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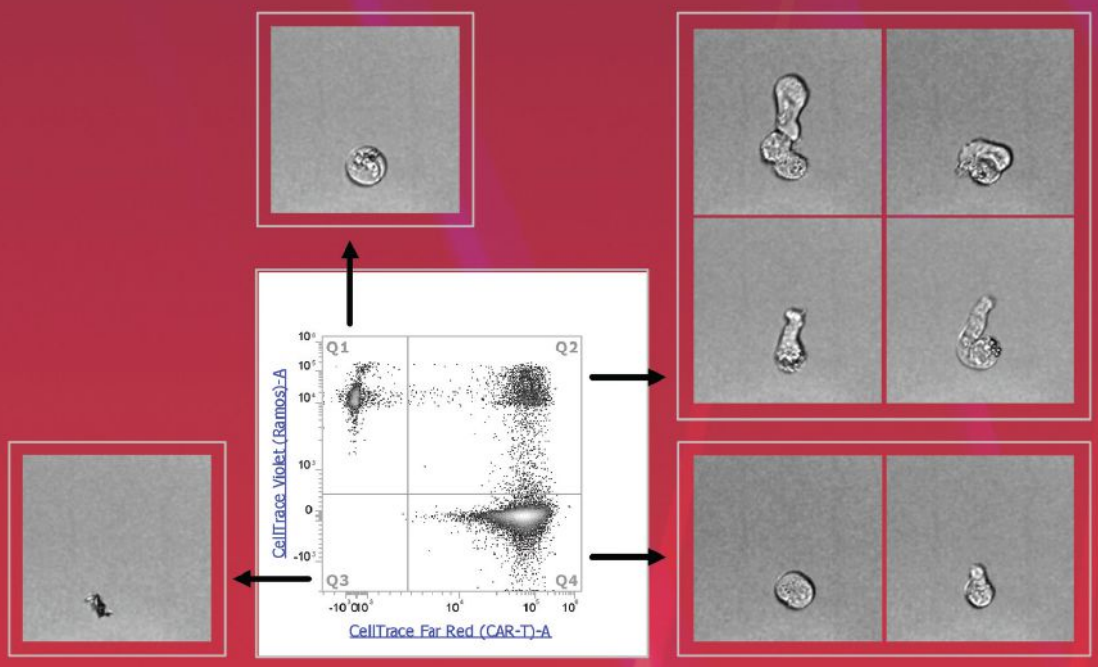

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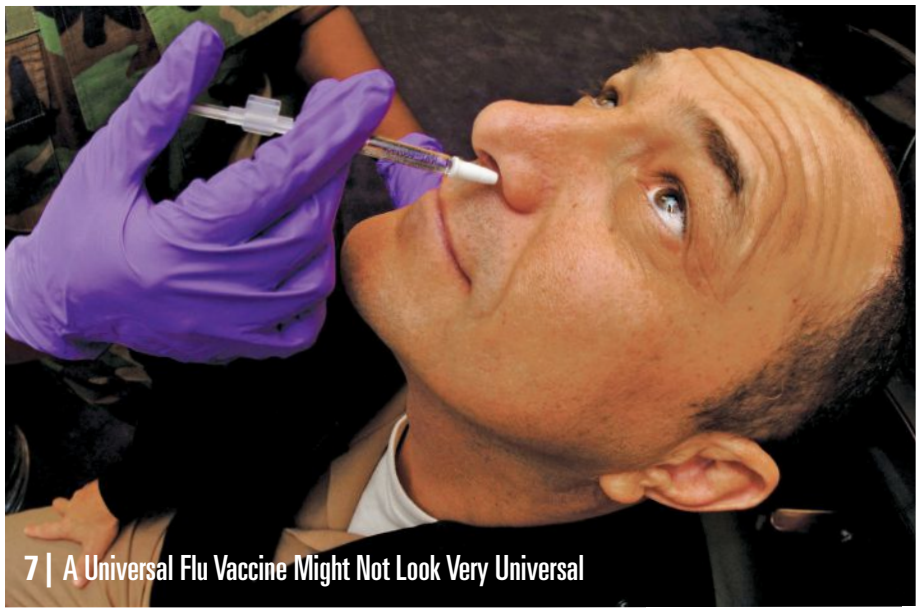

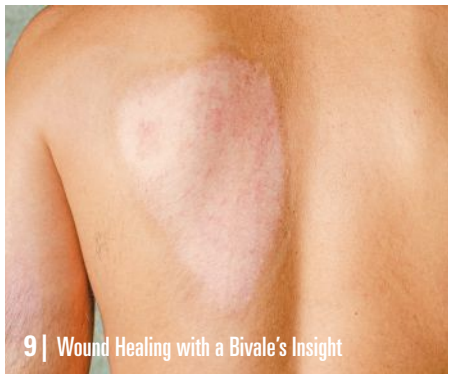

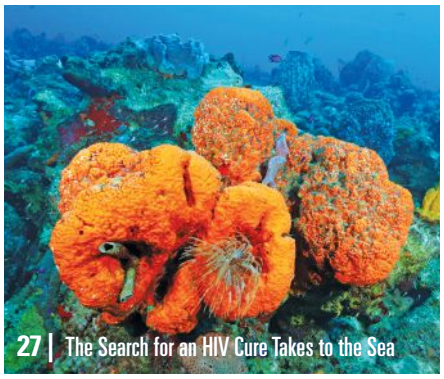

editor's
insight+opinion

Getting Ahead of the Next Pandemic

THE WORLD HAS BEEN STEWING IN THE COVID-19 PANDEMIC for close to three years now. A monkeypox outbreak where diagnosed cases number in the thousands seems poised to veer into a pandemic sooner rather than later. Scientists and science journalists are already declaring the 21st century the Pandemicine, where the cumulative effects of human activity, habitat destruction, and climate change will cause new viral pandemics or create zoonoses at a previously unheard of rate.

Even in a best-case scenario, it seems unlikely that humans can completely halt the age of pandemics. But as the United States is mired in another viral outbreak with monkeypox, we should take this opportunity to prepare for the next epidemic.

Scientists aren't in the dark about which viruses could be coming for us next. A 2021 paper in *Proceedings of the National Academy of Sciences* ranked the viral families most likely to cause pandemics or new spillovers into humans using data from viral surveys from 2009-2015. The paper listed familiar viruses, some of which have already caused regional or worldwide pandemics — SARS-CoV-2, Ebola — and others that haven't quite captured the world yet. But even those are known viruses with familiar biology: They're Arenaviruses like Lassa virus, Filoviruses like Ebola or Marburg (the latter of which is currently in outbreak), insect-borne Bunyaviruses, and a handful of other Coronaviruses.

Dan Samorodnitsky, PhD
SENIOR EDITOR

All of these viruses have known biology, which means that we are already somewhat prepared. While we don't yet have treatments for many of them, the biggest lesson of the COVID-19 pandemic has been that treatment is wonderful, but detection and prevention are the first lines of defense.

Academic and industry scientists should start preparing now for these viruses to spillover. The biotech industry can start preparing affordable, accurate, rapid tests for broad viral families. Pharmaceutical companies can start R&D on small molecule inhibitors like Paxlovid. Academic scientists with the help of federal funding can keep efforts up on teasing out the underlying biology and life cycles of viruses that might spillover. Knowing how these viruses behave — what their vectors are, how they spread from animal-to-human or from human-to-human, what their potential incubation times are — is critical to limit spread at the start of an outbreak.

These aren't new ideas. The Centers for Disease Control already has hundreds of pages worth of planning for potential influenza outbreaks. This is good! We shouldn't have to start from zero every time we are faced with a potential new viral infection.

Not every virus that we know can spillover and cause a pandemic will actually do so. But there's absolutely no reason to sit and wonder what will happen when and if they do. ■

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immunology

Canceling Antibody Reboots

Rather than relying on cell lines and mouse models, researchers at the biopharmaceutical company Atreca study people who have cancer to identify novel immunotherapies.

INTERVIEWED BY NATALYA ORTOLANO, PHD

Researchers at Atreca use B cells from people with cancer to find potential antibody therapeutics in an unbiased manner.

CREDIT: ATRECA

REMAKES, BOOK-TO-MOVIE adaptations, and sequels are tricky. No matter what, someone will always say, "It's just not as good as the original." Atreca, a biopharmaceutical company that develops antibody-based therapeutics, shares that sentiment. Scientists at the company are searching for originality in cancer therapies by going to the source. On the assumption that the body knows best, they study antibodies against foreign invaders and rogue cells produced by an individual's B cells with the hope that they'll be better than their lab-made doppelgangers.

Antibody-based cancer therapies target proteins that are abundant in cancer cells but absent in normal cells, called tumor specific antigens (TSAs). Cancer researchers inject these antigens into animals such as mice and sharks to induce an antibody response. Then they take a blood sample and find antibodies that target the TSA and adapt them for therapeutic use in the human body.

The animal to human discovery platform is effective. The FDA and EMA have approved more than 100 monoclonal antibody therapies to date for treating cancer, autoimmune disorders, and infectious diseases. More than 80 more are in clinical trials (1).

Scientists at Atreca think that researchers are leaving some antibodies on the table since most of the monoclonal antibodies used to treat cancer seek out the same handful of targets. Tito Serafini, cofounder and chief strategy officer of Atreca, finds novel TSAs and effective antibody therapeutics that work for many patients with different types of cancers.

How do researchers at Atreca find new antibodies?

We let the human immune system guide us to new antibody targets in cancer cells. Immune systems are constantly scanning the environment looking for what is different, whether it's from an infectious disease or cancer.

There are lots of tumor antigens generated by cancer cells that the immune system hasn't seen before. Immunotherapies target these antigens to enhance the cancer immune response. To find antibodies relevant to human biology, why not look at the immune response in a patient with a tumor? That's what we do. And we're only interested in an active immune response.

For example, when someone gets an mRNA vaccine, the immune system immediately generates antibodies against the new foreign mRNA. Long lived plasma cells go to the bone marrow and make antibodies over a long period of time and become memory B cells that float around in the blood just waiting for the antigen to show up again. Looking through all the memory B cells floating around would be like looking for a needle in a haystack, so we look at a specific type of B cell that is released after affinity maturation — when antibodies gain increased affinity and anti-pathogen activity — that we can easily find the blood. This way, we don't need a tumor biopsy. We only need a blood sample to find a subset of antibody-producing B cells that are potentially useful to us in building therapeutics to target tumor cells.

We have this huge collection of patient blood samples taken at different times over the course of an individual's cancer progression. We process several of these longitudinal samples for one patient and look for antibodies present throughout the course of disease. Then we test if the antibodies respond to tumors from other patients by checking if they can bind to tissue samples from patient tumors. If they do, then we synthesize the antibody and test it in the wet lab. We determine if the antibody can effectively target cancer cells in a dish and in animals. Then we start doing experiments needed to apply for an investigational new drug application and start clinical trials.

Tito Serafini is the cofounder and chief strategy officer of Atreca.

CREDIT: ATRECA

> "To find antibodies relevant to human biology, why not look at the immune response in a patient with a tumor? That's what we do."
>
> *– Tito Serafini, Atreca*

What is the most exciting project or finding you've worked on since you cofounded Atreca?

Our most advanced therapeutic, ATRC-101, is an antibody identified in a patient with lung adenocarcinoma. It targets a novel ribonucleoprotein (RNP) complex that was never described in the literature before we discovered it. The immune system is often directed to RNP complexes that include RNA viruses. In 2020, we started a phase Ib clinical trial and so far, ATRC-101 is very well tolerated. We see clear correlation between clinical activity and target level in the patients; not all patients have the same amount of novel RNP complex in their tumors. We've had a complete response in combination therapy with the FDA-approved immunotherapeutic pembrolizumab, which is used to treat a variety of cancers such as breast and skin cancer.

A 78-year-old woman with metastatic melanoma was previously treated with pembrolizumab alone and combination therapy with inhibitors against cancer-causing proteins B-raf proto-oncogene serine/threonine kinase (BRAF) and mitogen-activated protein kinase kinase (MEK). Each of the prior treatments stabilized her disease, but she had a complete response to our therapy. It's really exciting that a woman who probably wouldn't have qualified for a lot of clinical trials due to her metastatic melanoma is now cancer free as far as we can tell so far.

What does the antibody target?

The proteins and mRNA that make up the RNP are in every cell, but the target of ATRC-101 is specific to cancer cells. We don't know the exact epitope. It could be a tumor-specific post-translational modification to one of the proteins. The modification could change the shape of the complex or how the proteins interact and reveal the antigen.

The complex is interesting. It has hallmarks of being a biomolecular condensate, which is a membrane-free organelle. If it's a complex that forms in response to a certain type of stress, introducing a stress such as chemotherapeutics could reveal the antigen and make it more effective. It's pretty exciting. ■

This interview has been condensed and edited for clarity.

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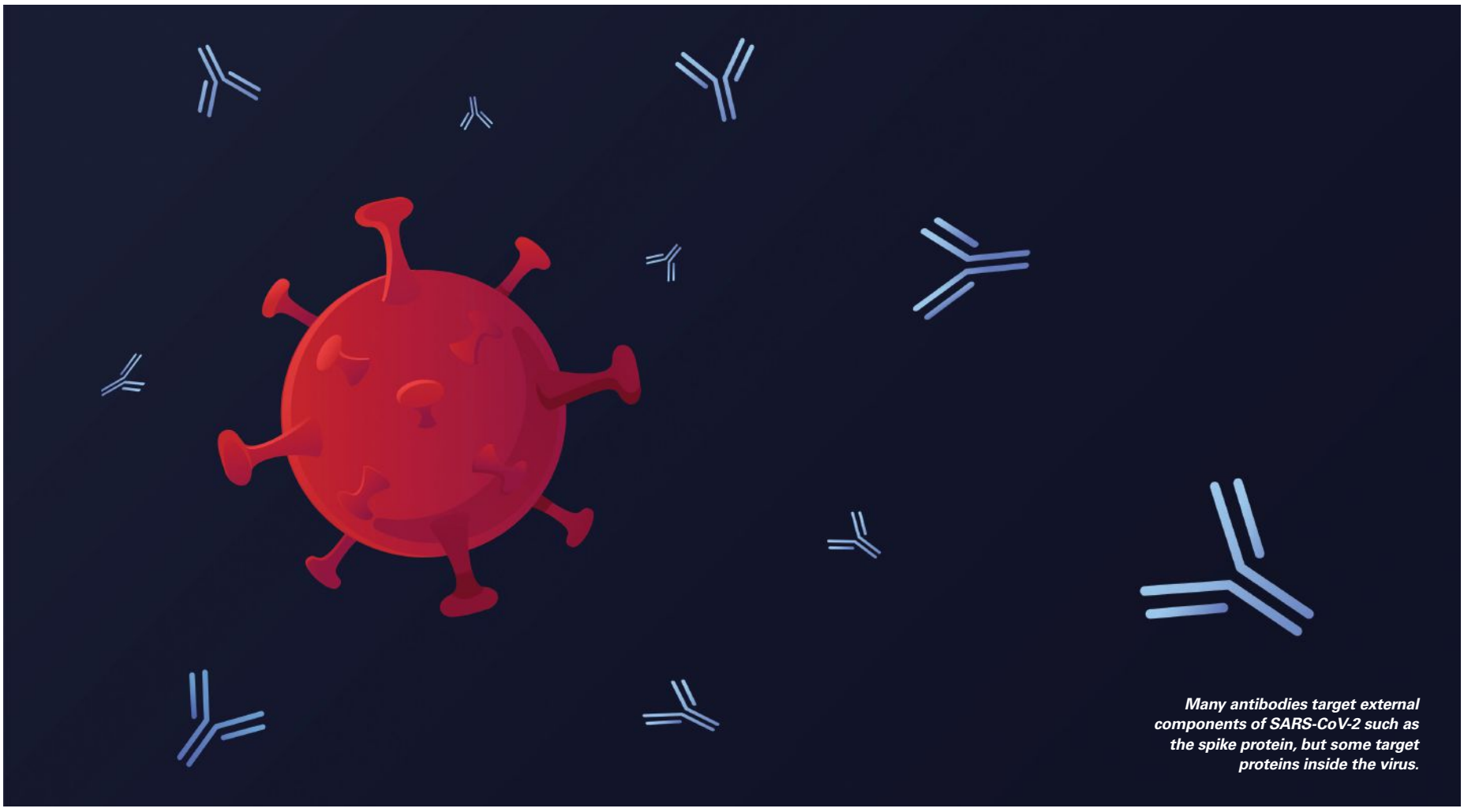
It's What's Inside That Matters

COVID-19 researchers focus their studies on antibodies targeting exposed parts of SARS-CoV-2, but in a new study, a group of researchers showed that antibodies targeting proteins inside the virus are just as important for a robust immune response.



Many antibodies target external components of SARS-CoV-2 such as the spike protein, but some target proteins inside the virus.

BY NATALYA ORTOLANO, PHD

THE IMMUNE SYSTEM readies an arsenal of antibodies the moment it encounters a virus. These antibodies often target the first thing vigilant surveilling immune cells touch. In the case of SARS-CoV-2, that's often proteins that decorate the virus' exterior, such as the spike protein, which allows the virus to enter cells. But the SARS-CoV-2 genome also encodes approximately 25 proteins that are likely found inside the virus (1).

In a study recently published in *Cell Reports*, researchers found antibodies against these viral proteins in blood taken from 21 people with severe COVID-19 (2, 3). They could even predict who lived and died amongst people with severe COVID-19 based on the patient's noncanonical antibody profile.

"Other studies have looked at antibody characteristics such as this, including antibodies to the spike [protein] with respect to COVID outcomes. Where the study goes further is that they also evaluate targets you wouldn't normally think about," said David Martinez, an immunologist from Yale School of Medicine who was not involved in this study. "The author's take a step further and begin to turn over stones that people haven't necessarily turned before."

