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CHEMICAL & ENGINEERING NEWS

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*“A motto for
my group
is that we
'explore,
discover,
treat, cure,
teach, and
repeat.’”*



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Regenerative engineering: Polymeric chemistry and materials science for regeneration

Priestley Medal address 2023

by Cato T. Laurencin, 2023 Priestley Medalist



PRIESTLEY
MEDAL PROFILE

CATO T. LAURENCIN



I'm very proud and honored to receive the 2023 Priestley Medal, the highest honor of the American Chemical Society. I am here to discuss with you regenerative engineering, which is my field. I am Dr. Cato Laurencin. I am the University Professor and the Albert and Wilda Van Dusen Distinguished Endowed Professor of Orthopaedic Surgery at the University of Connecticut. I am professor of chemical and biomolecular engineering, professor of materials science and engineering, and professor of biomedical engineering.

I work in the area of musculoskeletal tissue regeneration, relying heavily on principles of chemistry and materials science. At this point, I and my team have been able to create every tissue found in the lower and upper extremity, including bone, ligament, tendon, blood vessel, nerve, cartilage, and skin. In my talk, I will focus on my work in bone as a paradigm for the work across all musculoskeletal tissues.

But first I want to say, I am very thankful to my family for their love and support. My autobiography is entitled *Success Is What You Leave Behind*, and one of the theses behind the book is the importance of family in our lives. So to my wife, Cynthia, and my children: Thank you. I love you.

Next, I would like to acknowledge and thank Dr. Robert Langer, my science mentor and my life mentor. And I want to acknowledge and thank the late Dr. Henry Mankin, my clinical mentor, who allowed me to open my laboratory at MIT while training in orthopedic surgery.

Regenerative engineering is a field that I first talked about in a piece in *Science Translational Medicine* a decade ago, when the editors of *Science* magazine came to me and asked me to share my views about the future of tissue regeneration. In that piece, I said the future of tissue (re)generation lies in a new field called regenerative engineering, with biomaterials playing an important role.

We can define regenerative engineering as “the convergence of advanced materials science, stem cell science, physics, developmental biology, and clinical translation toward the regeneration of complex tissues, organs, or organ systems.”

Regenerative engineering is a convergence field. And we can define convergence as the coming together of insights and approaches from originally distinct fields—such as nanomaterials and nanoparticle technology; stem cell science; biophysics, such as bioreactor work; work in developmental biology—for example, how a newt or a salamander regenerates a limb; and clinical translation.

As surgeons, we’re constantly working in tissue regeneration through the procedures that we perform.

I will take you through our work on bone that I’ve devoted my life to over the course of a number of years. The work has involved polymeric matrices, polymeric micro-nano matrices, polymer-ceramic matrices, and polymer micro-nano ceramic matrices—all for bone regeneration.

BONE REGENERATION

When we first started looking at bone regeneration, the ultimate goal was to create a degradable, porous matrix that would allow bone regeneration to take place. Ideally we’d like to implant it in a location, have regeneration take

place, have complete healing take place, and then have that matrix go away. And at the time we started to explore this, we were just learning how to grow bone cells outside the body. And so when I first talked about regenerating bone, there was a bit of skepticism in terms of moving the area forward.

But I and my team were able to do it. We started with creating polymeric microspheres. We can control their chemistry to modulate their degradation characteristics. And we can engineer microsphere shape, surface, and size by controlling polymer concentration, stirring speed, and surfactant concentration.

And then when we heat these microspheres above the glass transition temperature of the polymer, we can create sintered microsphere matrices. By precisely controlling microsphere size, we can control the mechanical properties of these different matrices to allow them to be suited to be placed in different locations.

