Advancing medicine, layer by layer

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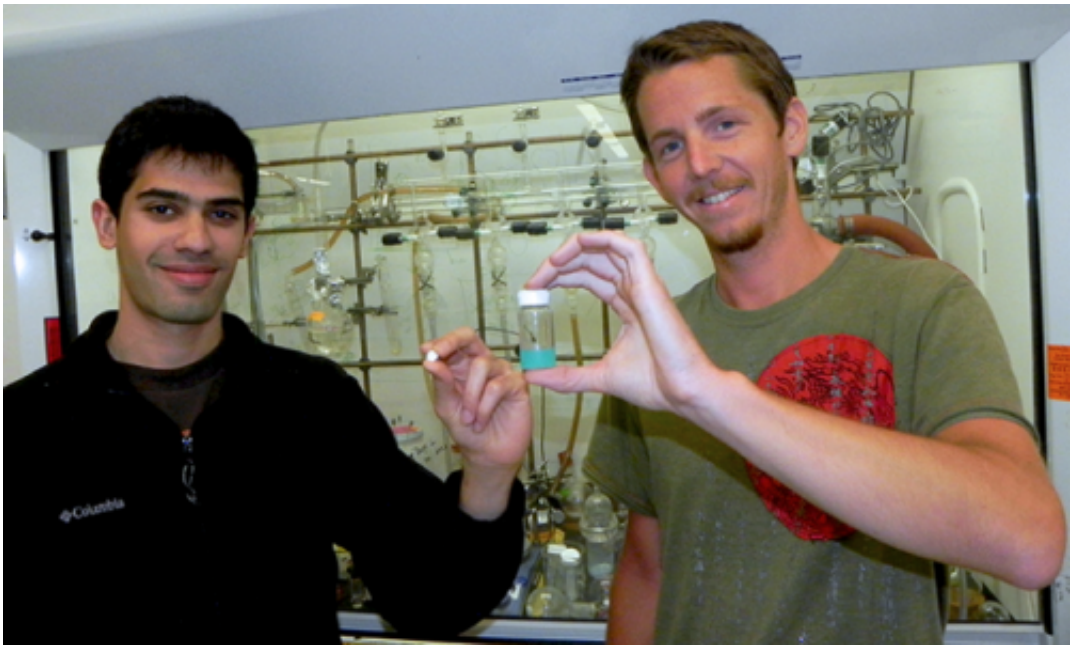

Nisarg J. Shah (left) and Stephen W. Morton collaborate on research to improve bone implants and cancer treatments. Shah holds a 3-D-printed implantable polymer scaffold, while Morton holds a jar of nanoparticles for targeting triple-negative breast cancer cells. Credit: Denis Paiste/Materials Processing Center

Personalized cancer treatments and better bone implants could grow from techniques demonstrated by graduate students Stephen W. Morton and Nisarg J. Shah, who are both working in chemical engineering professor Paula Hammond's lab at MIT.

Morton's work focuses on developing drug-carrying nanoparticles to

target hard-to-treat cancers—such as triple-negative breast cancer (TNBC)—while Shah develops coatings that promote better adhesion for bone implants.

Their work shares a materials-based approach that uses layer-by-layer assembly of nanoparticles and coatings. This approach provides controlled release of desirable components from chemotherapy drugs to bone growth factors. Use of natural materials promises to reduce harmful side effects.

"We have all of these different areas in which we are seeking to address different problems related to human health, certainly in the context of cancer research which is a very big part of the lab now," Shah says. "In addition to that we are also looking at how we can improve ways in which various patient diseases and injuries are managed in a way that will improve current clinical standards."

However it could take from five to seven years to move from preclinical success in lab animals through human clinical trials to public availability.

"Layer-by-layer allows us to introduce very specific materials on the surface of various substrates, be it a nanoparticle, be it an implant, right from the nanoscale to the macroscale," Shah explains. "We were able to introduce all kinds of different properties by depositing very specific materials on substrates, modifying their surface properties and eventually having them do very specific things in the context of applications."

Targeting hard-to-treat cancers

When delivered through time-staggered release from a liposome-based nanoparticle, the chemotherapy drugs erlotinib and doxorubicin shrunk tumors in mice, Morton and colleagues reported in a recent paper. A

layer of hyaluronic acid promotes nanoparticle passage through the body, while folate attached to their shell helps the nanoparticles bind to receptors on cancer cells. The study targeted two hard-to-treat cancers: TNBC and non-small cell lung cancer. Morton was lead co-author with Michael J. Lee in biology professor Michael B. Yaffe's group at MIT; Shah was one of several other co-authors. Both Hammond, the David H. Koch Professor in Engineering, and Yaffe, the David H. Koch Professor in Science, are members of the Koch Institute for Integrative Cancer Research at MIT.