The researchers searched for antibodies in blood taken 24 hours after a patient was admitted to the intensive care unit with a confirmed case of COVID-19. These patients were part of the first wave of COVID-19 before new variants emerged or vaccines were available to the public. Although all of the patients had the same increase in antibodies against canonical external targets of SARS-CoV-2 compared to healthy patients, seven of the 21 patients died.

Aniruddh Sarkar, a bioengineer from the Georgia Institute of Technology and Emory University and coauthor of the paper, used an antibody profiling platform he developed to do a deep dive into the antibodies floating in the COVID-19 patients' blood.

"Our flavor of engineering is more like micro- or nano-scale devices and assays. That's basically our way of generating large amounts of data from very small amounts of samples. These precious samples that we get from the patients can give us as much data as can be extracted from them," said Sarkar.

The researchers exposed the antibody-rich blood from patients with severe COVID-19 to three canonical antigens, such as the spike protein, and four noncanonical antigens, including nonstructural protein 13 (NSP13), a helicase conserved across coronaviruses that is required for SARS-CoV-2 to replicate. This protein is now a target for COVID-19 therapeutics in development.

With this massive set of data in hand, Sarkar turned to Jishnu Das, an immunologist and computational biologist from the University of Pittsburg Medical Center. "This is where Jishnu and his computational magic of sorts comes in," said Sarkar.

Das expected COVID-19 survivors to have higher levels of canonical antibodies targeting the outside of the virus than the seven patients who died, but he was surprised to find that noncanonical antigens were just as high in survivors.

> "The most logical thing to evaluate moving forward following this study would be to try to incorporate some of these noncanonical sites potentially into next generation vaccines."
>
> *– David Martinez, Yale School of Medicine*

Levels of noncanonical antibodies predicted life or death in patients with severe COVID-19 equally as well as canonical antibody levels did.

Das believes that the key to finding the predictive power of noncanonical antibodies was analyzing blood taken only from individuals with severe cases of COVID-19.

"It is possible that in a mild case, the host immune system has minimal encounters with internal protein antigens. But in severe cases, presumably there are a lot of dead cells. There's a lot of lysis so there are these internal proteins floating around in the blood," said Das.

These noncanonical targets, particularly NSP13, could be important for developing a pancoronavirus therapeutic since Sarkar and Das found the same noncanonical antibody profiles in nine healthy patient samples collected before the pandemic began, likely developed in response to other coronaviruses that cause the common cold.

"The most logical thing to evaluate moving forward following this study would be to try to incorporate some of these noncanonical sites potentially into next generation vaccines. Should we consider including NSP13, a highly conserved protein on these types of coronaviruses, into vaccination strategies that could potentially target parts of the immune system to essentially direct the immune response at these highly conserved epitopes? I think it offers an additional layer of considerations that we might want to think about in the face of what appears to be a bevy of ever evolving variants," said Martinez.

For now, Sarkar and Das are focusing on repeating their findings in other patient cohorts to convince researchers that considering noncanonical targets such as NSP13 for next-generation COVID-19 treatments is the logical thing to do. ■

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A Universal Flu Vaccine Might Not Look Very Universal

New research on vaccines that cover multiple influenza viruses arrives frequently, but biological, evolutionary, and communications challenges remain.

BY DAN SAMORODNITSKY, PHD

BREAKTHROUGHS DON'T happen overnight. Years of research, millions of dollars, and uncountable work hours go into even seemingly overnight successes. For example, the COVID-19 mRNA vaccines went from sequence to injected into arms in a lightning-fast year, but they stood on the shoulders of decades of work. Influenza, on the other hand, has managed to dodge the vaccine strategies that researchers have used to target other viruses, despite having lingered in human lungs for thousands of years.

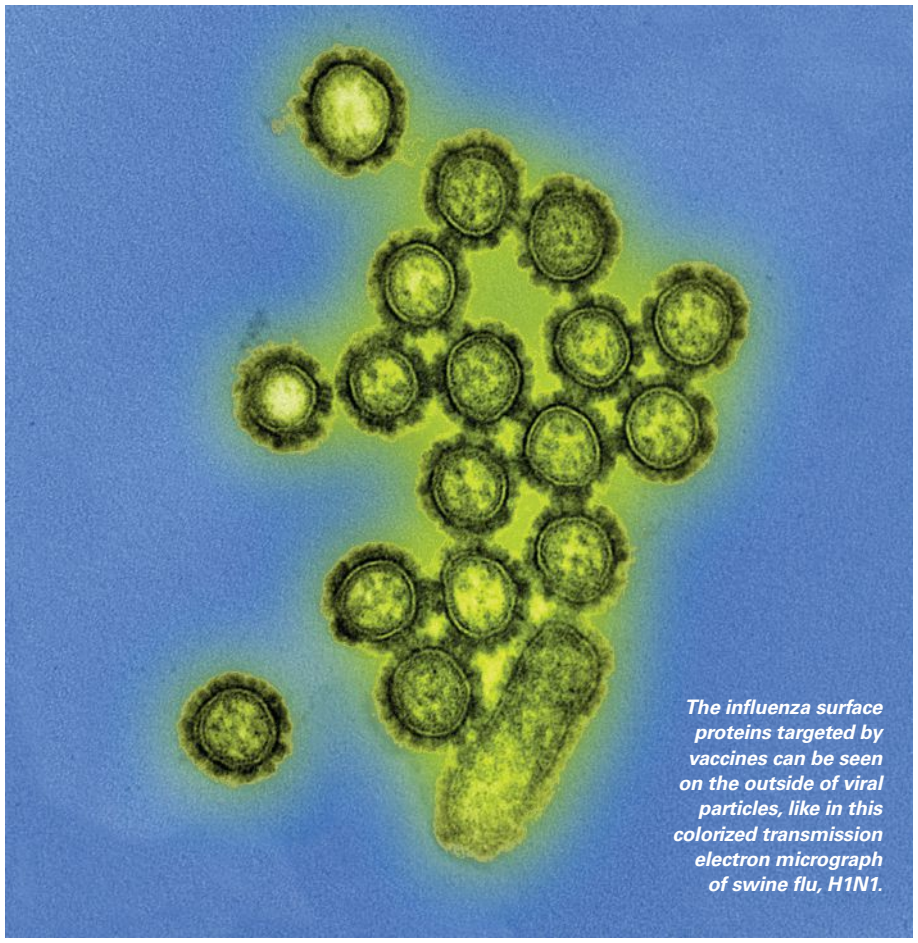
Every year, flu vaccines need to be updated since the virus mutates at such a rapid clip that each successive year's vaccine is rendered mostly ineffective by the following season. Using data from infection patterns in various parts of the world, virologists and epidemiologists make educated estimates of the type of influenza virus to vaccinate against. This is difficult guesswork for the biologists, a logistical challenge for vaccine manufacturers who must produce billions of doses every year, and a game of whack-a-mole for healthcare workers who struggle to get 40% of the US to take the vaccine, blunting its effectiveness even further.

A universal flu vaccine that could be used for years without requiring updates would solve many of these problems. In June, a group of researchers led by Jeffrey Taubenberger of the National Institute of Allergy and Infectious Diseases (NIAID) announced in *Science Translational Medicine* a successful test of a vaccine in mice and ferrets that protected against a wide variety of influenza subtypes (1).

Flu viruses are broadly sorted into two categories based on the type of hemagglutinin protein (HA or H) and the type of neuraminidase protein (NA or N) they carry. HA proteins dot the outside of the virus on the head of a stalk, like a sunflower, and help the virus attach to target cells. NA destroys the receptor that the virus uses on the way into a cell, which helps newly created virus particles burst out to infect new cells (2).

Since these proteins are the most visible to the immune system, they are under the most evolutionary pressure to mutate and evade detection. HA and NA are also encoded on different parts of the virus's segmented genome, allowing each of the 18 different HAs and 11 different NAs to mix and match. On top of that, influenza's RNA polymerase is famously inaccurate and constantly introduces mutations into new viruses. These factors all contribute to the flu's constantly shifting identity: H1N1, the 1918 pandemic flu, H5N8, an avian flu that rarely infects humans, and H5N1, another avian flu that frequently infects humans, are a few of the variants commonly in the news.

For the NIAID vaccine, Taubenberger's team used specifically chosen subtypes with the hope of generating immune responses against viruses with different H- and N-types. The team created a cocktail of inactivated viruses containing the viral subtypes H1N9, H3N8, H5N1, and H7N3, all inactivated using beta-propiolactone, a common preparation for creating inactivated viral vaccines. They then tested the ability of this cocktail to protect mice and ferrets against a variety of genetically dissimilar viral subtypes and monitored other markers of health such as weight loss, lung damage, and detectable viral RNA.



The influenza surface proteins targeted by vaccines can be seen on the outside of viral particles, like in this colorized transmission electron micrograph of swine flu, H1N1.

CREDIT: NIAID

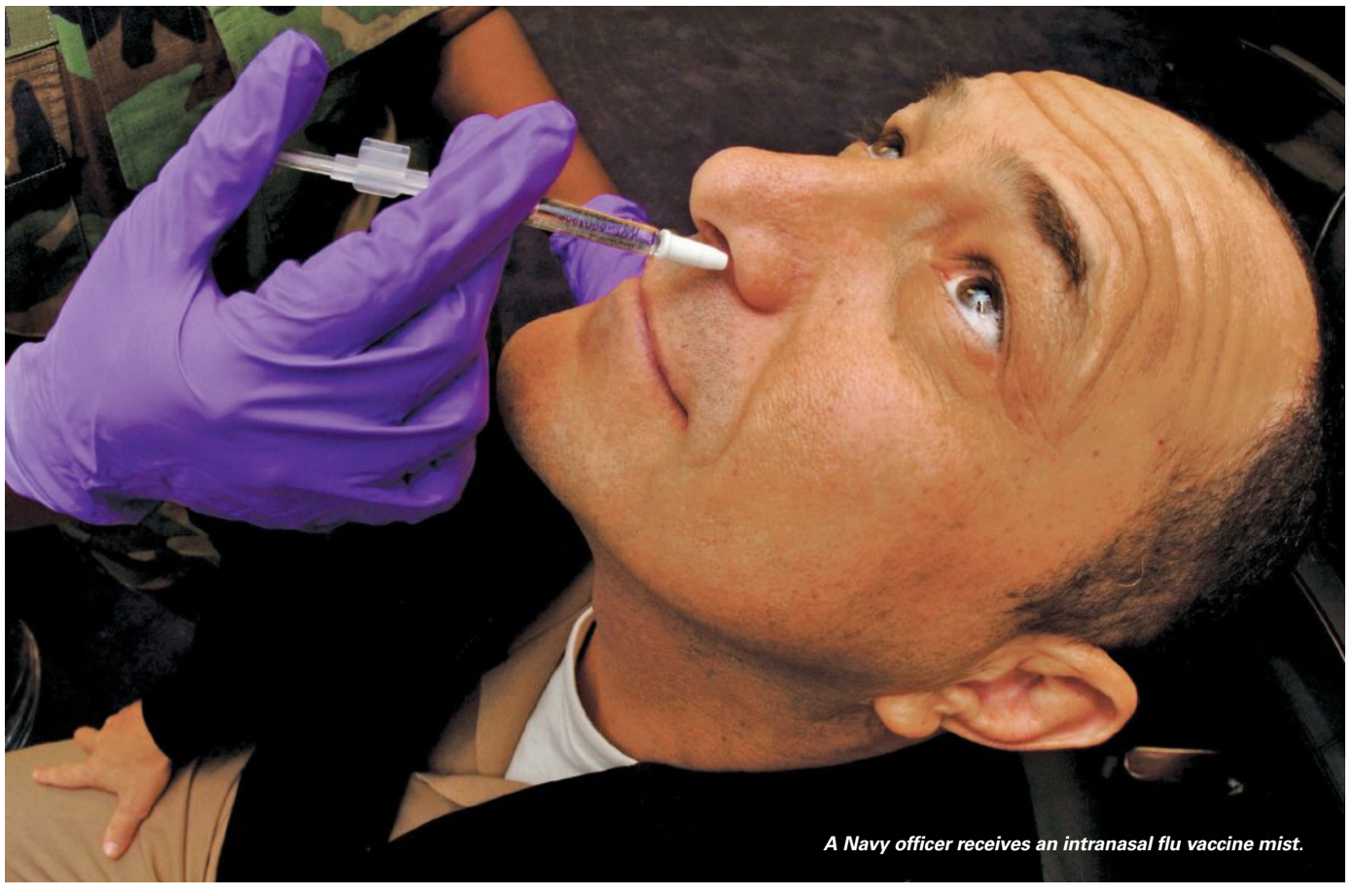
H5N8, an avian influenza variant, killed 10% of the test animals that received the vaccine intramuscularly and 30% that received it intranasally. Other than that, all animals survived all other viral challenges for the 14-day duration of the trial, including an H2N7 strain that shared only about 45% of the HA sequence with any strains in the vaccine, and an H3N2 strain that only shared 43% of the vaccine's NA sequences.

Vaccinated test subjects were also far more likely to maintain weight after being infected. During the course of their illness, most animals dropped at most 10% of their initial weight, and most returned to a healthy weight by the end of the test round, although animals infected with an H10N7 strain or an H6N1 strain lost between 15-20% of their weight. Even those animals had a 100% survival rate.

Vaccinated animals had 90-100% less detectable viral RNA in their blood than animals that were inoculated with buffer without a vaccine. Unvaccinated animals sustained damage to their lungs when infected. For example, H1N1, the 1918 pandemic flu strain, produced visible swaths of necrotized bronchioles and pulmonary edema. In comparison, immunized animals showed "minimal histopathological changes, including mild, focal bronchiolitis, an absence of alveolitis, and no viral antigen in alveolar epithelial cells," according to the authors.

Buoyed by these results, researchers conducting a Phase I clinical trial have begun using a vaccine similar to the one tested by Taubenberger's group in mice and ferrets. The trial is expected to be complete in March 2023. But what happens if the results are positive? Will a universal human flu vaccine become available?

"A universal flu vaccine could be anything, right?" said Richard Webby, a virologist at St. Jude's Research Hospital in Tennessee and the director of the World Health Organization Collaborating Centre for Studies on the Ecology of Influenza who wasn't involved with the study. "When the term was first floated, it was really meaning something you got once and it protected you from everything. That was always sort of the Holy Grail.



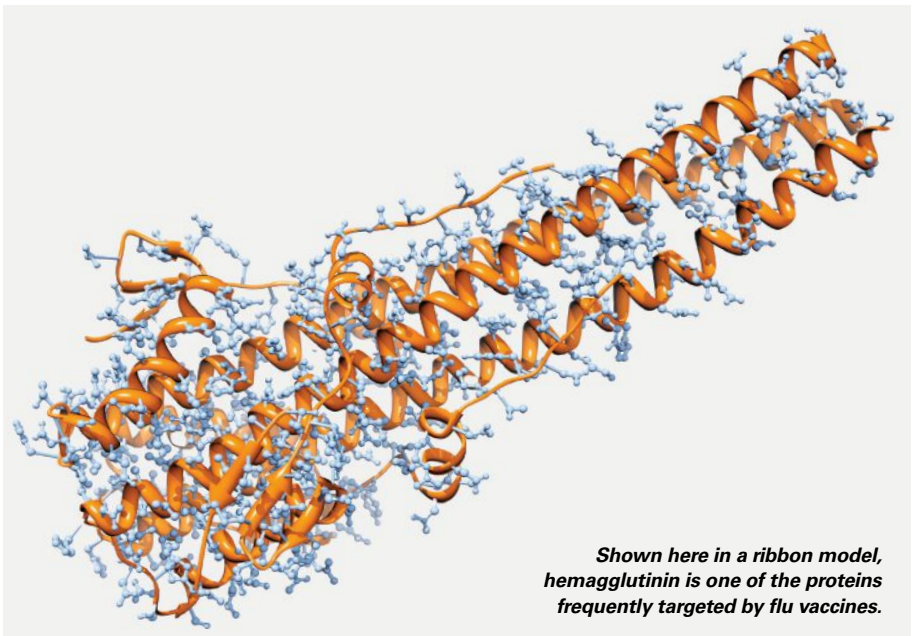
A Navy officer receives an intranasal flu vaccine mist.

"But flu is a very, very different virus than those where vaccines are one and done in terms of how it changes, in terms of the protective efficacy, in terms of how fast it replicates. So, I think that's still a very, very tall ask. And I haven't seen anything that would suggest we're close to having a vaccine like that."

Current vaccines are potent, offering strong antibody-based protection when they match the correct H- and N-subtypes of that season's viruses. Since those targets frequently change, other universal vaccine approaches have tried targeting more stable regions of the virus, a common one being the stalk region that holds the HA protein. Unfortunately, the stalk is simply harder for the immune system to spot so the vaccines just don't work as well. "Most of the universal approaches that target something else in the virus come with a little bit of drop of potency," said Webby.

With these challenges, some researchers are reconsidering what it means for a vaccine to be universal. Given the frequently shifting nature of influenza viruses, asking just one vaccine, even one that can protect against multiple strains, to cover every possible patient at every point during life might be asking too much.

"There're lots of different strains of influenza," said Nicholas Heaton, a virologist at the Duke University School of Medicine who was not involved with the study. "There're people who have different exposure histories. There are types of vaccines that brand new babies need, versus young adults, versus the elderly. Those are probably all different populations that all need different vaccines based on their exposure histories, based on the status of their immune responses, based on the strains that are circulating at that time."



Shown here in a ribbon model, hemagglutinin is one of the proteins frequently targeted by flu vaccines.

Since influenza is so variable and many different viruses cause flu-like diseases, another illness could make an apt comparison. "Maybe the best analogy I can give you is the 'cure for cancer,'" said Heaton. The phrase "cure for cancer" has been thrown about since Richard Nixon was president, with seemingly each successive administration promising a "cure" or a "moonshot."

