then there are molecules you can deliver to engage those pathways more directly and hopefully lead to a therapy that will act faster and more robustly.” ■



The Evolution of Genetic Engineering

A Q&A with Charles Gersbach

Charles Gersbach, the Rooney Family Associate Professor of Biomedical Engineering at Duke University, leads a lab that is centered on developing and applying genome engineering tools—most notably CRISPR-based technology. CRISPR-Cas is a bacterial defense system that allows bacteria to use RNA molecules and CRISPR-associated (Cas) proteins to target and destroy the DNA of invading viruses. The discovery of this technique sparked a genome editing revolution as researchers explored how the tool could be used to specifically target and edit DNA in human cells. In the years since its discovery, Gersbach and his lab have used this technology to study genetic diseases like Duchenne muscular dystrophy, precisely control gene expression and even develop tools that can make CRISPR more accurate.

How did genetic engineering come to be your area of research?

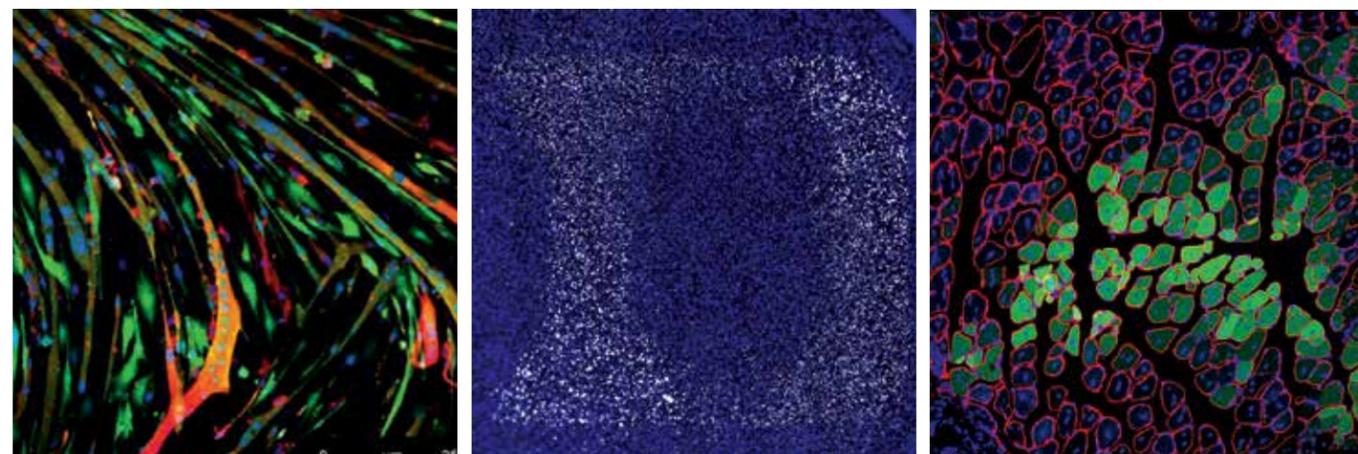
I knew that I wanted to go down an academic career path where I could help advance regenerative medicine and gene and cell therapies. I wanted to work on something that could affect a lot of different areas within bioengineering, and I realized that gene expression was a central component of a variety of research.

There were different wings in the building where I worked in graduate school, each focused on regenerative medicine as it related to a particular organ system. In different parts of the building, you could look at posters for cardiovascular

research or musculoskeletal research, or stuff pertaining to diabetes or neurological work. All of these topics essentially involved trying to get cells to do something interesting. As a very simplified example, if we want cells to behave like cartilage cells, we essentially bang on them using mechanical force and hope that they turn on the cartilage genes, and if we want cells to behave like neurons we electrically shock them and hope that kicks the neuronal genes into gear. In all these cases you're applying external stimuli and hoping that internally the right genes get expressed. If the goal across all of these areas is to get the right genes on and off, I was curious if there was a better or more direct technology to go in and program gene expression.

CRISPR is a relatively new technology. How did that end up being the focus of your lab's work at Duke?