For an earlier study, led by postdoc associate Zhou J. "Jason" Deng in Hammond's group, Morton was part of a team that demonstrated progress in fighting TNBC with a layered nanoparticle. They used biodegradable biopolymers and FDA-approved liposomes to create nanoparticles made of a drug-carrying core and an outer layer containing short interfering RNA (siRNA). The siRNA binds to a gene on the cancer cell and blocks it from producing a protein that kicks out chemotherapy drugs. Shah also was part of that team.

"We're trying to design these systems that release therapies in combination that work together in a fashion that has this enhanced benefit. We're designing these systems with a focus on materials to release them in ways that will engage a cancer cell and kill it in a more efficacious fashion, where the drugs work together and do so with a more potent effect," Morton says.

In several studies published beginning in 2011, Hammond and colleagues showed how coatings could be laid down layer-by-layer to target tumor cells and control drug release from the core. This approach has the advantage of increasing drug strength against the tumor cell and decreasing harmful side effects. In the siRNA work, Deng, Morton, and colleagues identified poly-L-arginine (PLA) as a promising candidate because it offered the ability carry a large amount of siRNA, as well as

offering film stability and low toxicity to normal cells. In the study, they estimated their nanoparticles contained about 3,500 siRNA molecules per layer with approximately 95 percent surface coating. An additional layer of hyaluronic acid gave the nanoparticles "stealth" ability to travel through blood to the tumor site in live animal studies. "The result here demonstrates that a target gene within the tumor can be effectively silenced following a single, systemic administration of siRNA LbL nanoparticles," they wrote.

Strengthening implants, improving drug delivery

Shah was lead author of several papers on the bone implant studies, showing in a 2013 *Science Translational Medicine* report that layered coatings containing bone morphogenetic protein–2 (BMP-2) and hydroxyapatite (HAP) produced stronger bonding of implants to bones in mice. Morton also was part of that team.

"In a small percentage of people, the implant doesn't bond very well with the existing host bone tissue and it causes the implant to fail," Shah explains. Significantly, the coatings promoted growth of new bone tissue directly on the implants, indicating a potential to replace the cement seam that binds current implants to natural bone. Another step that can be included in the layer-by-layer technique is adding antibiotics or antimicrobial polymers that can prevent infection.

Morton says he joined the Hammond-Yaffe collaboration after Yaffe's group had shown that administering erlotinib and doxorubicin in a staggered fashion boosted the effect of each chemotherapy drug against cancer—but when administered independently, they didn't work as well. "In free form, whenever you apply it to a biological system such as a mouse or human, the drugs get rapidly cleared and don't go where they need to go," Morton explains. "We were trying to find better ways to deliver these drugs in a way that would promote this nice synergy that

they observed in culture."

Morton made the nanoparticles himself, worked with colleagues to analyze lab cultures and conducted experiments on mice in the Koch Institute. The experiments showed tumor shrinkage in mice after 32 days of receiving the nanoparticles releasing both erlotinib and doxorubicin in time-staggered fashion. In contrast, tumor growth continued in both untreated mice, as well as mice given just a single drug, doxorubicin. The animal studies involved injecting human cancer cells into mice. A fourth-year graduate student, Morton has another year to defend his thesis and complete his doctorate.

Researchers in the Hammond lab last year developed a spray-based technique for applying layers on top of nanoparticles generated by the PRINT (Particle Replication In Non-wetting Templates) process, which was pioneered by Joseph DeSimone at the University of North Carolina at Chapel Hill. Morton was the lead author of that paper, which showed that coating the nanoparticles with hyaluronic acid functionalized them to adhere to CD44 receptors on TNBC cells (BT-20).

"Bringing PRINT and spray-LbL technologies together enables fabrication of medicine with exquisite control over particle composition, geometry, and surface properties, providing an exciting platform for large-scale manufacture of highly-controlled multi-functional particles," they report. Both the spray coat and PRINT technologies are being commercialized.

Morton and Shah also collaborated last year on a study of layered nanoparticles targeted against osteosarcoma, a form of bone cancer that has a low treatment rate. Their experiments showed tumor shrinkage, and in some cases, elimination, in mice from treatment with nanoparticles carrying a combination of chemotherapy (doxorubicin) and tumor targeting (alendronate). "To achieve this, a polyelectrolyte,

poly(acrylic acid) (PAA), was functionalized with a bisphosphonate, alendronate, and subsequently electrostatically assembled in a nano particle coating," they reported. Using clinically safe materials, mice treated with nanoparticles targeted at osteosarcoma tumor cells exhibited reduced tumor volume compared to the uncoated doxorubicin-loaded liposome control nanoparticles.