"The idea was we could cure cancer. And the reality is that there're different types of cancers with different types of mutations and different people. And it's probably not a one-size-fits-all-solution. And I think those types of ideas are going to apply to influenza vaccines."

Calling a vaccine universal or simply better or stronger matters. Vaccine uptake hovers around 40% of the population in the US, when 80% should be getting it (3). So even creating a 100% protective influenza vaccine would still not really achieve universality. A better goal, according to Heaton, may be to stop aiming for moonshots and simply aim for better.

> "We would hate to set ourselves up for a situation where we make a flu vaccine that's 10 times better than what we have now. It's still not universal, and then that's seen as a failure."
>
> *– Nicholas Heaton, Duke University School of Medicine*

"We would hate to set ourselves up for a situation where we make a flu vaccine that's 10 times better than what we have now. It's still not universal, and then that's seen as a failure," he said. ■

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dermatology

Wound Healing with a Bivalve's Insight

To avoid the pain, scars, and complications of traditional skin grafts and sutures, scientists take inspiration from mussels, some of the stickiest, most resilient animals on Earth.

BY DAN SAMORODNITSKY, PHD

A WOUND IS A WET AND messy place. The healing process can be long and painful, and while skin grafting has developed into a common procedure, it can leave scarring and stenosis behind. An alternative to staples, glue, or sutures to hold skin grafts in place that could promote healing and even potentially deliver medicine in one fell swoop would be an appealing development.

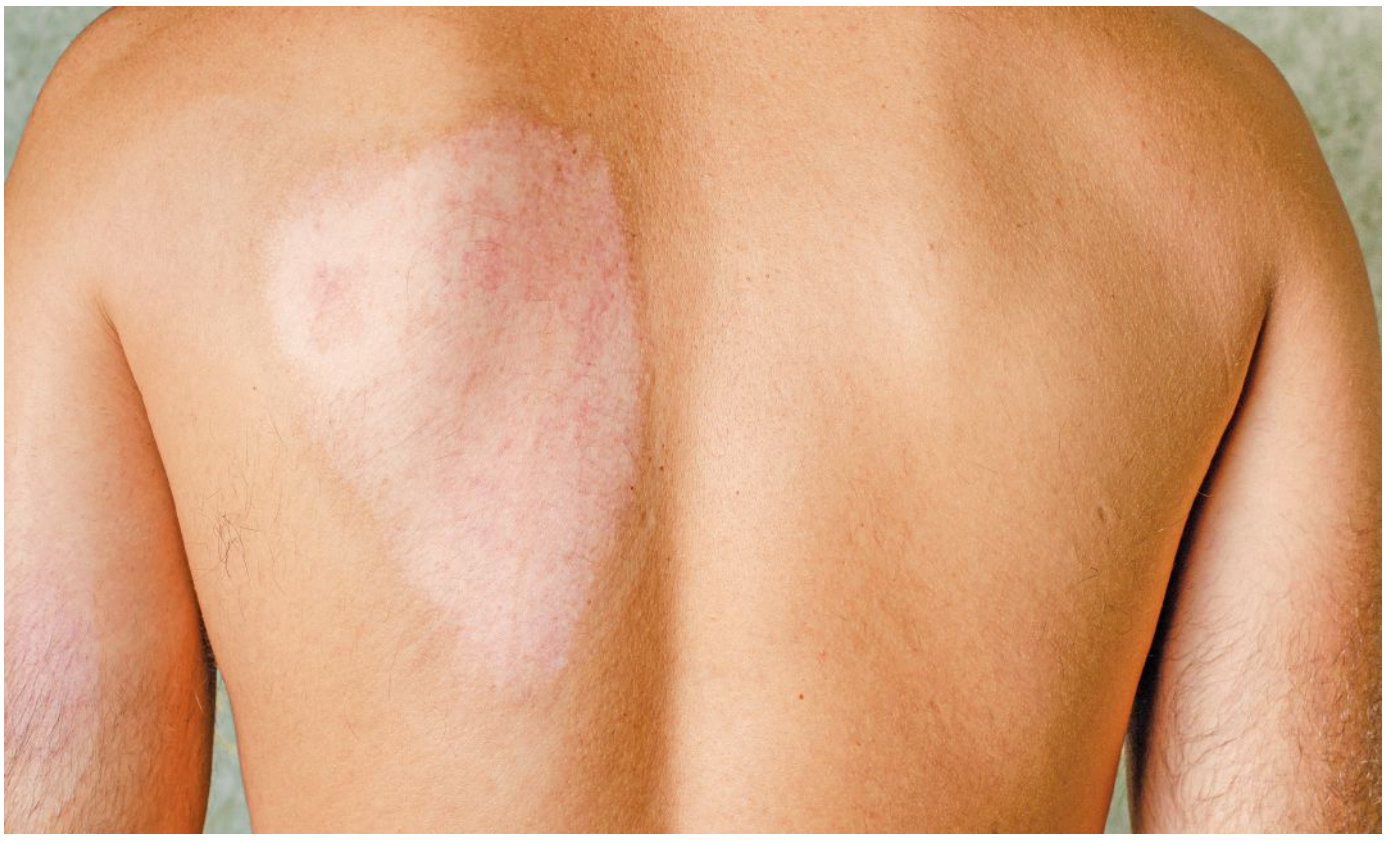
Mussels, the bivalves that cling heroically to seaside rocks withstanding wave after punishing wave, have something sticky figured out despite all the moisture they put up with. They stick to rocks by secreting mussel adhesive proteins (MAP) from their "feet." Because of their incredible stickiness under the wettest of conditions, their biocompatibility, and their easy biodegradation, MAPs have become hot targets for medical research, appearing in applications as diverse as hydrogels, nanofibers, and drug delivery systems.

To improve drug delivery in the complex space of a healing wound, researchers based at the Pohang University of Science and Technology reported in the Chemical Engineering Journal that they had created a new kind of skin graft made from a complex mixture of MAPs and other molecules (1).

"This is an interesting use of the technology," said Bruce P. Lee, a biomedical engineer at Michigan Technological University who was not involved with the study. "This is research that's highly sought after. There're a lot of people doing this."

MAPs contain L-DOPA, a noncoded amino acid derived from tyrosine that often replaces tyrosine in proteins as a stabilizer in cells. MAPs' inherent stickiness is proportional to the number of L-DOPA residues they contain. The study authors converted every occurrence of tyrosine in the coded sequence of MAPs into L-DOPA to increase their grafts' stickiness even further. They expressed the resulting MAP sequence in *E. coli*, purified the protein, and dried it into a powder instead of harvesting minute amounts of protein directly from mussels, which is a laborious and difficult technique tried in the past.

The researchers next mixed the MAP proteins with hyaluronic acid (HA), a polysaccharide used by a wide cross section of all life as a lubricant and shock-absorber. HA is also ubiquitous in cosmetics and medicines since it stabilizes broken skin structures and promotes rebuilding the extracellular matrix after a wound. Lee pointed out that HA is a good choice for a skin graft application with some pricing drawbacks. "It's a naturally available polysaccharide from your body, but then it's relatively expensive compared to synthetic polymers," like heparosan, a synthetic peptide often used to replace HA in drug delivery applications.



Wounds and grafts can leave scars and other long-term challenges, but new concepts in skin grafts may overcome these problems.

To test the MAPs' ability to promote a scarless, quick-healing skin graft, the researchers created a coacervate, a mixture of droplets composed of proteins and other biomolecules in a liquid-liquid suspension. These suspensions have been used as drug delivery systems in the past because of their ability to stably encapsulate and deliver drugs; one notable example is the localized delivery of antitumor radionuclides, where the heat of the body melts the suspensions and releases the therapeutics (2).

For this study, the researchers made coacervates with MAPs, differing amounts of HA, and two medicines: allantoin and epidermal growth factor (EGF). Allantoin is a topical moisturizer common in skin creams that assists with wound healing, and EGF is a naturally occurring mammalian protein that also helps in wound healing by increasing cell proliferation (2).

The scientists measured the rate at which both drugs diffused into surrounding areas using an in vitro model. The group immersed the coacervate in a buffer and allowed the drugs to diffuse out of suspension. Afterward, they separated the drugs released from the coacervate and quantified them using liquid chromatography.

Two interesting things happened. First, the two drugs diffused at different rates. 100% of the allantoin was released within four days, while EGF diffused slowly, with only 80% of the total load diffusing in 14 days. This suggests that these grafts can deliver both quick- and slow-acting drugs.

> "Plastic surgeons will use skin grafts because they know they're going to get 100% closure. And usually, they work really, really well."
>
> *– Robin Martin, freelance reconstructive surgery consultant*

Next, the team cultured fibroblasts with coacervates and measured their effects on cell growth. The dual delivery of drugs rapidly increased the proliferation of cells over those cultured with coacervates that didn't contain drugs or coacervates holding either of the drugs individually. Cells cultured with the dual drug coacervates proliferated at almost 160% of the rate of cells grown in the presence of empty coacervates.

They then built an in vitro model of wound healing by placing two populations of keratinocytes side by side in a dish and measuring the rate at which they closed the gap. The dual drug coacervate closed 98% of the gap in 24 hours compared to allantoin alone (83% closed) or EGF alone (35% closed, which was indistinguishable from cells cultured with coacervates that didn't contain drugs).

There are multiple goals involved in creating new kinds of skin grafts that stick in different ways and can be outfitted to deliver medications. A skin graft that sticks well without a suture can heal a wound or surgical incision without producing a scar.

"Plastic surgeons will use skin grafts because they know they're going to get 100% closure. And usually, they work really, really well," said Robin Martin, a freelance scientific consultant in wound healing and reconstructive surgery who was not involved in the study. Surgeons use a variety of approaches to get grafts to stay in place. In an area that

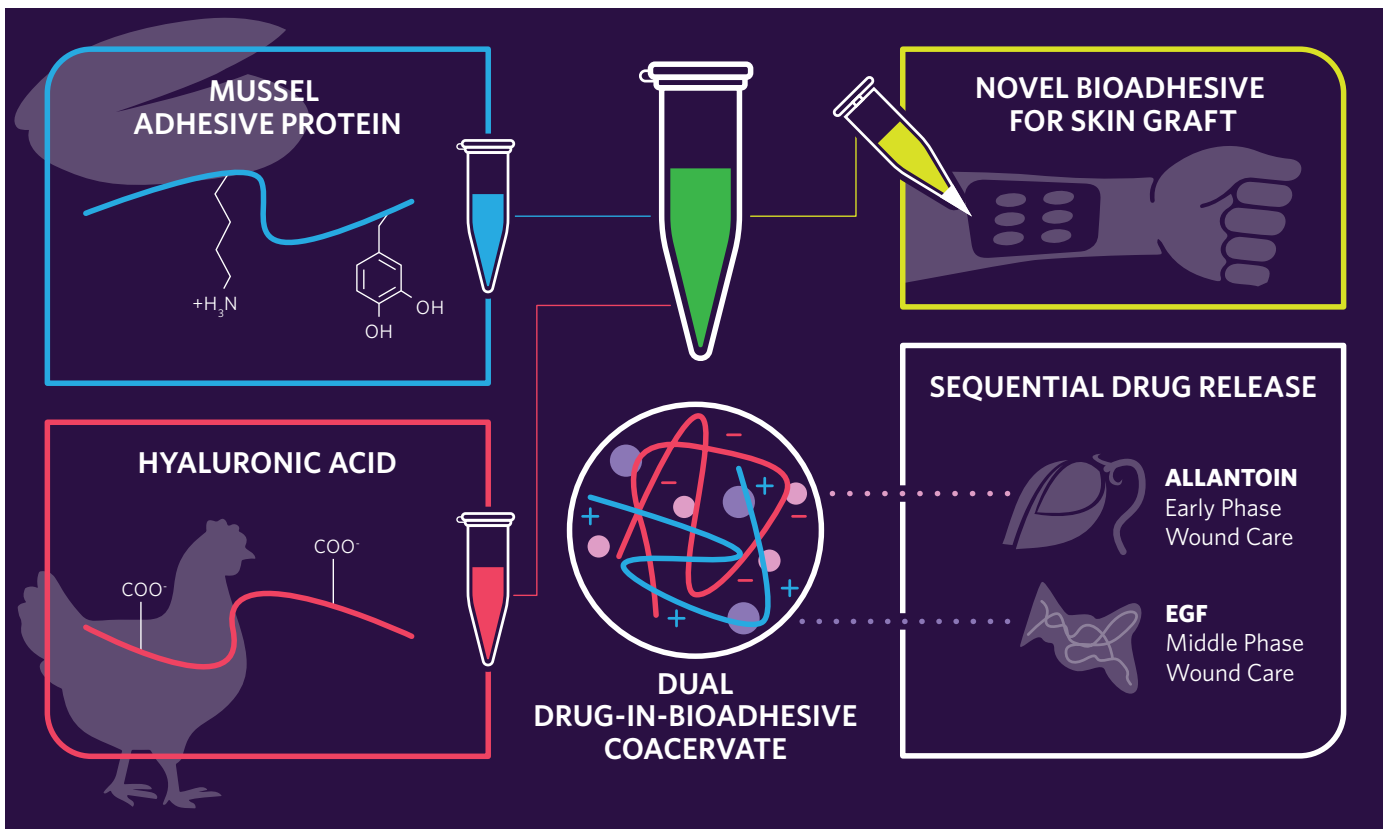
doesn't see much motion, a suture will do just fine. In high motion areas, they often use fibrin glue, a surgical formulation that creates fibrin clots. This is useful but has drawbacks. Most importantly, it must be prevented from seeping into the bloodstream where it can cause off-target clotting. Since MAP does not promote clotting on its own, it can simply biodegrade after grafting.

The researchers next compared the ability of a MAP skin graft to heal and then biodegrade in a mouse model. To test this, they cut a circular piece of skin and then reattached it in the same place using either a suture, fibrin glue, an empty MAP coacervate, or the full coacervate with both drugs included. There was significant scarring when sutures were used, although less occurred with fibrin glue and the empty coacervate. The dual drug MAP coacervate, however, reduced scarring significantly, and unlike the fibrin glue, fully degraded after 10 days of healing.

In addition to aesthetic concerns, scars can be stiff, painful, and can reduce a patient's range of motion, particularly if they develop on an area like the neck, knee, or elbow. While this can be problematic for adults, these drawbacks are especially worrisome in children where scar tissue does not grow and stretch like undamaged skin. This can cause lifelong difficulties after a wound heals.

Mussels have been a source of inspiration for biomedical engineers for some time, especially those working on wound healing in wet places. For instance, in 2021 a research group based in Hong Kong used another mussel-derived molecule called N-(3,4-dihydroxyphenethyl) methacrylamide to improve the stability and coloration of tooth restorations used by dentists, which often stick well at first but degrade over time in the moist environment of the mouth.

But a laboratory incision in one animal doesn't translate directly to another. "The mouse model, the dermal model, is anatomically very different from the human. So, I think there's still a way to go to have demonstrated that it's suitable even for a larger animal like a pig," said Lee. ■



CREDIT: IMRAN CHOWDHURY

Researchers developed a new coacervate including mussel adhesive proteins that shows promise for improving skin grafts.

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Tattooing Toward Human Health

From ancient medicines to equipping humans with new senses, tattoos are more than just permanent marks on the skin. They may boost immune function, and they have the potential to effectively deliver therapeutics through the skin.

BY STEPHANIE DEMARCO, PHD

In every corner of the world for thousands of years, people have decorated their skin with colorful, permanent pigments. Despite how common tattoos are, scientists still know very little about how they interact with the body.

"Most of the research on the biology of tattooing is still sort of the old fashioned, 'Oh my God, is it cancerous? Oh my God, are you gonna get an infection?'" said Christopher Lynn, a medical anthropologist at the University of Alabama. "The idea that tattooing is this dangerous thing is not really attested to by modern hygiene and sanitation."

Tattoos are already used in the clinic to mark where doctors will apply radiation treatments, to correct skin discoloration, to mark potential tumors or other pathologies in endoscopic surgery, and for applying permanent makeup (1).

Now, researchers in fields spanning from chemical engineering and dermatology to medical anthropology are investigating tattoos not only as therapeutic in nature, but also as a means for better drug delivery and as "smart tattoos" to monitor and diagnose diseases. Tattoos are more than skin deep.

FROM THE ICEMAN TO TODAY

When two hikers stumbled upon a body frozen in the Italian Alps in 1991 in a rocky gully about 3,200 meters above sea level, they had no idea that they had just discovered the oldest example of a tattooed human to date (2). Affectionately known as Ötzi, or the Iceman, the 5,300-year-old mummy has several tattoos decorating his body. Unlike the pictorial tattoos popular today, Ötzi's tattoos consist of lines and crosses, and most are in locations that would have been covered by his clothes such as at the base of his spine, ankles, and around his knees. Because of this, researchers hypothesize that his tattoos were likely therapeutic in nature, and the fact that almost all of his tattoos line up with traditional acupuncture points to relieve pain supports this theory (3). Two other prehistoric mummies from Siberia and Peru also show evidence of tattoos on common acupuncture points.

Acupuncture is thought to have originated in China in 100 BCE (4). Through the process of inserting thin needles into the skin, an acupuncturist induces a microtrauma, which elicits a localized inflammatory response to promote healing.

Multiple indigenous groups around the world have traditions of tattoos on acupuncture points. Lars Krutak, a tattoo anthropologist at the Museum of International Folk Art in New Mexico, has documented medicinal tattoo practices in more than 30 cultures, from the Ainu in Japan, the Berber in Morocco, to the Chippewa in the United States and Canada (2). When he began working with the St. Lawrence Island Yupiget people in Alaska, he noticed that after people made significant hunting kills or when serving as pallbearers, they would get tattoos on their joints to prevent the spirits of the recently deceased from entering their bodies.

"If you were to be possessed by one of these powerful spirits, I was told that you would suffer extreme arthritis and severe pain in those joints," said Krutak. "When I started lining up these primary tattoo locations, it was surprising to me that they lined up with classical acupuncture points to relieve rheumatism and arthritis in the primary joints."