The first paper that described using CRISPR in human cells was published in 2013, so we were working with technologies that preceded it. The first was engineered zinc finger proteins, which could be used to modify a specific region of the genome. At the time people were using zinc fingers to make



LEFT: Genetically engineered cells, including cultured muscle cells. MIDDLE: A cell line engineered to express a light-responsive gene in a user-defined pattern. RIGHT: Engineered cells that were implanted into mouse muscles to participate in muscle fiber repair (right). (Credits: Pratiksha Thakore and Lauren Toth).

transcription factors that that could turn genes on and off and regulate gene expression, but the technology was really difficult to work with so it hadn't really taken off, and there were only a few labs in the world that were working with it. I thought that if I trained with one of them then the world would be my oyster, so I trained in San Diego for three years and used that expertise as my pitch to start a lab at Duke in 2009.

Two years after I started at Duke a new technology came out, called TALENs, which was another tool that could make proteins to target specific sequences in the genome. It was a lot easier to use than zinc fingers, but before it had a chance to take off, CRISPR came along.

CRISPR is super easy to use, so my old dream that I was going to be one of the only people in the world that knew how to do this stuff went out the door. At the time I was thinking, "Great, I'm totally obsolete only four years into my academic career". Except we'd built up a lot of expertise and model systems for using these tools, and I was lucky to have some really brilliant and hard-working students and fellows in the lab, so we were able to quickly grab the CRISPR reagents and use them for our own work. On January 3rd, 2013, the first two papers using CRISPR in human cells were published online in Science, and three weeks later we had already obtained the plasmids and done CRISPR-based experiments in our own lab that worked. Our first CRISPR paper came six months later.

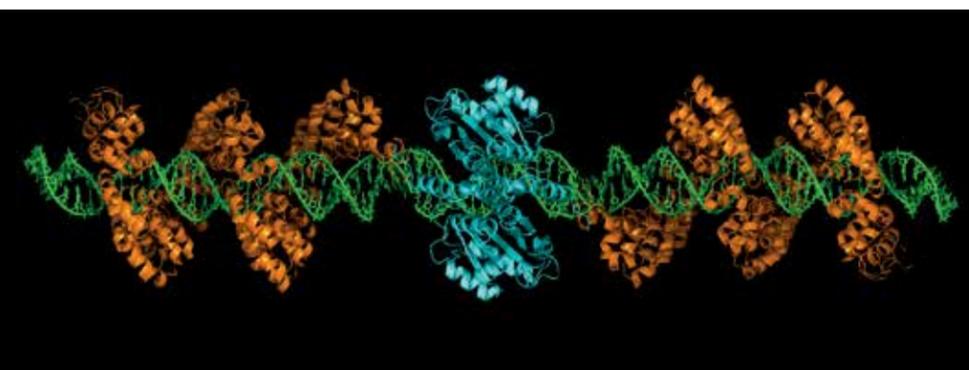
Why did you choose to focus on using CRISPR to study Duchenne muscular dystrophy?