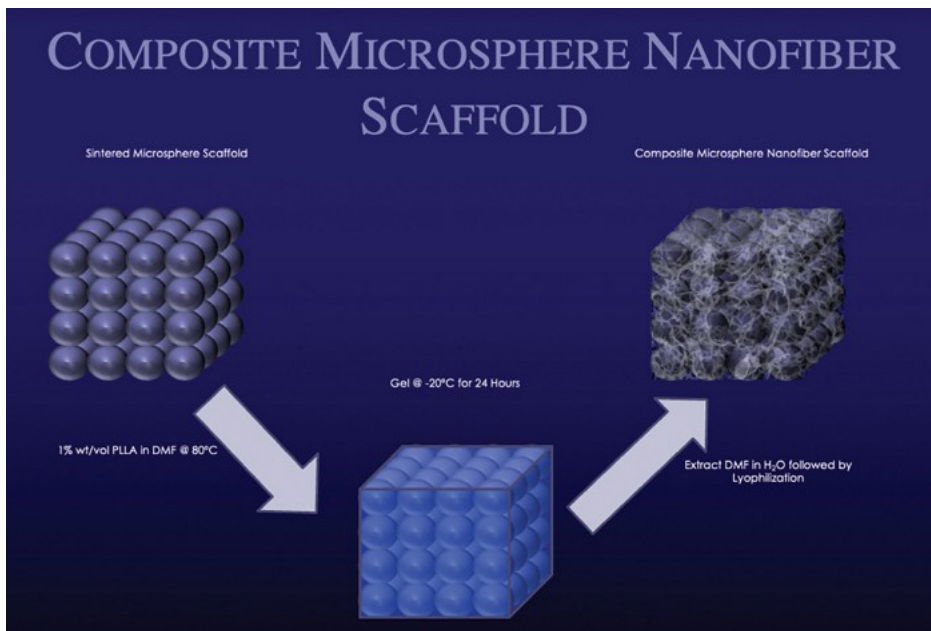
Sintered matrices can be quite versatile and can come in different sizes and shapes. In one set of experiments, we created an in vivo ulnar—forelimb—defect in a rabbit. The in vivo ulnar defect in the rabbit is an excellent model for surgical experiments because we have the radius bone that can act as an external strut. We can actually implant these without using any types of plates or screws, and we have been able to demonstrate the ability of these different matrices to regenerate bone.

Ectopic bone formation in mice Nano Matrix versus Micro Matrix



In vivo 3D micro CT scans showing ectopic bone formation using nano-and micro fiber matrices

Matrices with nanosize fibers (red, left) promote bone growth to a greater extent than microfiber matrices (red, right).



Infusing a microsphere matrix (left) with nanofibers yields a composite microsphere nanofiber scaffold (right) that fosters tissue growth.

At the same time, I and my team became very interested in nanoscale mechanisms. Now, in terms of materials, we know that the increased surface-area-to-volume ratio of a material in nanoparticulate form, or just the high surface area of a nanomaterial, provides more sites for adhesions of proteins. But overall, the nanoscale environment creates a more biomimetic environment, allowing for rich focal adhesion areas.

We were very fortunate to write the first paper showing the suitability of using nanofiber matrices for engineering tissues. And it's a highly cited paper, with over 3,000 citations. I reflected on the fact that nanofibers have a lot of physical characteristics, like collagen. And it would be interesting for us to be able to explore whether we can utilize nanofibers for the regeneration of different types of tissues. And so this first paper opened the field of using polymeric nanofiber matrices for tissue regeneration; it was published in the *Journal of Biomedical Materials Research*.

Interestingly, in 2012 the *Journal of Biomedical Materials Research* had its



It has been great to see our ability to work with stem cells and really develop the technology to allow us to be able to utilize them in a variety of different settings.

100th volume celebrating the top 25 papers, and our paper was chosen for the cover. I think this speaks to the importance and impact of the work.

Next, we decided to compare our nano matrix to our microsphere matrix. We created a mouse muscle pouch, and we placed our nano matrix in some animals and our micro matrix in others. And what we found was the nano matrix actually produced more bone than our micro matrix, as you can visualize here (shown). But we also found that the nano matrix didn't have the mechanical properties to be really suitable to be used in load-bearing areas, like the micro matrix was.

So we decided to work to get the best of both worlds. We started with our sintered microsphere matrix, and then we added a nanofiber matrix using a gel nanofiber technology method. Combined together, this created a microsphere nanofiber matrix.

And when we seed cells on these different matrices—specifically, osteoblast cells—we can compare alkaline phosphatase expression, osteopontin expression, osteocalcin expression, and scaffold calcification. The microsphere nanofiber matrix results in higher levels of expression across the board than microspheres alone.