The practice of tattooing at acupuncture points continues around the world today, and it has ventured into the professional acupuncture space. Douglas Wingate, an acupuncturist at Oregon Health and Science University and a licensed tattoo artist, found that "tattooing essentially stimulates the acupuncture point to a greater degree than standard acupuncture does." He has found that placing a tattoo at an acupuncture point is about equivalent to ten acupuncture treatments at that site, which is often enough to resolve someone's pain.

Wingate has found that acupuncture with tattooing seems to work best in people with chronic pain conditions such as tattooing the ear to treat people with decades-long shoulder or back pain. Patients can pick whatever kind of tattoo they would like as long as it fits over the specific acupuncture point to be treated. In one memorable instance, a patient came to Wingate looking for relief from chronic headaches. As was his usual process, Wingate located the appropriate acupuncture points and administered the tattoo.

"[She] had the first period of time where she didn't have headaches at all in years," said Wingate. But what the patient didn't tell Wingate was that her headaches were due to a Chiari Malformation, which is a painful, congenital condition that causes part of the brain to poke out of the bottom of the skull. It can be treated with surgery in severe cases.

"If she would have come to me and told me that, usually my talk that I have to have with somebody is… acupuncture and tattooing is not going to change that," said Wingate. "I was very surprised when she did have such a positive response and was able to get through without pain until she was able to get in for that surgery."

Wingate has seen much success with his tattoo and acupuncture treatment combinations, but he is also interested in studying this phenomenon more formally. For now, he's excited to provide people with a beautiful tattoo and some relief.

While many traditionally medicinal tattoos line up with acupuncture points, not all of them do. Researchers have seen that tattoos can have medical benefits that have nothing to do with pain.

CREDIT: DOUGLAS WINGATE

Douglas Wingate places tattoos over specific acupuncture points to help relive patients' pain.

CREDIT: DOUGLAS WINGATE

Small tattoos placed on an acupuncture point on the thumb may help relieve uterine pain.

CREDIT: DOUGLAS WINGATE

Based on indigenous traditions of tattooing over acupuncture points, Douglas Wingate finds that many patients suffering from chronic pain conditions find relief from tattoos on acupuncture points.

EXERCISE FOR THE IMMUNE SYSTEM

Before the beautiful final result, people must go through the undeniably painful process of getting a tattoo. In the face of a stressful situation like this, the body ramps up production of stress hormones and dampens the immune system, readying the fight or flight response. Despite the pain and the stress, once people get their first tattoo, many go back for more. How multiple tattoos influence the body's stress and immune responses, however, remains an open question.

"Cultures around the world see tattooing as a way to toughen up the body or make it stronger, and I think of that very biologically," said Lynn. "I want to understand how that health happens… How can your cultural practice and, ironically, this injury to your body make you healthier?"

To investigate the connection between tattooing and the immune system, Lynn and his team sampled saliva from people with different levels of tattoo experience (5). They measured cortisol, an immunosuppressant, and immunoglobulin A (IgA) antibody levels just before and immediately after people got a tattoo. Cortisol levels in the blood peak in response to stress, which triggers the dampening of the immune system, indicated by a drop in IgA levels.

Lynn and his team reported that people with little tattoo experience had decreased IgA levels after their tattoos, indicating a stress-induced suppression of the immune system. For people with lots of tattoo experience, however, the researchers saw an increase in IgA levels immediately after the tattoo, indicating that there was no immune suppression in frequent tattoo-getters.

"Think about it in terms of exercise," said Lynn. "We oftentimes will push ourselves and expect to feel pain or feel sore, and the idea is to push our bodies so that ultimately, we're not continually reinjuring it, but we're building up our muscles…and in the process, we're building up our immune systems."

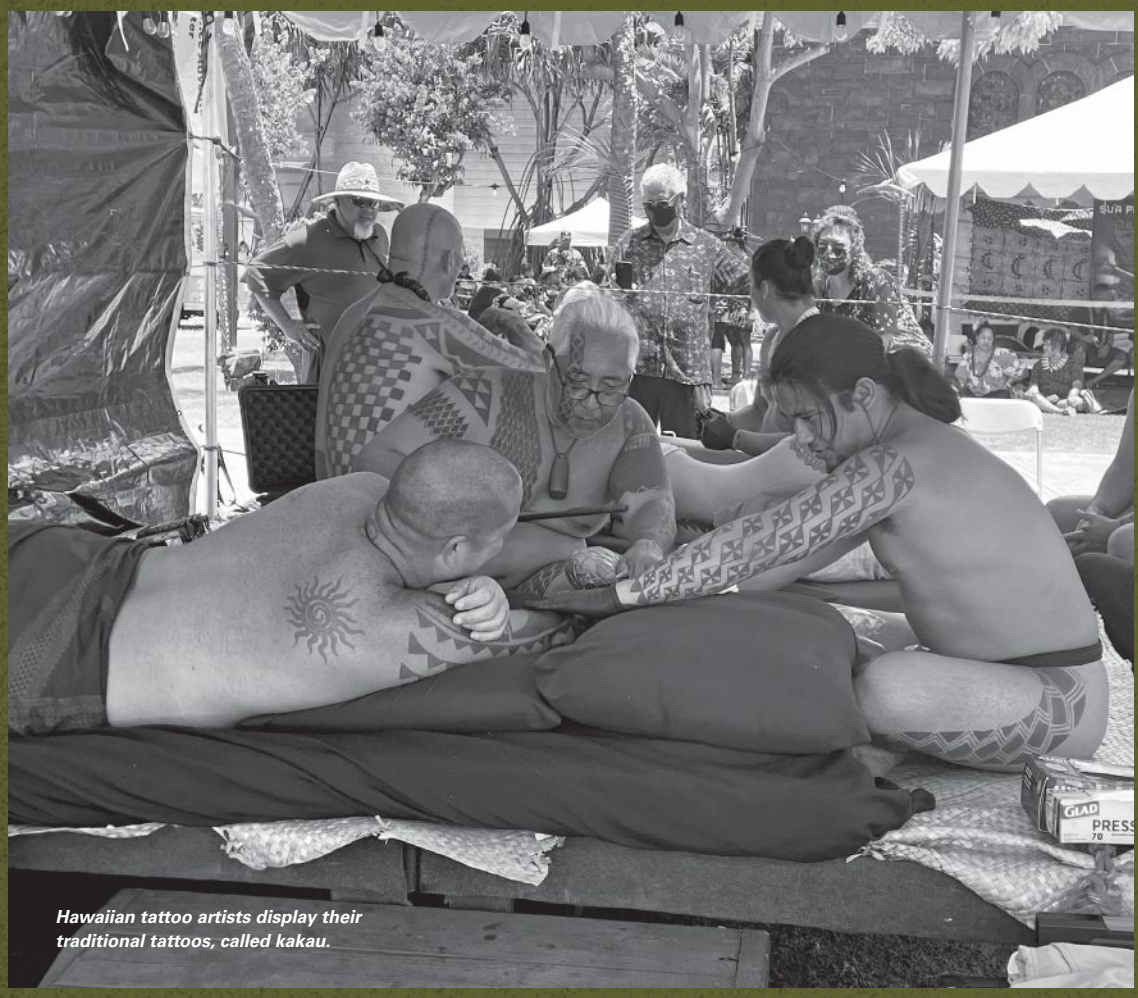
Lynn and his team have since replicated these results in a Samoan population (6), and they have additional data from an even larger population that await analysis. Lynn plans to assess additional immune biomarkers and common health biomarkers like cholesterol levels in the context of tattooing.

In particular, he and his team are studying how the levels of these biomarkers change in a person undergoing a very painful and long tattoo experience such as the traditional Samoan pe'a. This tattoo uses the hand tapping tattooing technique, which relies on handheld tools to pierce and transfer the ink into the skin. Hand tapping is more painful than a tattoo applied with a typical electric tattoo machine and can take as many as 20 to 30 hours to complete. The final tattoo covers the entire lower torso and thighs.

"We're trying to understand not just the on-off switch, but the interaction of mechanisms within physiology," said Lynn. These large, traditional tattoos also hold important cultural significance for the wearer. Lynn is interested in uncovering how the cultural meaning of a tattoo influences an individual's immune response.

While it may be tempting, Lynn does not recommend that people go out and get a tattoo for the sole purpose of boosting their immune systems. Exercise and eating well accomplish the same thing just fine.

"It's one thing to go and get a tattoo because it's cool and you'll have an immune response. It's another thing altogether to get a tattoo that says, 'I am of service to my community, and I am wearing something that my ancestors have worn for at least 1000 years. And it's extraordinarily visible and very painful,'" said Lynn. "There's an added psychological benefit to that, and that's what I'm trying to piece together now."



Hawaiian tattoo artists display their traditional tattoos, called kakau.

CREDIT: CHRISTOPHER LYNN

"If you were to be possessed by one of these powerful spirits, I was told that you would suffer extreme arthritis and severe pain in those joints. When I started lining up these primary tattoo locations, it was surprising to me that they lined up with classical acupuncture points to relieve rheumatism and arthritis in the primary joints."

– Lars Krutak,
Museum of International Folk Art

The traditional tattoo for Samoan women is called the malu. It is administered over the course of hours using the hand tapping tattoo technique.

CREDIT: CHRISTOPHER LYNN

"If you have a nice blue whale on your shoulder or on your arm, it's very likely that the lymph nodes in your armpit are also blue."

– Ines Schreiver,
German Federal Institute for Risk Assessment

BLUE LYMPH NODES

The dermal layer of the skin, the dermis, resides just underneath the outer epidermal layer and contains blood and lymph vessels along with hair follicles and sweat glands. When a tattoo artist pokes into the skin with ink-laden needles, the cells in the dermis spring into action.

Once the tattoo ink enters the skin, macrophages gobble up the insoluble pigment particles as if they were invading microbes (7). When the pigment-containing macrophages eventually die, they release the pigment particles back into the dermis, and other macrophages engulf them. Scientists think that this process of "capture-release-recapture" is what leads to the permanence of tattoos. But sometimes pigment particles can evade capture and trickle into the lymphatic system (8).

"If you have a nice blue whale on your shoulder or on your arm, it's very likely that the lymph nodes in your armpit are also blue," said Ines Schreiver, a tattoo toxicologist at the German Federal Institute for Risk Assessment (BfR).

But macrophages are not the only immune cells in the skin (9). Keratinocytes, often called skin sentinels, induce an inflammatory response when skin is cut or injured. Specialized macrophages called Langerhans cells interface with the lymphatic system and promote an immune response in the skin. The dermal layer also contains many antigen-presenting cells like dendritic cells and T cells that are ready to respond to an infectious threat.

The interaction of tattoo pigments and these immune cells is normally benign, but sometimes the immune cells in the skin overreact and produce an allergic reaction. Schreiver and her team investigate how different pigment particles in tattoo inks interact with the immune system to determine what it is about certain pigments that causes these reactions.

Using skin biopsies of people who had allergic reactions to tattoo ink, Schreiver and her colleagues noticed that bright red and pink pigments made of organic azo compounds, which are pigments often used in the textile and printing industry, seem to cause the most allergic reactions (10).

Prior research using patch tests noted that the red pigments might not cause these allergic reactions themselves, but in the skin, they may breakdown into smaller metabolites or degradation products that could trigger an immune response. UV light, for example, can easily cleave azo pigments into smaller molecules.

"There are by now a few hints, but not really proof yet, that maybe tattoos that are more exposed to sun are the ones that are reacting when they're red," said Schreiver. To better understand the interaction between immune cells and tattoo pigments, Schreiver and her team are developing a 3D-skin model for tattooing (11).

Schreiver and her team recently used their model to understand how tattoos interact with UV light. A prior study reported that mice tattooed with black pigment and then exposed to UV light developed skin cancer more slowly than mice that were exposed to UV light but not tattooed (12).

To determine the mechanism underlying this UV-protective effect, Schreiver and her colleagues tested how UV light affected human skin that had been tattooed with black, white, or orange pigments (13). To their surprise, the white and black pigments protected the dermal cells in the model from UV-irradiation.

"If you think about it, it actually sounds logical afterwards because everything that's beneath this black layer and also with other pigments that are very good in absorbing or scattering light, they protected the underlying cells," said Schreiver.

She and her team are now adding additional components to their 3D skin models such as macrophages to get as close to human skin as possible and better understand how tattoo pigments interact with human skin.

TATTOOING VACCINES

While some tattoo pigments elicit an allergic reaction, researchers wondered whether they could tip the immune scales in the opposite direction and use tattooing as a mode of vaccination. With the skin's unique composition of immune cells, it is an excellent place to mount a multi-faceted immune response of antibodies and immune cells (14). Capitalizing on this, researchers have developed multiple methods to target immune cells in the skin, including a gene gun (which shoots DNA-coated gold particles and was originally developed to genetically modify plants), microneedles, a jet injector (a high-pressure stream of liquid containing vaccine components), and a tattoo machine (15).

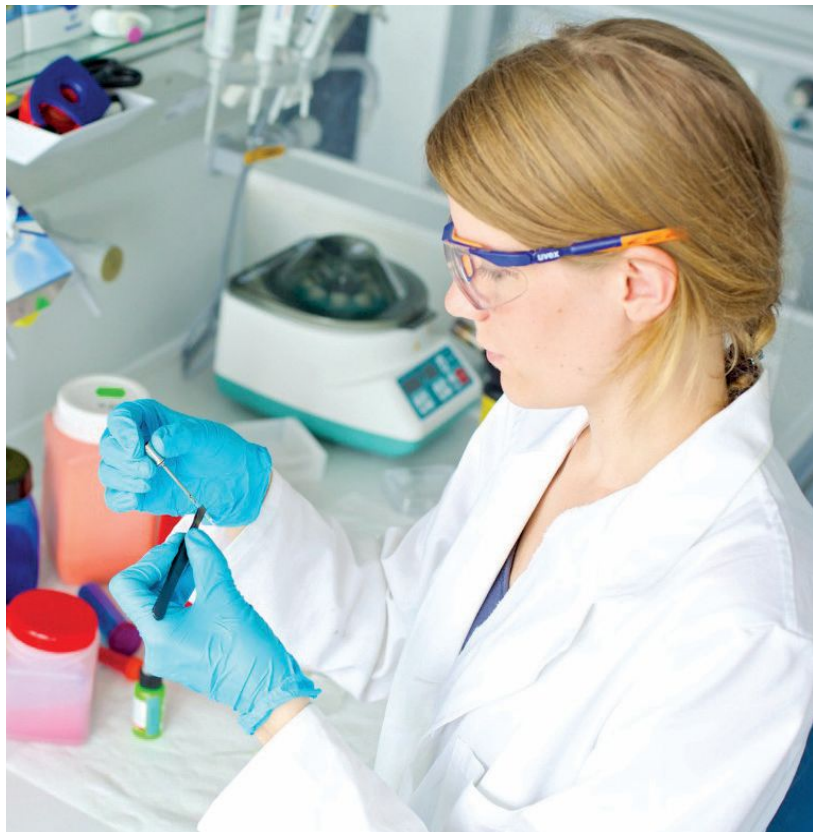
Researchers have mostly investigated the potential of tattoo-based vaccination in the context of DNA vaccines because DNA vaccines delivered via the skin generally induce a stronger immune response than vaccines injected into the muscle. While there are multiple DNA vaccines in clinical trials for HIV, human papilloma virus (HPV), Zika fever, and cancer, the only DNA vaccine approved for human use so far is India's ZyCoV-D DNA vaccine against SARS-CoV-2.

In a study comparing intramuscular vaccination to tattoo-based vaccination, researchers found that the tattoo delivery strategy led to a faster T cell response to HPV and protection against an influenza virus challenge (16). More T cells encountered the vaccine antigen via tattooing than when the same vaccine was given via a standard injection into the muscle.

"People study tattoo delivery as a way to deliver more DNA over larger areas," said David Weiner, an expert in DNA vaccines at the Wistar Institute. Tattoo delivery "can cover larger areas of the skin, and so you can deliver a lot more product."

Samir Arbache, a dermatologist at São Paulo Federal University, uses tattoo machines to treat a variety of dermatological conditions including alopecia and idiopathic guttate hypomelanosis, a disease that causes the loss of pigmentation in spots on the skin (17). Arbache started the company Traderm, which commercializes tattoo medical supplies, and so far, he has trained more than 2,000 doctors on how to use tattoo machines for drug delivery and vaccination.