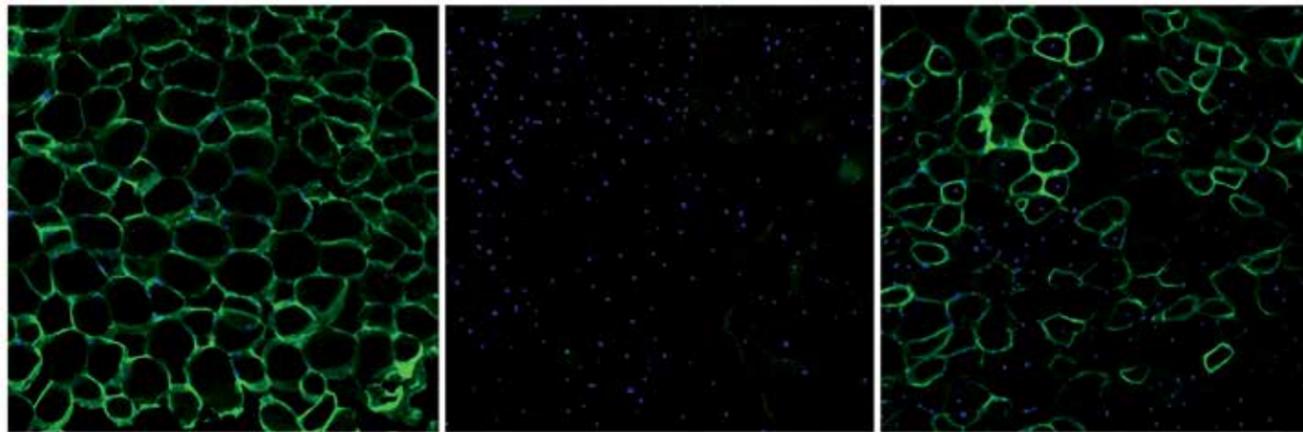
The first project my lab started in 2009 was gene editing for Duchenne muscular dystrophy using the zinc finger technology. DMD is the most common inherited genetic disease, but in 2009 there really weren't any therapies or options available for to help people with the disease, which causes progressive degeneration of their muscles. I was also a brand new assistant professor, so I wanted to do something that was two steps ahead of what everyone else was looking at, but it had to be a tangible enough goal so that we could make clear progress, and gene editing for muscular dystrophy was in line with those criteria.

Previously, people had looked at ways to modify stem cells and put them back into muscle, but replacing all the muscle cells in the body isn't really feasible. The also had modified viruses to deliver genes to muscle, but the gene that is mutated in this case is also one of the largest genes in the human genome, so it doesn't fit into typical gene therapy vectors. Instead, the idea of fixing the copy of the gene that is already in the genome, rather than trying to deliver it, seemed like an attractive opportunity. So we worked on that approach, and actually used zinc fingers and TALENs until CRISPR-based gene editing was discovered and we saw how easy it was to use.

Our work with Duchenne muscular dystrophy is ongoing. Most recently we published a paper in Nature Medicine that showed that a single systemic treatment could correct the DMD mutation for more than a year in mice.

Cartoon crystal structure of the TALEN gene editing technology. (Credit: Gersbach Lab)





LEFT: CRISPR gene editing used to correct a mouse model of Duchenne muscular dystrophy, with the dystrophin protein visualized in green. Sample cross-sections of normal muscle tissue. MIDDLE: Muscle tissue from a mouse with muscular dystrophy. RIGHT: The same type of dystrophic mouse treated with CRISPR. (Credit: Christopher Nelson)

What are the things that you can't do today with CRISPR that you want to be able to do?

I think it would be useful to do gene editing without cutting the genome. The solution to that problem may involve a new version of CRISPR, or it may involve something entirely different. But we're learning more about these systems every day—ten years ago it took two years to do a project involving gene editing, and now you can

Looking ahead with CRISPR, what are you most excited about?

The very first clinical trials for CRISPR in humans have started this year. We're seeing the first trials for cancer, for sickle cell disease, and for rare, inherited forms of blindness. And we'll have the results from those trials in the next year or two, so that's super exciting and definitely near the top of the list.

I also think we're at a place now where CRISPR has been fully integrated into the drug development process. Not just CRISPR as a therapy, but CRISPR to make model systems and cell lines to help design better drugs. I think

I think CRISPR will dramatically impact medicine in a way that is above and beyond how CRISPR is used as a therapy.

do it in a week or two. There are lots of flavors of CRISPR, where Cas9 is the most commonly used protein, but researchers have discovered dozens of other types of Cas9s and even other CRISPR proteins and complexes, including Cas12, Cas13, Cas14, Cas3 and even Cascade, and each of these systems lends itself to a unique use.

CRISPR will dramatically impact medicine in a way that is above and beyond using CRISPR directly as a therapy. In fact, we started a company, Element Genomics, to do this by facilitating drug development with these tools, not to necessarily use CRISPR as the therapy. That's subtle, so people don't necessarily appreciate that things like CRISPR are working in the background to make biological research more advanced to help come up with new drugs. ■

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