Next, we moved to create a combination of polymers with ceramics. Now the rationale in terms of using a hydroxyapatite ceramic in our work was that it is the main mineral component of bone: it's osteoconductive, bonds directly to bone, and provides mechanical reinforcement. But at the same time, it has some drawbacks. The material is brittle, has low tensile strength, and has low resistance to impact loading. And so we were extremely interested in creating polymer-reinforced composites with ceramics as a better material for engineering bone.

The goal was to achieve the best of both worlds in terms of strength, formability, ease of use, and biodegradability. Now in our first studies, we combined our polymer and our ceramic and created our matrix, and we were initially incredibly happy. But then we placed matrices in vitro and subsequently in vivo, and we found that the ceramic component completely dissociated from the polymer component. And so we had to find another way.

So what we decided to do was to go back and directly synthesize hydroxyapatite—low-crystalline hydroxyapatite—as a part of our composite microspheres. We integrated the hydroxyapatite component as part of the emul-

sion process that we used to create our sintered microsphere matrices. And again, we sintered above the glass transition temperature of the polymer to create three-dimensional matrices for bone.

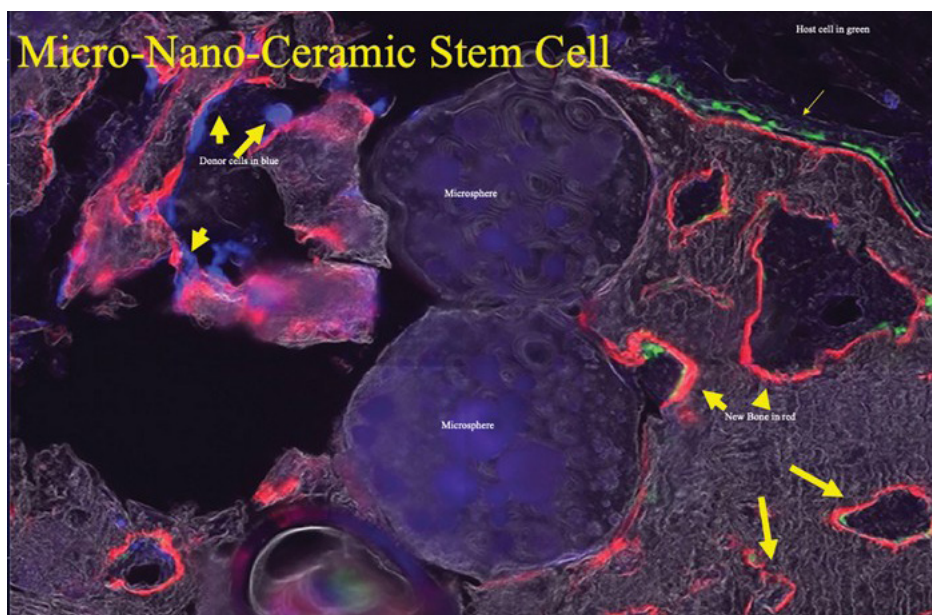
And we can actually see some nice biomechanical results based upon the hydroxyapatite concentration and the stirring speed in creating our emulsion. We can really modulate the compressive modulus and get fairly high levels of compressive modulus, depending upon changes in hydroxyapatite concentration and stirring speed.

THE ROLE OF STEM CELLS

At the same time, our work in regenerative engineering has involved stem cell technology. We can now have facile isolation and characterization of stem cells. We have adult, we have embryonic, we have induced pluripotent types of cells, and we've now developed a fourth type that we can discuss. We work with adult stem cells because we think those are stem cells for all. They can be harvested from your own body.

We've worked with human adipose- derived stem cells, and we can isolate them with an appropriate enzymatic digestion and centrifuge. It has been great to see our ability to work with stem cells and really develop the technology to allow us to be able to utilize them in a variety of different settings.

One of the areas that I and my team have explored and have really led has been the concept of inductive materials. The dogma states that one needs a



Adding stem cells to this custom-made multicomponent matrix stimulates bone formation (red).

morphogenetic factor to allow for the differentiation of cells; we and others believe that one can have direct differentiation of cells by contact with a material.