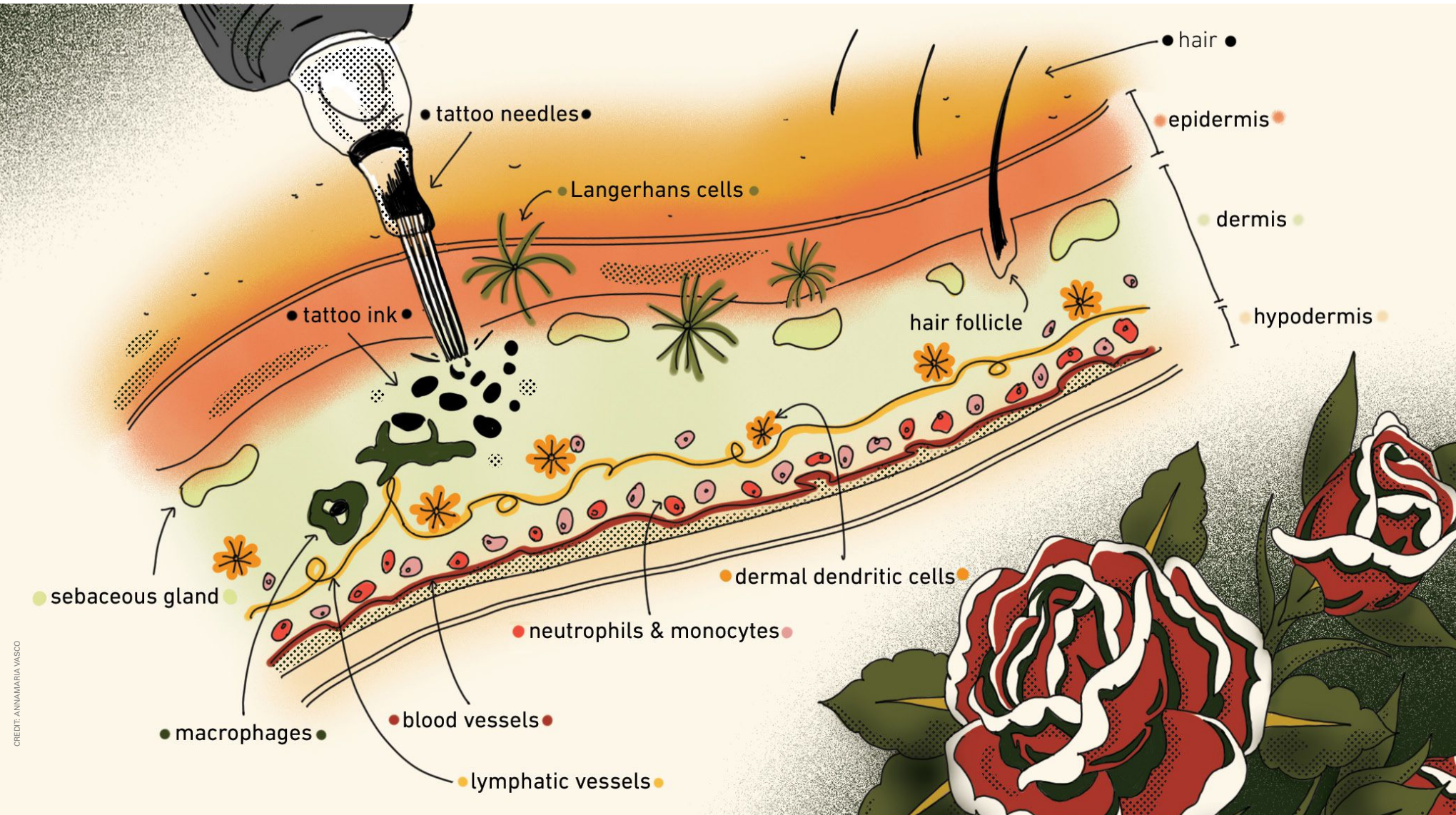
"The future is vaccination," said Arbache. Tattoo vaccine delivery is "becoming very popular [in Brazil]. Several doctors began clinical trials, and we need to spend some time [with] regulatory" groups, he added.



CREDIT: GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT (BFR)

Ines Schreiver studies how the pigments in tattoo ink interact with the immune system.

The skin is home to a menagerie of immune cells, including Langerhams cells in the epidermis and macrophages in the dermis among others. When ink from a tattoo needle enters the dermis, macrophages recognize the pigment particles as foreign and engulf them, capturing the pigment in the dermis. This capture by macrophages is what gives tattoos their permanency in the skin.



CREDIT: ANNAMARIA VASCO

Carson Bruns, a chemical engineer, develops tattoos with color-changing pigments that act as permanent sensors in the skin.

CREDIT: GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT (BFR)

"It's not very fun or easy to get your whole face tattooed, so we're also really working on new methods of tattooing that are much faster and less painful. We want to make tattooing more efficient so that treatments like that don't seem so scary."

– Carson Bruns, University of Colorado Boulder

SMART SENSORS

After Carson Bruns, a chemical engineer at the University of Colorado Boulder, got his first tattoo at age 19, he quickly became a fan of body art. But he didn't marry engineering and tattoos until he started research as a chemist building color-changing molecular machines.

"I just had this idea that maybe some of the color changing compounds I was used to working with could be exchanged for the colorants that are used in normal tattoo pigments," said Bruns. "As I started working on it and started my lab, we quickly realized that there were probably a lot of biomedical applications of this idea too."

Bruns and his team started out by designing what they called "solar freckles," which are small, tattooed dots containing a pigment that changes color in response to UV light (18). These dots could tell the wearer when it's time to apply more sunscreen. The solar freckle tattoos worked well when the researchers tested them on dead pig skin, but they wanted to see if it would work in humans. Bruns volunteered.

"It was really exciting. I was very thrilled when the first time I tested it on my skin and I saw the tattoo change color," said Bruns.

Bruns has now expanded on the idea of solar freckles to design another sensor, in this case for measuring exposure to high energy radiation (19). These sensors would be useful to people likely to be exposed to radiation, like astronauts in outer space, sailors on nuclear submarines, and people undergoing radiation treatment for cancer.

"The thing we've been working on the hardest is a UV protective tattoo," said Bruns. "What we're doing now is developing a completely invisible tattoo ink. So, it won't change the color of your skin, but you would get this tattoo wherever your skin is exposed frequently to sunshine."

Because most skin cancers occur on the face and the hands, Bruns suggests that people could get tattooed in those areas, and the tattoo would act as a permanent sunscreen. But people are likely going to be reluctant to tattoo their entire faces.

"We consider that the main barrier to people actually adopting this technology if it becomes available. It's not very fun or easy to get your whole face tattooed, so we're also really working on new methods of tattooing that are much faster and less painful. We want to make tattooing more efficient so that treatments like that don't seem so scary," Bruns said.

While Bruns uses tattoos as interfaces between the body and the environment, Ali Yetisen, a chemical engineer at Imperial College London designs tattoos that sense the inner workings of the human body. Yetisen and his team used existing color-changing reactions and modified them to produce tattoo inks that change color in response to changes in interstitial fluid, the fluid that transfers nutrients and waste products between capillaries and cells.

"We developed electrolyte-based sensors. So, these are based on sodium, potassium, calcium, magnesium, and zinc," said Yetisen. By monitoring these biomarkers, the tattoo sensors could tell people about their liver and kidney function or if they have a hydration imbalance (20). "For example, if you're exercising, your electrolyte levels may change in the blood," he added.

There are still significant hurdles. One is figuring out how to reverse the sensor so that once it reacts to a stimulus in the body, it can reset and be ready to react again. A second is how to implant the sensors to make them as biocompatible as possible. While all of their tattoo sensor research has taken place in animals, Yetisen and his team plan to start tests in humans later this year.

Like Bruns, Yetisen knows that user acceptance will be a large impediment to the feasibility of his tattoo sensors.

"Maybe [for] chronic conditions such as diabetes, it can be useful, but if someone were to use it for a couple of weeks, maybe it may not be the best approach," he said. Since these tattoos would serve a medical function more than an artistic one, Yetisen hopes that people in cultures that may have a stigma against tattooing will have a different perspective on these sensor-based tattoos.

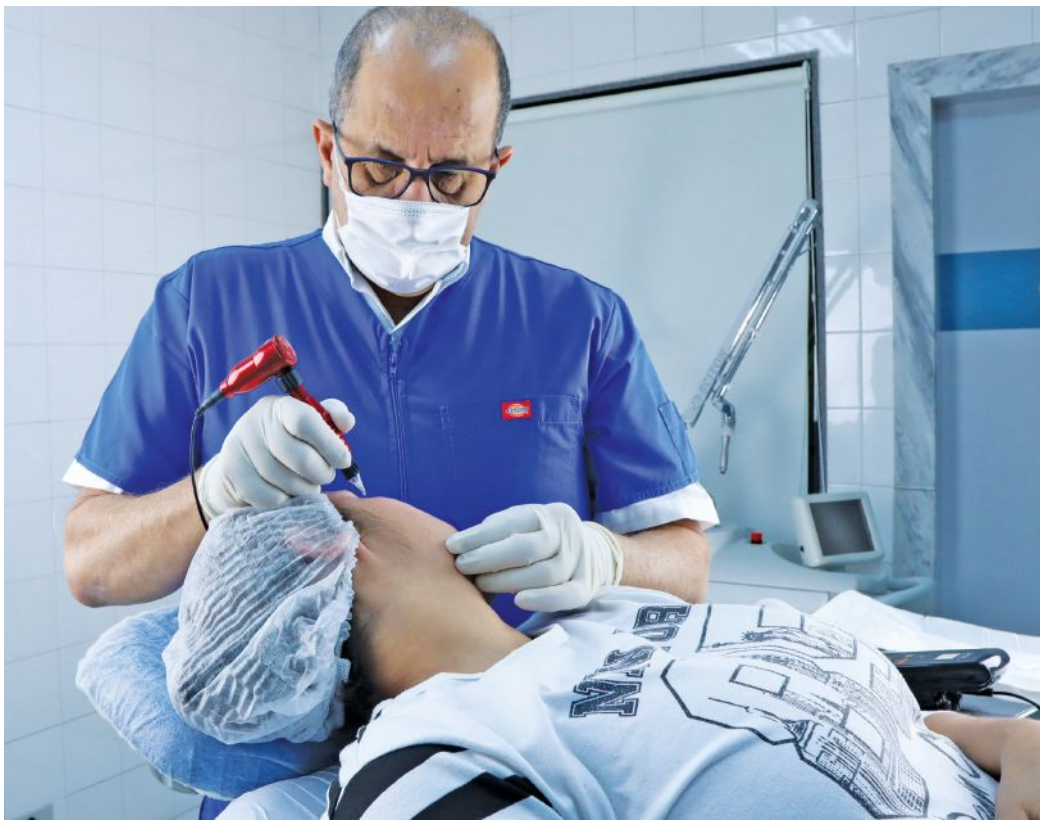
Both Bruns and Yetisen see tattoo-based sensors as an important middle ground between wearable sensors and surgery. While surgery often permanently fixes a problem, it is invasive. Wearables are convenient and able to be taken on and off, but they can cause discomfort such as skin conditions like atopic dermatitis. And devices that sit in the skin but are open to the outside environment have a risk for infection.

"What excites me is thinking about what types of technologies we can implant in the body by tattooing so as to avoid a more invasive intervention like surgery," said Bruns.

Yetisen echoed this same sentiment. He sees tattooed sensors extensions of human senses.

"Our bodies have been evolving over centuries now, and to [get to] the next level, it is going to be the integration of new types of materials, including electronic technologies inside the skin to create new senses that can transcend the ability of a conventional human," he said.

From Ötzi's delicate line tattoos to next-generation tattooed sensors, tattoos have been a part of human existence from the beginning and will likely be with us long into the future, enhancing human health and telling a story in vibrant color.



CREDIT: SAMIR ARBACHE

Samir Arbache uses a tattoo machine to deliver drugs to the skin to treat dermatological conditions in his clinical practice.

CREDIT: ALI YETISEN

Ali Yetisen designs tattoos that can sense electrolyte changes in the body. He hopes to develop a tattoo-based sensor that one day could monitor chronic conditions like diabetes.

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CRISPR

CRISPR Turns White Adipocytes to Brown Adipocytes

Implanting brown fat into mice fed a high fat diet helps them lose weight and become glucose tolerant. Will it do the same for humans?

BY NATALYA ORTOLANO, PHD

WHEN PEOPLE GET COLD, they shiver. Skeletal muscles use energy to make small jerky movements that warm up the body. Newborn babies can't shiver, but they do have a built-in heater: globs of brown fat stored behind their shoulders.

There are two main types of fat cells, or adipocytes: lipid-storing white adipocytes and metabolically active, heat-producing brown adipocytes. People lose brown fat as they age, leaving them with mostly white adipocytes. But brown fat helps adults with type II diabetes and obesity just as much it does cold newborns. Obese mice with glucose intolerance akin to that seen in diabetes regain glucose tolerance and lose weight when given brown fat implants.

Implanting brown fat into humans is trickier though. Because adults don't have many brown adipocytes, it's difficult to isolate enough to use therapeutically. But the study authors found a loophole. They used CRISPR to knock out nuclear receptor interacting protein 1 (*NRIP1*), a gene that prevents white adipocytes from acting like brown ones (1), allowing white fat cells to act more like their brown cousins. When the researchers implanted the brown-like white adipocytes into mice fed a high fat diet, the mice became more glucose tolerant than mice implanted with unmodified white adipocytes.

While this is an interesting first step, diabetes researchers Philip Scherer from the University of Texas Southwestern Medical Center and Daniel Drucker from the Lunenfeld-Tanenbaum Research Institute, who were not involved in the study, warned that it's a long way from the clinic.

Michael Czech is a diabetes researcher from the University of Massachusetts.

CREDIT: UMASS CHAN MEDICAL SCHOOL COMMUNICATIONS

"Translation is hard. It's very easy to help animals; it's much more difficult to help humans," said Drucker. "It was a very nice study and very imaginative. I give it full marks for an animal study. And who knows, in 10 or 15 years, we might be using gene editing to fix a large number of disorders that today might be beyond reach."

Silvia Corvera and Michael Czech, both diabetes researchers from the University of Massachusetts, and coauthors of the study, argue that the way they edited the cells makes the therapy more translatable. Rather than exposing the cells to a lentivirus carrying a plasmid encoding Cas9 and the guide RNA (gRNA) that will lead the enzyme to NRIP1, they added the protein and gRNA directly.

"This technique reduces, at least in theory, the off-target effects of CRISPR because the fact that the Cas9 protein gets degraded within about 48 hours or so means it's not still in the cell for days and days or weeks or months, and certainly not when we implant the cells back into the mouse. By that time, the Cas9 and the sgRNA are long gone," said Czech.

The process is quick too. Corvera said that they could return personalized brown-like adipocytes back to a patient within a month of the first time they stepped in the clinic. First, the researchers would extract and culture white adipocytes from a biopsy of the patient's fat, which takes about three weeks. Then they need a week to knock out *NRIP1* and convert the white adipocytes to brown-like adipocytes.

"It's important to think about this in terms of the costs involved and the time because diabetes is a very common disease. In the long run, we're thinking off-the-shelf cells would be what one would need. In other words, we would want to be able to [use a patient's own cells]. But in addition to that, we'd want to be able to also modify the cells from an individual so that they could be implanted into a different individual," said Czech.

Scherer still sees a long road ahead for the researchers. "There's a high hurdle before this could be done more systematically at the clinical level, but on the other hand, things are moving quite rapidly in that area. CRISPR/Cas9 technology is being used already to correct specific mutations in some settings. So, it does look like this is going to be the future. And the next step for somebody like them now would actually be to provide proof of principle. Maybe not so much in the clinical setting, but perhaps in a in a nonhuman primate setting," he said.

Czech and Corvera agree that their brown-like adipocytes aren't ready for a human clinical trial just yet. They are gearing up to test their CRISPR technique in nonhuman primates next. They hope to answer some more questions about the brown adipocyte cells themselves and how they could be used more broadly as a therapeutic.

"It's also very important that we continue to try and understand what these cells are and what they are doing when we put them in the mice and in the monkeys. What exactly are they secreting? And how or why are they so potent systemically? We really don't know," said Corvera. ■

Silvia Corvera and Michael Czech developed a potential CRISPR-based therapy to treat type II diabetes.

CREDIT: UMASS CHAN MEDICAL SCHOOL COMMUNICATIONS

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>
> *– Silvia Corvera, University of Massachusetts*

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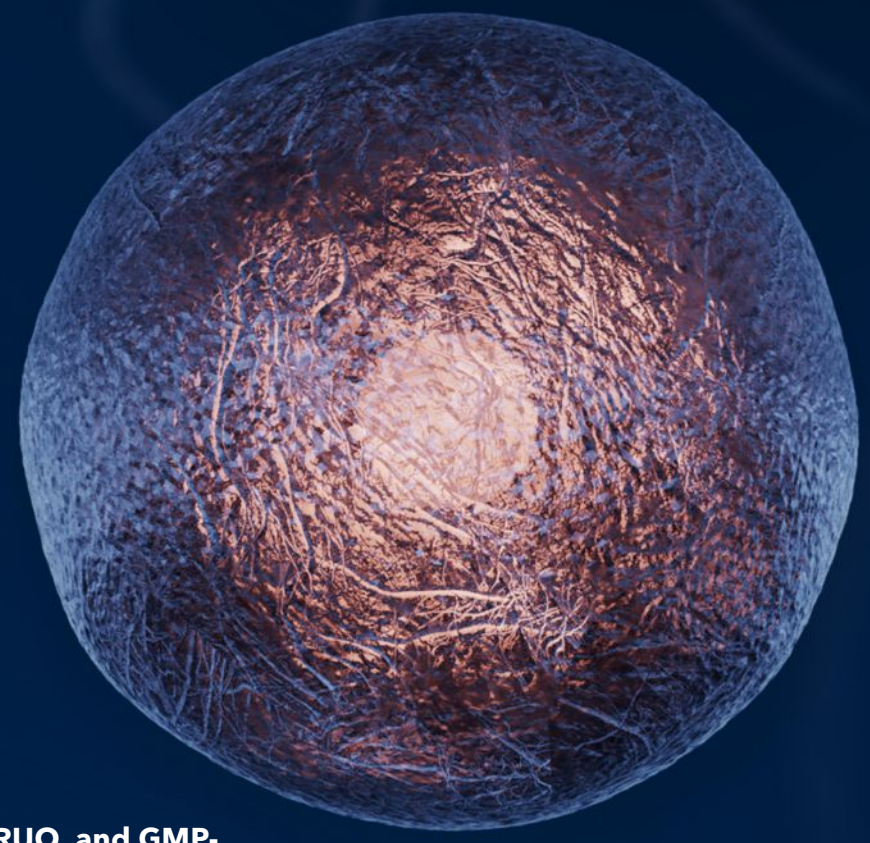