Restoring bone growth

Shah, who successfully defended his PhD thesis in May, uses the layer-by-layer technology for regenerating tissue damaged by injury or congenital defect, as well as better bonding of implants—such as in artificial knee or hip bones—to natural tissues.

"We've also looked at taking these scaffold constructs that can be put inside the body at the site of an injury," Shah says. "We've coated the scaffolds using the layer-by-layer approach, depositing one polymer layer, followed by one layer of biological drug that can induce the differentiation of stem cells that are present within the body to form cells that can start secreting very specific kinds of tissue." Once activated, stem cells can generate blood vessels or bone, and heal defects in the body.

Hammond and Shah patented some of their work and a startup, LayerBio, is attempting to commercialize some aspects of the work in bone tissue engineering and delivering drugs from bandages. Those bandages could aid diabetic patients or wounded soldiers. Shah is acting as a consultant to the company. He also will continue in the Hammond Lab as a postdoc to oversee a new project.

In the lab, Shah assembled nanoparticles, made bone scaffolds and coated scaffolds and implants using layer-by-layer technology. An important component is a polymer that breaks apart in the presence of

water, a material property called hydrolytic degradability. That allows the scaffold to dissolve naturally as new bone forms to replace it. The polymers can be modified to break down faster or slower.

The next step from a research perspective is to reproduce the results found in small animal studies of mice and rabbits and in larger animals, such as dogs or goats. "We're confident in the technology, so we know what we need to do in order to do these large animal studies to prove that ultimately we can use them in patients. This is a necessary step for any therapeutic-based approach," Shah explains.

Morton hopes there might be enough interest in the folate-decorated nanoparticles with the dual-drug combo of erlotinib and doxorubicin to jump to human clinical trials without larger animal studies. "That could be a possibility as well," he said.

Continuing collaborations with Brigham and Women's Hospital and Massachusetts General Hospital are testing the folate-dual-drug platform against tumors in mice caused by TNBC cells implanted in them. The primary cancer cells were isolated from women who've had the cancer.

"There really isn't a specific therapy for triple-negative breast cancer (TNBC)," Shah explains. One possibility might be an expedited approval process through the FDA to get the new approach to clinic even faster (perhaps two years), because there is a tremendous need for a specific therapeutic strategy for TNBC. "This would be first in class in that sense," he adds.

Morton has another year to go to complete his doctorate. Shah and Morton both work a lot with animals: They use fluorescent labeling of proteins, drugs, nanoparticles, and substrates to track what happens once they are implanted in test animals, particularly how they are distributed in the different parts of the body. "We've looked at that extensively,"

Shah says. Tumor progress, for example, is tracked using micro CT—essentially a CAT scan of the animal. The same imaging can be used to track bone formation.

Although their earlier studies didn't evaluate their nanoparticles for toxicity to non-cancer cells, one previous study of cancer in mice showed nanoparticles accumulated in the liver, kidneys, and brain. "We will be evaluating the off-target toxicity, but it's also allowed us to engage in collaborations for treating other types of diseases," Morton says. A new collaboration with a Koch Institute clinical investigator, Scott Floyd, is looking at glioblastoma, a brain cancer. The researchers will be studying toxicity and looking for genetic cancer targets in glioblastoma tumors, in order to deliver inhibitors that are specific to that cancer. "The beauty of siRNA is that you can target it to essentially any gene. You can modify the sequence that you incorporate into your siRNA, and then you can target it to whatever gene you want to shut down or control the expression of," Morton says. "In combination with traditional chemotherapeutics, for instance, you can really design a number of different combinations that are pretty powerful."

Delivering a knock-out punch

It isn't clear how long the inhibiting effect of siRNA stays active against a target cancer cell, Morton explains. "That's why these combination therapies are nice," he says. "If you can induce this kind short-term loss of protein, or whatever it is that's causing the problem, then expose it to a second drug for the knock-out punch, that may be all you need. But I think there is still a lot to be flushed out in the community as to how long different siRNAs and different gene targets are able to be suppressed."

Because no two cancer patients have the same genetic profile, they may have the same type of cancer, but with different genes driving the aggressive growth. Based on genetic screening to identify the specific

drivers for individual patients, siRNA can be engineered to target them specifically. "Our technology can deliver these drugs very well and it can do so in a way that will incorporate independently all of these different types of therapeutics for personalized medicine," Morton says.

"Certainly once that driver is identified, we can go back and design specific kinds of therapy for those patients," Shah says.

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