We've explored this concept using our polymer-ceramic matrix. As you'll remember, we directly synthesize our ceramic on our polymer to create a polymer-low-crystalline-ceramic matrix that we have shown to be biodegradable, formable, osteoconductive, osteointegrative—and also, we can really control the mechanical properties.

When we place these materials in appropriate in vitro and in vivo environments, the matrices are able to release calcium and phosphate. And it's the calcium ion release in these low-crystalline-hydroxyapatite-polymer composites that really drives what we see in terms of induction.

If we place adipose-derived stem cells onto these polymer-ceramic materials, we can measure osteocalcin and bone morphogenetic protein 2. What we see is that there's secretion of osteogenic markers by these adipose-derived stem cells over time, which is consistent with their differentiation toward a bone phenotype.

If we examine in vitro mineralization using low and high amounts of hydroxyapatite concentration in matrices, we can see that when we add adipose-derived stem cells with these matrices, we can get an exuberant amount of mineral formation. This is consistent with these adipose-derived cells that have now differentiated in the presence of the material with low-crystalline hydroxyapatite.

Next, we examined the matrix in vivo, implanting the polymer low-crystalline ceramic in an ulnar defect model.

What we see after 8 weeks is new bone formation being present and also rimming osteoblasts that are actually laying down new bone—again, in a setting in which we've only used our matrix plus adipose-derived stem cells.

The mechanism by which inductive effects take place using materials alone has been discussed by a number of groups, including our group and Luyten. We start with calcium phosphate-based materials that produce calcium and phosphate ions. They are able to accomplish different things. First, they are mitogenic; they cause proliferation. They regulate osteocalcin and osteopontin genes, among others. They also regulate extracellular matrix mineraliza-

tion with osteocalcin and osteopontin.

But the most exciting effect is a bone morphogenetic protein 2 autocrine/paracrine osteoinduction loop. It's not hypothetical, because we've demonstrated it. And this is where the cells themselves create and secrete bone morphogenetic protein 2, and that actually creates the autocrine/paracrine loop in which there is induction and differentiation taking place.

Next, we took our work to an in vivo mouse cranial defect model. The mouse cranial defect allows direct comparisons of different types of matrices on different parts of the mouse cranium. A parietal area defect is created. A microsphere matrix can be implanted on one side, and a microsphere nano matrix can be implanted on the other side.

We can use alizarin complexone as a quantification of the mineral formation between these different areas. And we can see that there is mineral formation on the microsphere matrix side, but there's really an exuberant amount of mineral formation by alizarin complexone on the microsphere nano matrix that we can see over time. This is consistent with what we have seen in vitro.

Next, we started to bring all these areas together. We took our low-crystalline-hydroxyapatite-polymer matrix, we created cranial defects in mice, and then examined micro and micro/nano matrices.

But at the same time, we decided to add bone marrow-derived stem cells in the matrix. We used a transgenic mouse host and donor model, where topaz (green) reported host collagen production, and cyan (blue) reported donor collagen production. So we have blue bone marrow-derived donor stem cells that are seeded onto these matrices with low-crystalline hydroxyapatite.

And this is a slide (shown) of our micro-nanofiber-ceramic stem cell matrix. Our micro-nano matrix encourages phenotypic expression, while our low-crystalline ceramic component encourages differentiation down a bone phenotypic line of our stem cells. We see microspheres that are present. New bone formation can be seen in red in different areas. We can see these donor cells in blue that are in different areas. The host collagen cells, in green, are also laying down bone. It is visually stunning to see that this micro-nano-ceramic stem cell matrix allows for great bone formation.

A motto for my group is that we "explore, discover, treat, cure, teach, and repeat." I am proud that my research and the research of my team has resulted

in so many tissues being designed and understood.

For instance, our work in soft-tissue regeneration was highlighted by National Geographic magazine in its “100 Scientific Discoveries That Changed the World” edition. The magazine highlighted the creation of what is now called the Laurencin-Cooper ligament, which has been successfully implanted in humans. And to put this in perspective, of the 100 scientific discoveries that were there, number 85 was space travel, number 15 was the cell phone. We were listed under number 30, which I think is about right in terms of the importance of the work.