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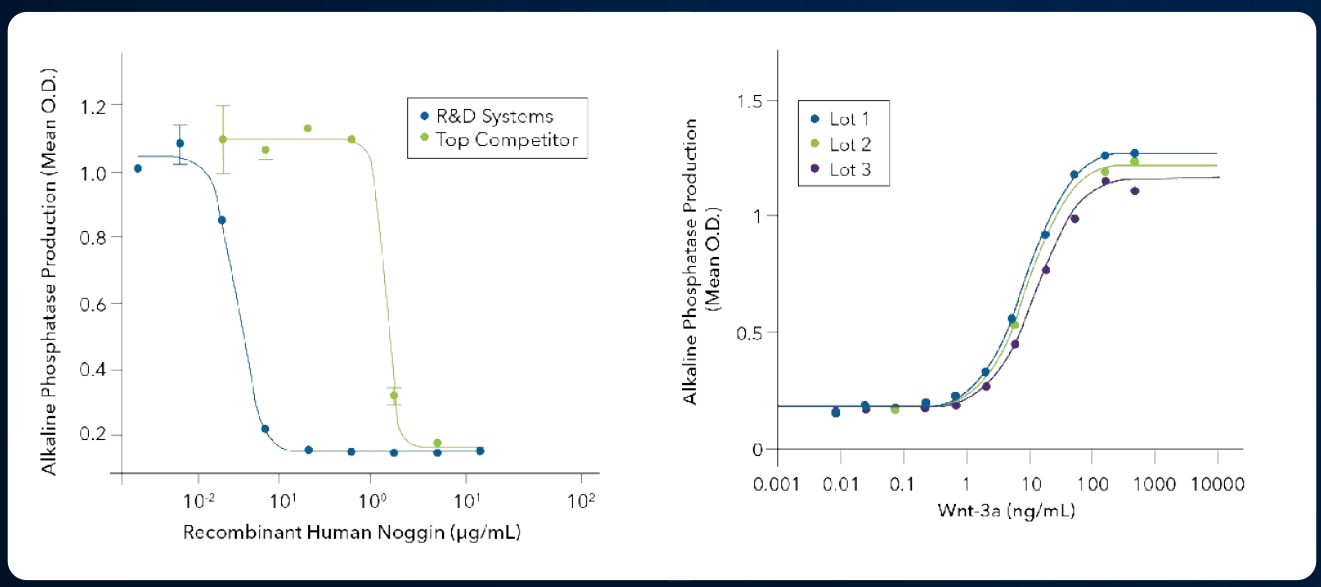

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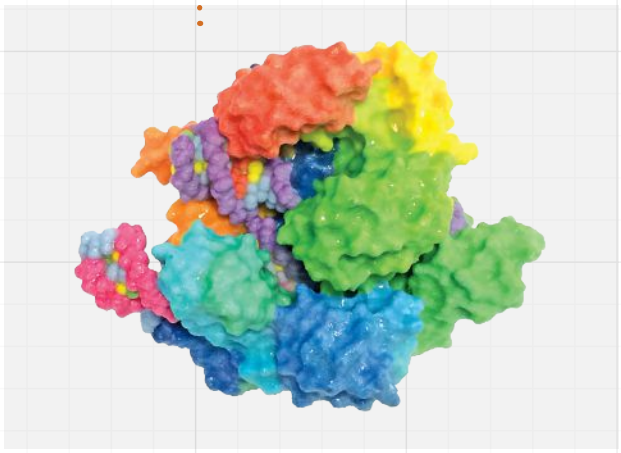
milestone

The Creation of CRISPR

BY MAGGIE CHEN

Before the introduction of CRISPR gene editing technology, editing the genome was a concept found in movies and rarely done in real life. While technologies such as zinc finger nucleases allowed for certain types of editing, they were hard to use and often hard to design. Over the past few decades, the discovery, characterization, and application of CRISPR-Cas systems has allowed for specific, targeted, and purposeful genome editing. Scientists have leveraged CRISPR-based gene editing systems to model diseases, develop therapeutics, and improve crop yields among many other applications.



The Cas9 protein travels with the CRISPR complex to a specific DNA sequence and cleaves it.

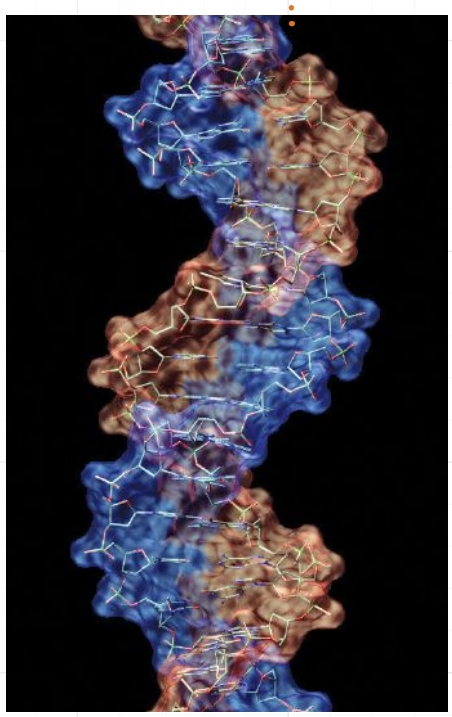
1990s | Prokaryotic immunity

In 1992, Francisco Mojica, a microbiologist at the University of Alicante, worked to understand how halophilic archaea, salt-loving prokaryotes, survived in harsh, briny environmental conditions. To look for cues, Mojica decided to investigate their genomes and uncovered short sequences of base pairs that repeated regularly.

"We immediately realized that they were not related to halo-adaptation because they were expressed or transcribed in every single salinity we tested," Mojica said. "We tried to understand the meaning of that and what was the role that they played."

These repeats, along with spacer sequences of similar lengths that separated them, were later christened clustered regularly interspaced short palindromic repeats (CRISPR) in 2002 (1). Further research revealed CRISPR systems in a number of other bacteria and archaea. To figure out how organisms used CRISPR, Mojica used a word processor to isolate the CRISPR base pair sequences and then FASTA software to match them to the existing sequences of other microorganisms.

"We saw that one of them matched a sequence in a phage that infected the host, and the host was *E. coli*," Mojica recalled. As a result, Mojica hypothesized that these repeats were involved in a bacterial type of immune system to protect them from foreign DNA sequences (2).



Prokaryotic DNA contains repeat sequences that scientists named CRISPR.

2000s | CRISPR-Cas

Following Mojica's discovery of CRISPR, other scientists quickly identified Cas9, a protein that they predicted to cut DNA, in 2005. They also noticed that the spacer sequences in between each CRISPR repeat shared a short common sequence at one end, which they later termed protospacer adjacent motifs (PAM) (3).

While several scientists had predicted that CRISPR-Cas was part of a novel DNA repair system, computational biologist Eugene Koonin at the National Center for Biotechnology Information took a different view. Koonin's team was interested in horizontal gene transfer — the movement of genes between organisms of the same generation, rather than between parents and children — between different kinds of bacteria and archaea. Through computational analysis, he and his team confirmed Mojica's finding that sequences in the spacers were shared by phages.

Based on these observations, Koonin's team developed "a detailed hypothetical scheme of how this thing was supposed to work in adaptive immunity," he recalled. He also drew potential parallels between CRISPR-Cas and RNA interference, where RNA molecules silence expression of certain genes, including those of invading viruses. This adaptive immunity hypothesis, published in 2006 (4), prompted molecular biologist Philippe Horvath, then at Danisco France SAS, to experimentally demonstrate that the CRISPR-Cas system provided resistance against phage infection in bacteria (5).

Erik Sontheimer, an RNA biologist then at Northwestern University, was also intrigued by Koonin's proposed schema of the CRISPR-Cas role in adaptive immunity. Together with graduate student Luciano Marraffini (now at Rockefeller University), he set out to determine whether CRISPR-Cas9 targeted DNA or RNA (6).

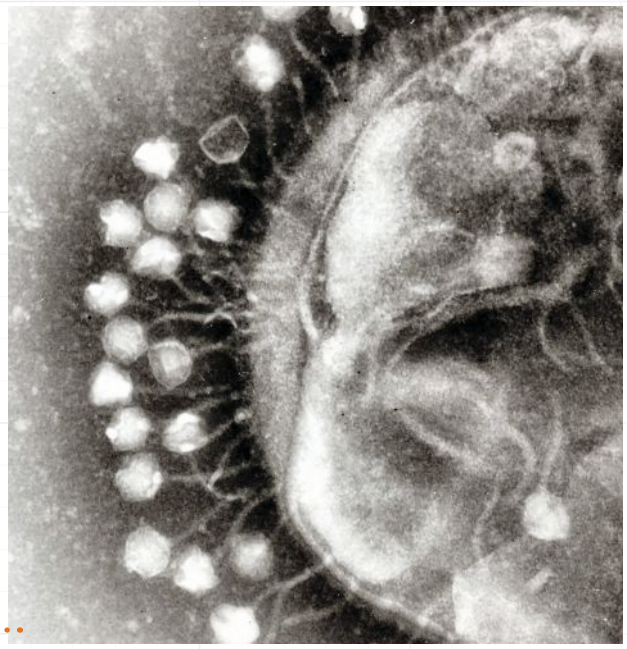
One of his key experiments incorporated a spacer region into a plasmid containing a non-coding region of DNA, which he introduced into cells in culture. If CRISPR-Cas9 targeted RNA, no interference would occur since the spacer would not be transcribed into RNA. "Yet, we still had CRISPR interference," Sontheimer explained. "The fact that we got CRISPR interference against the plasmid, even with a region that had no function and didn't code for anything — that's most consistent with DNA targeting."

2012 | CRISPR-Cas9 cleavage

While scientists had demonstrated that CRISPR-Cas9 was involved in adaptive immunity, "the mechanism was very unclear," said Virginijus Siksnys, a biochemist at Vilnius University. To figure this out, Siksnys's team began exploring what happened when CRISPR-Cas9 moved from one bacterium to another. "It showed that CRISPR-Cas9 systems are independent functional units that you can transplant between different bacteria," he said. "This transplanted functional unit also provided interference against invading phages."

Published in 2011, Siksnys recalled that this finding "to some extent, paves the way for transfer of CRISPR systems in eukaryotes" (7). Building on this work and other studies that uncovered the Cas9 cleavage mechanism and RNA guide mechanism, Siksnys' group isolated the Cas9 protein and demonstrated in 2012 that it used an RNA molecule to guide itself to cleave a specific spot in DNA and that Cas9 could be reprogrammed simply by changing the guide RNA's sequence (8).

Siksnys submitted the paper for publication, only to be surprised at its rejection without peer review. In the meantime, biochemist Jennifer Doudna at the University of California, Berkeley and biochemist Emmanuelle Charpentier at the Max Planck Institute showed that the CRISPR-Cas9 system depended on a two RNA molecule system composed of a trans-activating crRNA (tracrRNA) and CRISPR RNA (crRNA) that directed the CRISPR-Cas9 system towards the appropriate DNA sequence (9). Doudna and Charpentier also demonstrated that the crRNA and tracrRNA could be synthetically combined into a single RNA chimera, which introduced the possibility of targeted, specific gene editing via CRISPR-Cas9. Their paper was published just prior to Siksnys' paper in 2012; Doudna and Charpentier later won the 2020 Nobel Prize in Chemistry for this work.

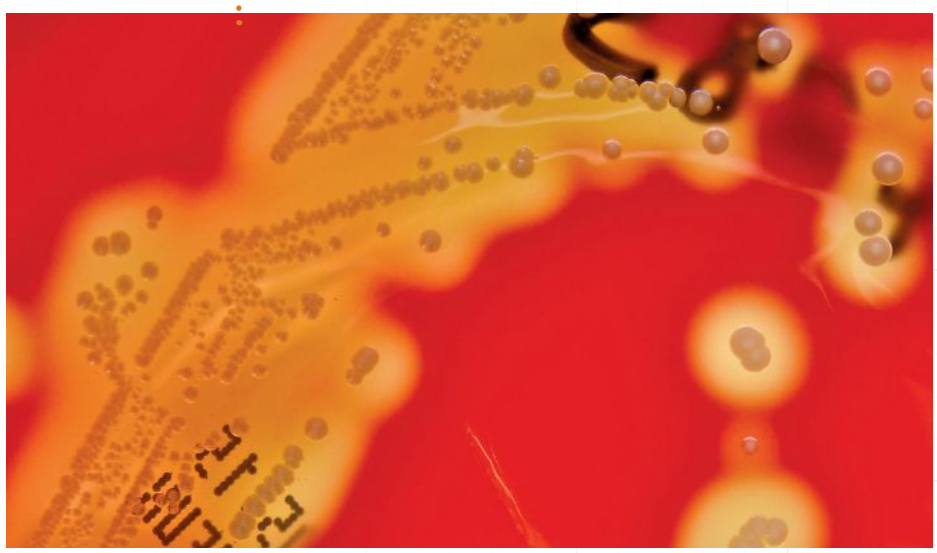


Scientists found similarities between CRISPR spacer sequences and phage sequences, indicating that CRISPR-Cas might be involved in adaptive immunity.

2013 | Editing the genome

With the biochemical mechanism of CRISPR-Cas9 uncovered, molecular biologist Feng Zhang at the Broad Institute of Harvard and MIT next applied the system to edit eukaryotic cells. Using CRISPR-Cas9 from *Streptococcus pyogenes* bacteria, Zhang and his team generated custom chimeric tracrRNA-crRNAs (now called guide RNAs, gRNA) as Doudna and Charpentier had described, and tested these against several gene targets, including mouse *Th* loci and human *PVALB*. Targeting many of these loci proved successful, indicating that CRISPR-Cas9 could be designed to target and edit a broad variety of genomic loci (10). These results, along with similar data from geneticist George Church at Harvard University (11), pushed CRISPR-Cas9 forward as a programmable and powerful tool for genome editing.

As CRISPR-Cas9 generated increasing interest, scientists began to investigate other CRISPR-Cas systems from a variety of species. They worked to classify CRISPR-Cas into several types (1-6) and classes (1-2), with each type relying on different Cas protein complexes and mechanisms and each class using structurally different effector molecules (Cas proteins). One system, CRISPR-Cpf1, requires a shorter crRNA as a targeting guide, making it easier and less expensive to synthesize a custom RNA guide molecule (12). In contrast with the *S. pyogenes*-derived CRISPR-Cas9 system, which generates blunt ends that are better for gene silencing, this system also generates cuts with sticky ends, leaving the door open for DNA sequence insertions (12).



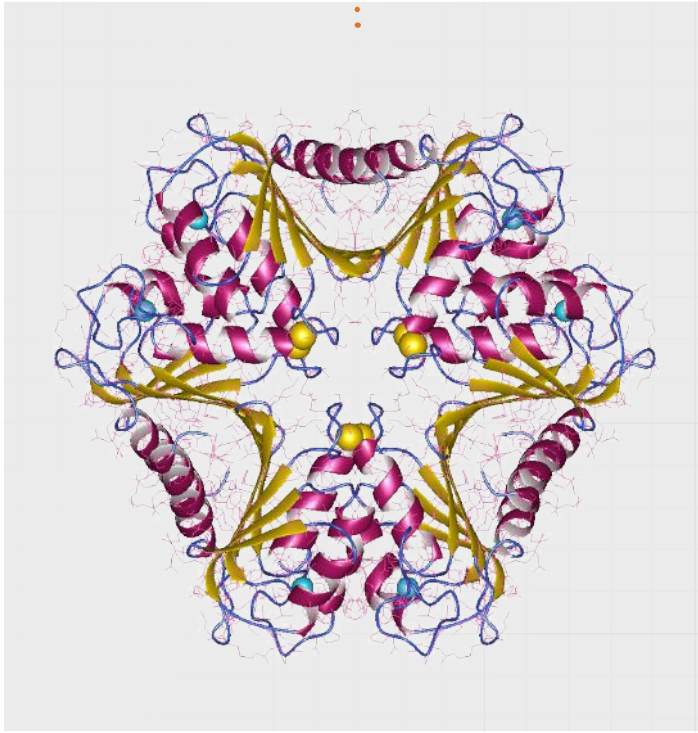
Feng Zhang used CRISPR-Cas9 from Staphylococcus pyogenes to edit eukaryotic cells.

2016 | Editing single base pairs

As CRISPR-Cas systems became a thriving field, scientists became interested in other kinds of editing. Biochemist David Liu at Harvard University and his team decided to use the sequence targeting capabilities of CRISPR-Cas9 to switch individual bases, a technique called prime editing.

To do this, Liu's team used a modified Cas9 (dCas9) protein that could bind DNA via guide RNA molecules but could not cleave the DNA sequence. They attached a cytidine deaminase enzyme, which switches cytosine to uridine, to the dCas9 (13). Using a custom guide RNA, the CRISPR-dCas9-deaminase system cleanly switched bases at specific locations in the DNA sequence. Later, Liu's group generated a similar system to switch an A-T base pair to a G-C base pair, further expanding the possibilities of genome editing at single base resolution.

Sontheimer's group continues to improve these base editors with the goal of applying them to treat certain genetic diseases. In some cases, they "want to actually change the sequence in a highly defined way, where you take the mutated gene that results in disease, and you correct it back to the wild type, healthy version that non-patients carry," Sontheimer said. "The important thing is that you want to specifically rewrite it; you don't just want to introduce a random insertion or deletion to break the gene."



A deaminase enzyme can switch out specific base pairs.