Applying science and technology involving chemistry and materials science to treating the human condition has been something I’ve been devoted to. I am proud that the work has been recognized by the orthopedic surgery profession with most of its major awards, including the Nicolas Andry Lifetime Achievement Award, the Kappa Delta Award, the Marshall Urist Award, and the Distinguished Contributions to Orthopaedics Award from the American Orthopaedic Association, which included induction to the AOA Awards Hall of Fame.

But equally or more important is that the work will have impact across multiple professions. I am proud that this work and this field have been recognized across medicine, engineering, science, and technology.

In medicine, I was proud to receive the Walsh McDermott Medal from the National Academy of Medicine.

In engineering, I received the Simon Ramo Founders Award from the National Academy of Engineering. That was for fundamental, critical, and groundbreaking scientific advances in the engineering of tissues; guiding technology and science policy; and promoting diversity and excellence in science.

And I received the Philip Hauge Abelson Prize from the American Association for the Advancement of Science, given for significant contributions to the advancement of science in the US.

Two awards have been the most impactful to me to date. The first is the Spingarn Medal. The Spingarn Medal is awarded annually for the highest and noblest achievement of a Black person in America.

To put this in perspective, other Spingarn medalists include George Washington Carver, Jackie Robinson, Martin Luther King Jr., Duke Ellington, Rosa

Parks, Charles Drew, and Maya Angelou. It's given by the NAACP. I am the 106th Spingarn medalist, and I am so honored.



At a White House ceremony in May 2016, President Obama presented Cato T. Laurencin with the National Medal of Technology and Innovation.

The other award is the National Medal of Technology and Innovation. It is America's highest honor for technological achievement, awarded by the president of the United States in ceremonies at the White House.

WHAT'S NEXT IN REGENERATIVE ENGINEERING?

A key area has been our work in the development of polyphosphazenes. I want to take this opportunity to acknowledge professor Harry Allcock, who took a call from me almost 40 years ago when I was a graduate student at MIT and began a long collaboration with me. Harry, thank you. The work we have done has really demonstrated that polyphosphazenes are hugely promising as biomaterials.

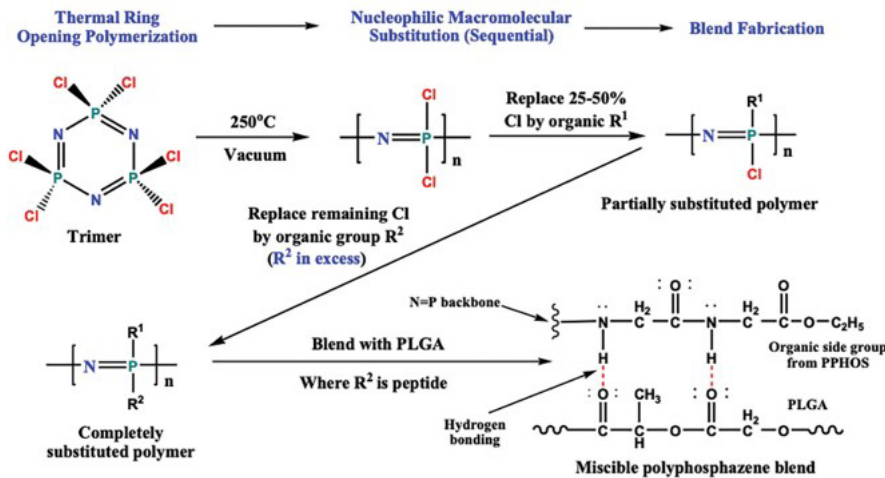
The advantages of polyphosphazenes are their design flexibility, their biocompatibility, their ability to be blended with other traditional polymeric biomaterials, and their erosion mechanisms.

We have been interested in the development of stable polymeric blends. The

steps (shown) are synthesis through a ring-opening polymerization, followed by nucleophilic sequential substitution, followed by blend fabrication. In our work, we've taken advantage of the hydrogen bonding between poly(lactic acid/glycolic acid) (PLGA) and certain degradable polyphosphazenes to achieve blends.

Approach and Theoretical Framework

Blending polyphosphazenes and poly(lactic acid/glycolic acid) produces biocompatible, biodegradable copolymers.