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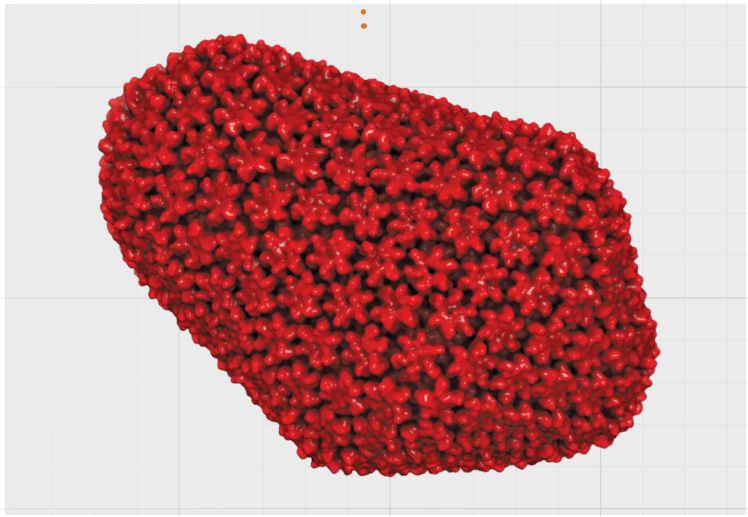
2020-present | Applying CRISPR-Cas

Leveraging these different types of CRISPR-Cas systems has led to myriad applications for treating diseases ranging from Alzheimer's disease to Duchenne muscular dystrophy. CRISPR-Cas can also be used for biosensing, cell engineering, and diagnostics. A bustling biotechnology niche has also rapidly emerged, with companies such as Intellia Therapeutics founded by Sontheimer, Caribou Biosciences cofounded by Doudna, and Beam Therapeutics cofounded by Liu and Zhang.

Another powerful application of CRISPR-Cas is in disease modeling, where researchers create models of diseases in isolated cells. This allows scientists to study diseases and search for promising therapeutic targets in cultured cells rather than relying on patients and increasing their burdens.

Nevan Krogan, a systems biologist at the University of California, San Francisco, used CRISPR-based methods in conjunction with structural biology studies to figure out the functional significance of certain genes. By using CRISPR-Cas9 in T cells, Krogan and colleagues screened more than 400 genes to identify their roles in HIV infection. The functionally significant genes could then be used in downstream studies as drug targets. "CRISPR is so powerful and can be used as a discovery tool," he said. "But then it can be pivoted and potentially used as a therapeutic tool as well."

The potential applications for CRISPR are limitless, ranging from detecting disease to treating it, from facilitating drug target discovery to expanding the nutrition and availability of food crops, and even going as far as reviving the extinct wholly mammoth by gene editing Asian elephants. Scientists will continue designing better CRISPR-Cas systems and finding new ones to advance these goals and develop new initiatives. "The story is continuing and goes on," Krogan said.



Nevan Krogan used CRISPR-Cas9 to study how HIV interacts with proteins in the cell.

Infusing CRISPR Therapeutics with Safety and Soul

Samira Kiani combines her passion for art and design with synthetic biology to create safer CRISPR-based therapeutics for the future.

INTERVIEWED BY STEPHANIE DEMARCO, PHD

LIKE A SCULPTOR'S HAND steering a chisel toward a stone, guide RNAs direct CRISPR's Cas9 protein to cut a piece of DNA. With multiple CRISPR gene therapies in clinical trials and some on track for FDA approval, CRISPR has come a long way from its humble origin as a bacterial defense system.

When it comes to gene editing, questions of safety, ethics, and potential off-target effects follow. If scientists could make CRISPR safer, then lifesaving CRISPR-based therapeutics could reach the patients who need them sooner.

Samira Kiani, a biologist at the University of Pittsburgh School of Medicine, is up to the task. By melding her background in medicine with her passion for art and design, Kiani uses synthetic biology to engineer safety directly into the CRISPR gene editing system in both her academic lab and biotech company, Genexgen. By creating safe CRISPR systems, she hopes to accelerate the delivery of CRISPR-based therapies into patient hands.

As a scientist and storyteller, Kiani doesn't stop at safety. She uses filmmaking and video games to center the ethical concerns surrounding CRISPR and gene editing. By bringing scientists and the public together in creative spaces, Kiani wants to disrupt the scientific status quo and facilitate discussions around the consequences of scientific discoveries for both science and society.

As a scientist, filmmaker, and storyteller, Samira Kiani uses synthetic biology to make CRISPR therapeutics safer and challenges scientists to think critically about the ethical implications of their research.

CREDIT: SAMIRA KIANI

How did you become interested in science?

I came to science from a nontraditional entryway. I went to medical school and was doing science in a liver research lab in the evening. I enjoyed my medical training, but I felt like there was something in it that was not for me. Then one day I sat with myself and said, "Part of me is always a scientist slash medical doctor. I can't really remove that from myself, but I also like design and storytelling and being creative." I wondered if there was any way of combining these interests, and I came across the field of synthetic biology. The idea of designing organisms from scratch, coming up with an idea for a function, and then building that function into that organism was very interesting. It was equivalent to a piece of art, painting, or poetry for me.

What made you want to use synthetic biology to make CRISPR-based therapies safer?

My serious work in synthetic biology began when I worked with Ron Weiss at the Massachusetts Institute of Technology Synthetic Biology Center in 2012. At that point, it was the birth of the CRISPR technology for gene editing. Ron tasked me with combining the principles of synthetic biology with CRISPR technology. He wanted me to build new genetic circuits using CRISPR to activate, repress, or develop certain functionality in cells.

After two years into my research there, my dad was diagnosed with pancreatic cancer. Pancreatic cancer is deadly, and the current

treatment regimens are not very effective. Many patients resort to new experimental therapies in clinical trials. I was really determined to get my dad into one of these clinical trials, but because my dad didn't live in the US, we couldn't get him onto one. That experience made me think; we spend a lot of time evaluating the safety of these therapies in phase one and two clinical trials, while patients need these treatments now. What if we could engineer safety into these new treatments from the get go?

That's when I zoomed out and looked at what I was doing with synthetic biology and CRISPR. I was controlling Cas9 and the guide RNA. What if I could use these tools to build safety and controllability into CRISPR-based gene therapies?

How do you make CRISPR safer?

For one, we looked at the Cas9 protein structure, and through bioinformatics, we determined which part of the Cas9 protein elicits an immune response in the body (1). We wanted to figure out if there was any way to engineer Cas9 so that it would be more immune silent.

We're also interested in moving away from CRISPR's function as a DNA cleaving agent. Instead of creating a permanent change in DNA, we use a "dead" Cas9 protein that is mutated so that it cannot cut DNA. We can still send it to any part of the genome we want, but when it gets to the destination, it just sits on the DNA. We fuse proteins that activate or repress gene expression to the dead CRISPR system, and it carries these proteins with it to the target gene. Once there, they do their natural job, which is either to activate the gene or repress it (2). This is a safer way of applying CRISPR to treat conditions that don't necessarily need us to disable the gene permanently.

What kind of research questions are you working on at Genexgen?

Genexgen is a next generation immunotherapy company focusing on how we can bring epigenetic editing to immunotherapy. Nature engineered the immune system to fight a number of diseases, so I'm interested in bringing in what I know about synthetic biology and gene editing to manipulate the immune system. For instance, we could use the CRISPR system that helps dial down and up the expression of genes that are responsible for the immune response such as the production of antibodies, inflammatory signals, or cytokines (3).

How do you integrate your interest in storytelling and art into science?

I'm realizing more and more that genetic technologies have a profound effect on society. These technologies are not going to be confined to our research labs, conferences, or peer to peer communications. They will readily impact the lives of many people.

I want to explore how we can innovate differently. Instead of just asking questions from a scientist's perspective, we should integrate the points of view of people from other disciplines including art, social sciences, and behavioral sciences and collectively start asking questions that lead to new types of innovations. Through filmmaking, theater, and collaborations with game designers, I want to expose scientists to other points of view and have them reflect on the ethics and societal impact of the research that we do.

CREDIT: SAMIRA KIANI

Through filmmaking and bringing scientists and artists together, Samira Kiani hopes to infuse more compassion and thoughtfulness into the practice of science.

> "The idea of designing organisms from scratch, coming up with an idea for a function, and then building that function into that organism was very interesting. It was equivalent to a piece of art, painting, or poetry for me."
>
> *– Samira Kiani, University of Pittsburgh School of Medicine*

CREDIT: SAMIRA KIANI

By channeling her passion for storytelling and synthetic biology, Samira Kiani creates films and art about the implications of genetic technology for the future.

We recently collaborated with Carnegie Mellon University Entertainment Technology Center to develop a virtual museum called Unmute that houses information about CRISPR and its benefits and harms. Through a collaboration with the Center for Game Design, we're developing residency programs for artists and scientists to come together.

What is your documentary film "Make People Better" about?

The film is about the future and where we are heading in terms of genetic technology. It zooms into that higher question of what our responsibilities are as human beings and as scientists to each other and to the general population. We use the case of He Jiankui, the scientist who created the twin CRISPR babies back in 2018, as an example to discuss why and how this happened. I see the film as a way to start conversations about what our responsibilities as scientists are. The film should come out by the end of this year or early next year.

Did making this film change how you think about your own research?

Absolutely. One important aspect that this film touches on is the mentor-mentee relationship in science and how there are certain norms in our field that are not necessarily working. There is a lot of emphasis on pushing forward, breaking the glass, and being first in science. I had been doing that to my students, but I've been rethinking that. The more important thing is to think about what type of future we're going to build for the next generation. Science needs to be more compassionate toward the people we are serving and to each other in the scientific community. It requires us to reflect more of our souls as human beings. Let's revisit the process of innovation and how we do science. ■

This interview has been condensed and edited for clarity.

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A Balancing Act: Identifying the Best Conditions for Transfection

Gene and cell therapy success relies on one of the earliest steps in viral vector biotherapeutic production.

CELL AND GENE THERAPIES FIRST gained approval from the United States Food and Drug Administration (FDA) in 2017 (1). Today, there are more than 20 FDA-approved cell and gene therapies that treat numerous ailments, including some cancers, eye diseases, and rare hereditary conditions (2). The promise of replacing deficient or faulty genes by introducing a new, functional copy via gene therapy or by infusing genetically modified cells via cell therapy has gained traction. Although still in its infancy, there are more than 700 gene therapies and 9,000 cell therapies in clinical trials (3).

Mirus field applications scientist Hayder Abdul-Razak has experience with the preclinical optimization of cell and gene therapies. He has engineered adeno-associated viruses and lentiviruses in various disease models to identify the optimal conditions for viral vector-based therapeutic production. Abdul-Razak estimates that 30% of the success in cell and gene therapy has to do with one of its earliest steps: transfection.

During transfection, scientists introduce a viral plasmid containing the gene of interest and maybe an additional helper plasmid for viral assembly into producer cells. The success of this step directly influences viral vector-based therapeutic production and the integrity of cell and gene therapy products.

What do you enjoy about viral vector optimization?

It is quite challenging. When I started working in this field, most things were unknown. We were trying to find real answers to big questions: Will the transfection work? Is it safe? We worked with a lot of different possibilities. I like this challenging atmosphere where we are always scratching our heads trying to get something to work.

What is the most important thing for scientists to know about executing a successful transfection?

The first thing to understand is the tools, including the cells, transfection reagents, cell culture platform, and more. In general, there are quite a few parameters that need to be adjusted, and the best way to do this is to try some different experiments to identify the optimal conditions for transfection. There are many transfection reagents on the market, and they vary in nature, mechanism, and mode of action.

How do Mirus transfection reagents differ from others?

Primarily, Mirus reagents are a nonliposomal and novel combination of lipids and polymers. Not only is this combination unique, but the components, including lipids and polymers, are also proprietary and exclusively formulated by Mirus. Our *Trans*IT-VirusGEN® Transfection Reagent is no exception; it is completely synthetic and animal origin free.

What makes the TransIT-VirusGEN® line suitable for therapeutic viral vector development?

In addition to the fact that *Trans*IT-VirusGEN® is an animal origin free transfection reagent, it is also available in three configurations targeted for use in research and development (R&D), preclinical process development, or clinical trials and commercial manufacturing. *Trans*IT-VirusGEN® reagents are also available in different sizes, but most importantly, there are no differences in the actual formulation or chemistry. Scientists can rest assured that all *Trans*IT-VirusGEN® options will perform similarly from R&D to good manufacturing practices (GMP).

How can scientists scale up their transfection experiments?

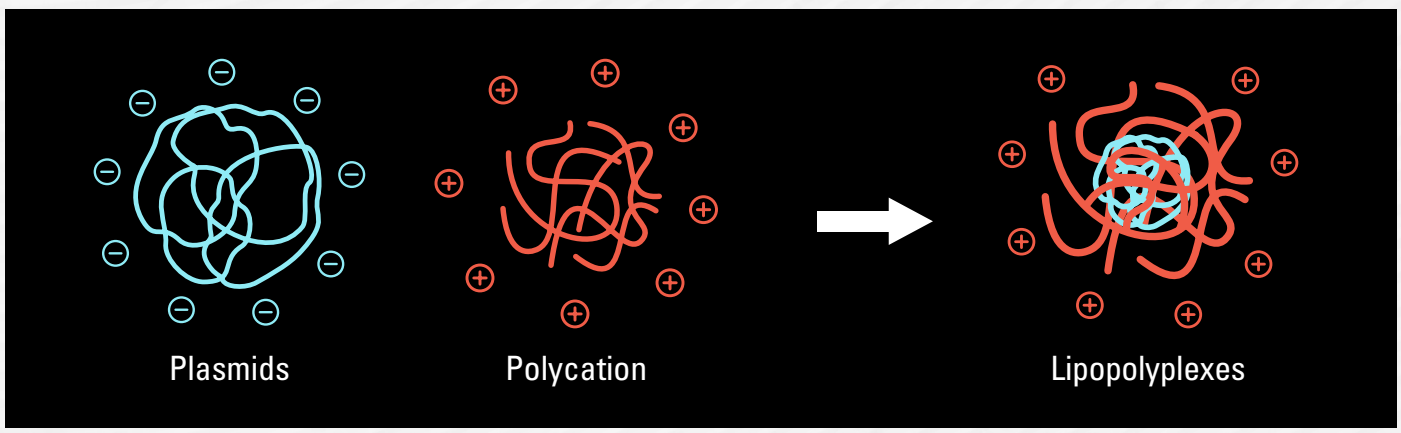
We recommend that researchers first try a small-scale, pilot experiment to optimize a few conditions. We initially guide them to use the recommended protocol conditions, but we highly advise testing a range. For example, our AAV protocol recommends a reagent to total DNA ratio of one and a half to one, for example, 1.5µl reagent to 1µg DNA, a starting total DNA amount of 2µg DNA per milliliter of cell culture, and four million cells per milliliter of culture at the time of transfection. To optimize, scientists can test ratios between 1:1 and 2:1, total DNA between 1.5 and 2µg, and cell densities between two to four million cells per milliliter. We recognize that this can lead to several variations but determining the optimal conditions empirically at a small scale is more feasible and goes a long way.

Scaling up is especially important for biopharma where we are talking about liters to hundreds of liters. The biggest thing for scientists to consider when scaling up their culture volumes is that they should scale up all of the numbers. We have many customers who have successfully managed to scale up with *Trans*IT-VirusGEN® Transfection Reagent. They start with small-scale experiments as outlined above and adjust everything in one or two rounds of optimization, and then scale up to different lateral volumes. Then that's it! It can be challenging for scientists to standardize all of the conditions, but our advice is to scale down everything into manageable experiments to set up, to optimize, and to standardize all of the conditions. From here, they can scale it to the desired volume, which should solve most problems.

Hayder Abdul-Razak, PhD
Field Applications Scientist
Mirus Bio, LLC

What are the advantages of using Mirus transfection reagents?

The workflow is very simple to follow. Scientists can transfect and if necessary, add enhancers into the culture all on the same or next day. If we can reduce the amount of work and personnel needed to attend to the cell cultures, it ultimately benefits everyone. Our customers like that everything can be done in fewer days. Other advantages include a flexible workflow compatible with many commercial culture media, scalable systems ranging from small academic laboratories to large scale GMP manufacturing, and applicability in diverse applications and platforms. This sets us apart.



Plasmids like those used in viral vector production possess a negative net charge. The TransIT-VirusGEN® *Transfection Reagent forms a unique lipid-polymer complex with DNA that confers a positive charge, allowing plasmids to efficiently navigate across cell membranes.*

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The Search for an HIV Cure Takes to the Sea

Small molecule drugs that trigger the death of cells storing latent viruses are a promising approach for treating HIV. Researchers identified a potent compound in a marine sponge — a more likely source than it may seem.

BY SARAH ANDERSON, PHD

MAINTAINING A WEED-free yard is a constant chore. Pesticides can keep weeds on the surface at bay, but they don't get rid of seeds and roots lurking underground that grow up at their whim.

Treating HIV has a similar problem. While combination antiretroviral therapy (cART) stops the spread of the active virus, it doesn't target latent virus reservoirs lurking in CD4+ T cells that evade the immune system. These reservoirs can spontaneously reactivate and replicate, requiring chronic administration of cART to manage HIV.

Forcing the dormant virus (or weed) to show itself in order to get rid of it once and for all would enable long-term remission of HIV (or a lawn effortlessly worthy of neighborhood envy). Researchers are developing small molecule drugs that can force the latent virus to turn on, causing the host cells to undergo apoptosis or an immune attack. While the cells are killed weedwhacker style, cART keeps the newly active virus from spreading and replenishing the reserve of dormant virus. Although several of these virus-coaxing drugs (called latency reversal agents or LRAs) have been tested as an HIV cure in humans, they haven't significantly reduced viral reservoirs and can carry toxic side effects.

In a recent study in the *Journal of Natural Products*, researchers reported a potent new LRA compound isolated from a sea sponge (1). Their work provides a starting point for the development of a new HIV therapy and illustrates the value of natural chemical diversity in drug discovery.

"It's another prime example of how we may now have a way forward with curing HIV that we never would've without mother nature giving us some hints," said Bill Baker, a natural product chemist at the University of South Florida who was not involved in the study.

Chemical compound libraries from natural sources have higher rates of antiviral activity than synthetic molecules, said Ian Tietjen, a molecular and cell biologist at the Wistar Institute and coauthor of the study. "A tree or a sponge doesn't have an immune system like you or me, so they rely a lot more on chemical compounds to fight off viruses," he said. "Nature has already done the work for us, so we should take advantage of that to look for antivirals."

The researchers did just that, screening an array of crude extracts and pure products from marine invertebrates for LRAs. "The marine environment gives you new chemical scaffolds with very promising biological activity," said Raymond Andersen, a natural product chemist at the University of British Columbia and coauthor of the study. "The power of looking in the ocean is that you find things that you wouldn't find otherwise."

Marine sponges are a rich source of unique and biologically active compounds.

The team observed that an extract from a *Phorbas* marine sponge collected from Howe Sound off the west coast of Canada showed latency reversal activity. They then separated the extract into multiple components and tested each one, repeating the process until they had isolated the active compounds. After extensive analysis of their chemical structures, the researchers identified the compounds as a class of molecules called sesterterpenoids.

The researchers tested the compounds in a CD4+ T cell line and found that two were more effective at activating latent HIV virus than the established LRA prostratin (another natural product derived from the bark of the mamala tree that Samoans have long used as an antiviral) (2). They observed that the activity of their compounds was blocked when they added an inhibitor of protein kinase C (PKC), indicating that these new LRAs work by activating PKC. Finally, the researchers tested the most potent compound, dubbed ansellone J, in CD4+ T cells donated from four people living with HIV and undergoing cART treatment. Ansellone J activated latent virus to a similar degree as prostratin, but at a 10-fold lower concentration.

The potency of ansellone J in real HIV cells from donors is a powerful demonstration of its therapeutic potential, according to Baker. "That's probably the real *coup de grâce* in this paper," he said. However, if the compound is developed into a commercial drug, its structure will need to be adjusted in order to synthesize it on a large scale and protect it as intellectual property, he added.

> "Nature has already done the work for us, so we should take advantage of that to look for antivirals."
>
> *- Ian Tietjen, the Wistar Institute*

Andersen's team is working on developing novel and superior analogs of ansellone J. "We basically start clipping parts off of the natural product until we find out what's the minimum structural requirement to give us the biological activity," he said. They can then add features to this core structure that enhance the molecule's synthetic feasibility, patentability, and pharmaceutical properties.

David Margolis, an infectious disease clinician and HIV scientist at the University of North Carolina who was not involved with the study, would like to see ansellone J tested in a greater number of HIV donor cell samples and compared to other common LRAs. Like previous PKC activators, the toxicity of the compound might be too high for clinical use, Margolis added. "Trying to find some way to get to more effective compounds that are also safe is a major challenge," he said.

While PKC activators can trigger dangerous non-specific immune responses, preliminary data suggest that combining low doses of LRAs that operate through separate mechanisms can increase the activity and decrease the toxicity of each, Tietjen said. He hopes that a more potent PKC-targeting LRA could lead to a safer drug cocktail.

For Tietjen, the search for a cure for HIV isn't over until the depths of the ocean have been explored. "We do have some leads; we have some things that we can test; we have some new therapies that we can continue to try," he said. "The next one is probably not going to cure HIV, but each one is pointing us to a closer and closer direction, and we are making progress." ■

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DDN EXPLORING DRUG DISCOVERY AND DEVELOPMENT

Food for Thought

by Natalya Ortolano, PhD
Illustrated by AnnaMaria Vasco

The words “diet” and “exercise” are synonymous with weight loss. Researchers now think that “pharmacotherapy” should be too.

Although decreasing food intake and increasing exercise can help people with obesity lose weight, it often isn’t enough to help them keep the weight off long term. Drugs may be the answer for maintaining weight loss and minimizing the risk for complications such as type 2 diabetes and cardiovascular disease.

Now, scientists are developing a slew of anti-obesity drugs. These drugs target enzymes that process fats in the gut to increase metabolic rates. They also modulate the levels of hormones in the brain such as dopamine and epinephrine to curb hunger and cravings. The most recently approved drug to hit the scene, WeGovy, looks and acts like a hormone that regulates levels of insulin and appetite through its effects on the brain and the gut.

CENTRAL NERVOUS SYSTEM (CNS) STIMULANTS

CNS stimulants increase chemicals in the brain such as epinephrine to suppress appetite. Stimulants are only approved for short-term use in people with obesity (1).

FDA approved CNS stimulant anti-obesity drugs:

- Phentermine (Lomaira and Adipex-P)
- Diethylpropion (Tenuate and Tepanil)
- Phendimetrazine (Bontril)
- Benzphetamine (Didrex and Recede)

Phentermine can be combined with topiramate, a drug commonly used to treat seizures and migraines, to reduce negative side effects such as dry mouth. These drugs are combined in an extended-release pill dubbed Qsymia, which is approved for long-term use. Exactly how topiramate promotes weight loss is unknown (1).

NEUROLOGICAL AGENTS

Bupropion/Naltrexone (Contrave and Mysimba) is an FDA-approved anti-obesity drug that suppresses hunger (1).

Bupropion increases the amount of dopamine and norepinephrine in the brain.

- Speeds up metabolism
- Reduces hunger and cravings
- Also prescribed for smoking cessation and depression

Naltrexone blocks opioid receptors.

- Inhibits endorphins, opioids that increase hunger
- Also prescribed for opioid and alcohol addiction

GASTROINTESTINAL AGENTS

Orlistat prevents the absorption of fats by inhibiting lipases in the stomach, small intestine, and pancreas. Patients can acquire orlistat via a prescription (Xenical) or over the counter in a lower dose (Alli).

GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONISTS (GLP1-RAS)

Produced by cells in the intestine, GLP-1 is a 30 amino acid hormone that regulates a variety of processes involved in diabetes and obesity, including insulin secretion and hunger, via its effects on the gut and the brain (2).

The GLP1-RAs liraglutide and semaglutide are greater than 90% similar to GLP-1. Several clinical studies have shown that both GLP1-RA drugs help sustain long-term weight loss (3-4).

Liraglutide (Victoza) is FDA approved for long-term use in adults and adolescents with obesity; it requires a weekly injection (3-4). A monthly injection of semaglutide (Wegovy) was approved in 2021 for adults with obesity (5).

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To The Bone

Researchers designed a lipid nanoparticle that sticks to bone minerals, increasing mRNA delivery and therapeutic protein expression in the bone.

BY SARAH ANDERSON, PHD

Protein-encoding mRNA drugs find a home inside the cell, but getting them there is no small feat. To shuttle mRNA across the cell membrane and protect it from degradation by nucleases, researchers use tiny lipid nanoparticles that encapsulate the mRNA and release it inside the cell (1).

Administering lipid nanoparticles to the bone where mRNA can stimulate the expression of proteins that combat bone disease and injury proves equally difficult. Bones struggle to take up nanoparticles due to the blood-bone marrow barrier, reduced blood flow and vasculature compared to other organs, and low attraction to biomolecules, hindering the delivery of mRNA cargo. Methods to efficiently supply lipid nanoparticles to the bone could help launch mRNA drugs for conditions such as osteoporosis and bone cancer.

In a recent study in the *Journal of the American Chemical Society*, researchers at the University of Pennsylvania developed a lipid that sticks to bone minerals, increasing nanoparticle accumulation and mRNA delivery to the bone (2). In addition to its therapeutic potential, their work provides a new approach for directing mRNA therapeutics to evasive environments.

To help the nanoparticles cling to bone, the researchers turned to bisphosphonate. This small molecule binds to the calcium ion in hydroxyapatite, a prominent component of bone's mineral makeup. They designed a lipid that incorporates bisphosphonate, which "kind of makes the bone act like a lint brush in that the particles can collect along it," said Michael Mitchell, a nanoparticle bioengineer at the University of Pennsylvania and coauthor of the study.

The team created a series of nanoparticles from 21 unique bisphosphonate lipids and encapsulated mRNA encoding a reporter protein. In screening the nanoparticles in cells, they identified one formulation that gave higher mRNA delivery than the same particle lacking the bisphosphonate group. They also observed that this nanoparticle showed much stronger binding to hydroxyapatite compared to its bisphosphonate-free counterpart. The researchers then intravenously administered the nanoparticle to mice and found that the addition of the bisphosphonate group increased particle accumulation and protein expression in the bone.

Finally, the researchers intravenously treated mice with lipid nanoparticles carrying mRNA encoding the therapeutic growth factor BMP-2. They observed that due to its enhanced uptake in the bone, the bisphosphonate nanoparticle increased the expression of BMP-2 both on the surface of the bone and deep in the marrow relative to the standard lipid particle. The results revealed a range of possible applications for the bone-loving nanoparticles, from driving the production of regenerative proteins for fracture healing to editing genetic material in hematopoietic stem cells in bone marrow.

The study is an exciting proof-of-concept for mRNA delivery to the bone, said Blanka Sharma, a biomedical engineer at the University of Florida who was not involved in the research. As some nanoparticles gathered in the liver of the mice, off-target effects — a widespread challenge in the nanomedicine field — should be investigated, she added. "The limitation is almost always how much of what we inject systemically is actually going to where we want it to go?" she said.

The researchers plan to evaluate toxicity resulting from the particles' biodistribution and to explore alternate administration routes. "Maybe in the near future, we can try some local injection delivery methods for reducing off-target effects," said Lulu Xue, a postdoctoral fellow in the bioengineering department at the University of Pennsylvania and coauthor of the study.

As scientists strive to deliver lipid nanoparticles to several specific organs, Mitchell hopes that the strategy of integrating a binding group into the design of the lipid can be harnessed beyond the bone. "This type of chemistry can be used to incorporate other small molecules into lipids that could target other cells and tissues," he said.

Whether they are optimized to target the tibia or adapted to access other parts of the body, there's no bones about the potential of these particles. ■

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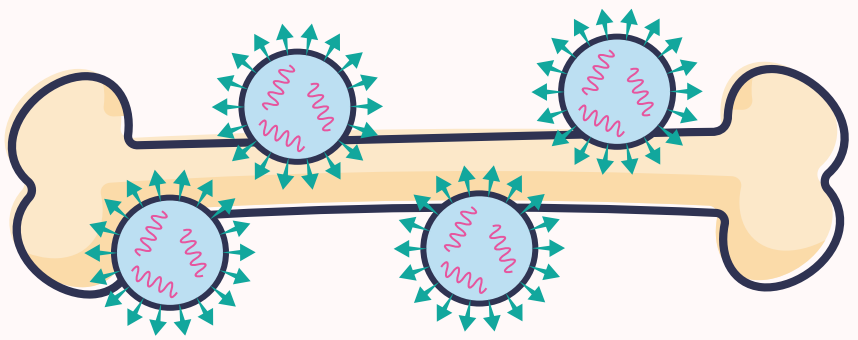

Lipid nanoparticles featuring a bone-binding bisphosphonate molecule help deliver mRNA to the bone.

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Oral Enzyme Delivery Comes Wrapped in Silk

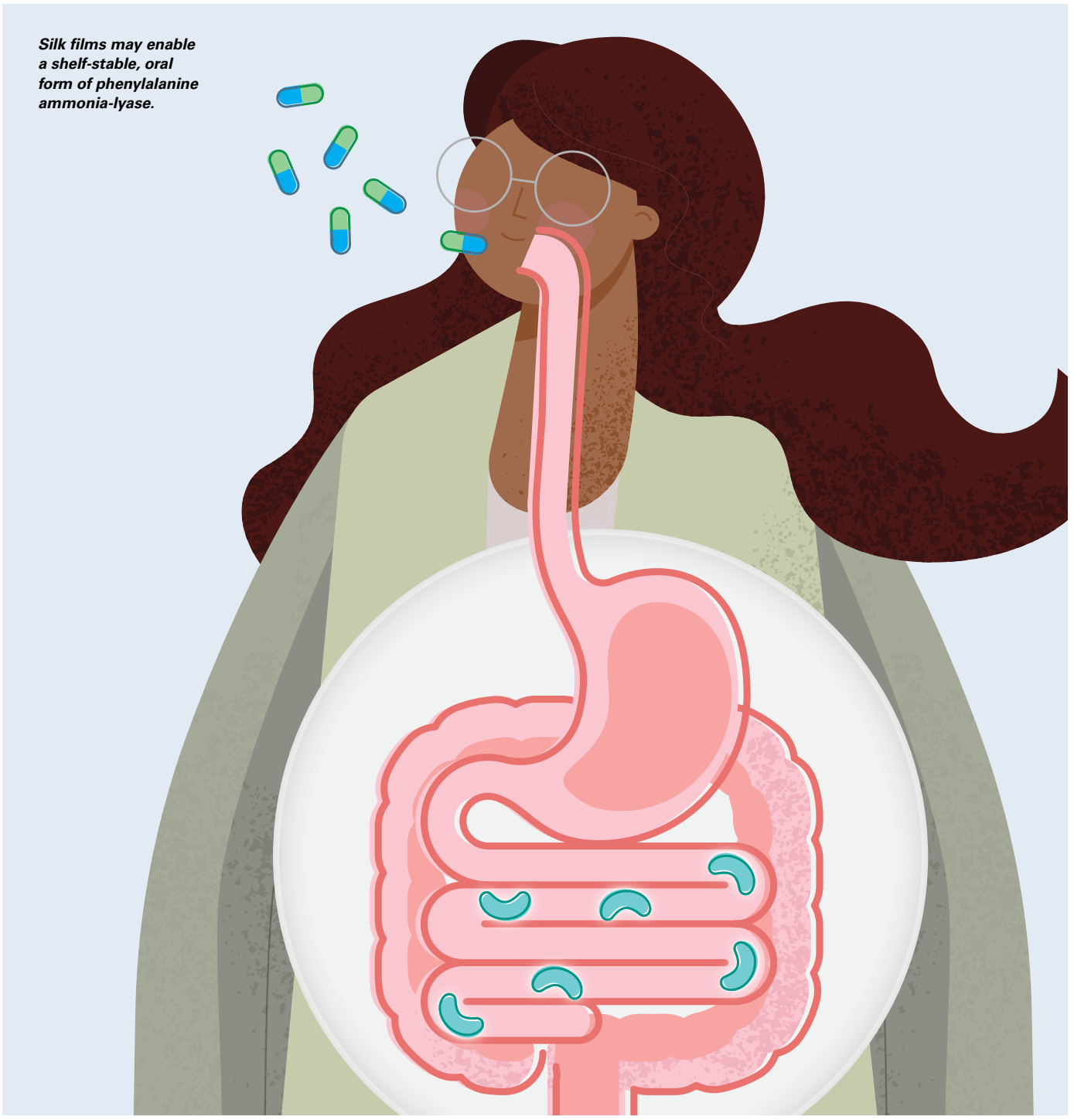
Researchers are harnessing the protective power of silk to develop a shelf- and digestive tract-stable drug for phenylketonuria.

BY SARAH ANDERSON, PHD

THE PHRASE "EVERYTHING IN moderation" applies even to essential amino acids. High phenylalanine levels due to impaired metabolism are the hallmark of the genetic disorder phenylketonuria (PKU). Scientists have hypothesized that an excess of phenylalanine disrupts signaling in the brain, resulting in developmental and cognitive disabilities.

Those with PKU adhere to a specialized diet to avoid ingesting sources of phenylalanine. They may also undergo treatment with phenylalanine ammonia-lyase (PAL), an enzyme that breaks down phenylalanine. PAL is currently administered as a daily injection, but the painful prick and injection site inflammation may cause patients to skip doses. The shot also needs to be refrigerated, restricting transportation and distribution.

While a shelf-stable, oral form of PAL would circumvent these limitations, the protein struggles to maintain its active structure at room temperature and under the extreme conditions of the gastrointestinal tract. In a new study in the journal *Molecular Pharmaceutics*, researchers at Tufts University developed a silky-smooth system to stabilize PAL for oral administration (1).



Silk films may enable a shelf-stable, oral form of phenylalanine ammonia-lyase.

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> "If you can develop strategies to stabilize protein therapeutics without the need for a refrigerator, it is a significant advantage from a cost, convenience, access, and global health standpoint."
>
> *- Matthew Webber, University of Notre Dame*

The team created films of silk fibroin from *Bombyx mori* silkworm cocoons and embedded PAL inside. "This enables silk to interact with the protein and create protective microenvironments that help to maintain the folding structure of the protein even in various conditions," said Luciana d'Amone, a graduate student in the biomedical engineering department at Tufts University and coauthor of the study. The silk is also biodegradable and does not elicit an immune response, making it compatible with drug delivery.

To assess silk's ability to stabilize PAL, the researchers measured its activity in various enzymatic samples. They first tested the shelf stability of different silk film formulations and identified a sweet spot in the relative amounts of enzyme and silk that gave maximum activity. In a long-term experiment at elevated temperature, they found that all formulations showed activity equivalent to or higher than the refrigerated enzyme after 42 days.

"If you can develop strategies to stabilize protein therapeutics without the need for a refrigerator, it is a significant advantage from a cost, convenience, access, and global health standpoint," said Matthew Webber, a chemical and biomedical engineer at the University of Notre Dame who was not involved in the study. "This technology has some promise there."

To evaluate the oral stability of the silk-wrapped PAL, the team exposed the leading silk film to simulated digestive fluids. The enzyme lost activity within hours in salivary and gastric fluid, but activity was preserved for 20 hours in intestinal fluid.

The poor stability in saliva and stomach acid indicates the need for additional stabilizing agents to enable oral PAL administration, according to Webber. "They would need to do something to protect it on the first half of the oral journey," he said. The researchers should also investigate if the enzyme can be absorbed from the intestine into the bloodstream and function systemically, he added.

The team is working on further stabilizing PAL against protein-chewing enzymes and fluctuations in ionic strength and pH in digestive fluids. "The gut is a pretty harsh environment," said Nikhil Nair, a chemical and biological engineer at Tufts University and coauthor of the study. "How do you keep things there for longer? That's a big challenge that we've been trying to think about more." One strategy is to encapsulate the silk film in coated gelatin that shields the film as it travels through the upper gastrointestinal tract and dissolves in the small intestine, Nair said.

The researchers next plan to test their platform in animal models to see if it helps clear phenylalanine that accumulates in the small intestine, and to find out if doing so provides a therapeutic benefit for PKU.

As scientists develop sturdier protein drugs for a range of conditions, they may use silk films to stabilize other enzymes against degradation. ■

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