The glycyglycine ethyl ester has been our main side group, but we have also used a phenylalanine ethyl ester and a phenylphenol side group.

Using a mutual solvent approach, we can fabricate stable blends. We can obtain unique phase distribution and domain sizes depending on the side groups.

When we compare degradation characteristics, we find that compared to more traditional polymers, these polyphosphazenes degrade to neutral pH by-products. What is exciting is the stable blends exhibit pH degradation characteristics that are in effect buffered by the polyphosphazenes.

Using electrospray ionization mass spectrometry, we have examined the mechanism of degradation of the polymeric blends composed of polyphosphazenes and polylactic acid. Using the bulky phenylphenol group, a unique erosion mechanism occurs where the polyphosphazene component remains.

This results in the development of a dynamic pore system, with microspheres composed of polyphosphazene. This has created the exciting potential of

implanting a block of polymer that self-erodes into a polymeric microsphere matrix. The matrix degrades, creating a porous network, which results in higher amounts of bone formation.

So the polyphosphazenes represent a class of extremely versatile polymers. The design flexibility of these materials is expected to meet the complexity and ever-changing requirements of regenerative engineering. The materials have superior in vitro and in vivo performance, with high regenerative and translational capability.

The work done in the development of these novel polymeric blends, I think, played a role in my receiving the Von Hippel Award, the highest honor of the Materials Research Society.

So, what's next in terms of this area of regenerative engineering?

We are now just past the 10th anniversary of our piece in Science Translational Medicine declaring the new field. We now have a textbook on regenerative engineering.

We have a journal, Regenerative Engineering and Translational Medicine. The journal is interesting in that we have people from surgery, from chemistry, from physics, from developmental biology all working together in the field. It's a very unique journal; also, each publication includes a lay summary of the work.

We have a new NIH training grant in regenerative engineering focused on the deep integration of fields such as chemistry and biology for next-generation solutions.

And since our piece in Science Translational Medicine was published, there have been a number of symposia in the area of regenerative engineering. And I think it's important to note that these are being put on by organizations, by other institutes throughout the country, and throughout the world. So it's great to see that this field has been able to spread.

Across the country there are programs and initiatives in regenerative engineering. Jian Yang, for example, who is at Penn State, has created a new ecosystem on regenerative engineering, called Create.

We have a society, the Regenerative Engineering Society. It's a community of disciplines that are coming together across chemistry, medicine, engineering, and science, all working in the area of complex tissue regeneration and em-

bracing these concepts of convergence. We encourage laypeople to be a part of it. Membership costs \$20 for those under 20.

The principles of the Regenerative Engineering Society are convergence, responsible science, and democratization of science. Meaning that it's a society and it's also a field, where inclusion and equity are important. And finally, it's a society and field where anti-racism is important.

I want to express again how honored I am to receive the Priestley Medal. It is not lost on me that I am receiving the medal in the 100th anniversary year of its awarding.

WHERE I CAME FROM

I want to take this time to pay homage to my ancestors who brought me to where I am.

First, I am here because of my parents—Cyril A. Laurencin, and Dr. Helen I. Moorehead Laurencin, who I loved and learned an incredible amount from. My parents taught me Black excellence, my parents taught me Black resilience, and my parents taught me Black resistance.

They taught me my history. As the “Negro National Anthem” states:

Stony the road we trod,
Bitter the chastening rod,
Felt in the days when hope unborn had died;
Yet with a steady beat,
Have not our weary feet
Come to the place for which our fathers sighed?
We have come over a way that with tears has been watered,
We have come, treading our path through the blood of the slaughtered,
Out from the gloomy past,
Till now we stand at last
Where the white gleam of our bright star is cast.

It was best said by Maya Angelou:

I'm a black ocean, leaping and wide,
Welling and swelling I bear in the tide.

.....

Bringing the gifts that my ancestors gave,



I give thanks to the generations before me. They have made me unapologetically strong, powerful, and dynamic.

I am the dream and the hope of the slave.

I rise

I rise

I rise.

I give thanks to the generations before me. They have made me unapologetically strong, powerful, and dynamic.

They are part of my motivation to not only pursue overwhelming excellence in science but to tackle the systems of racism and bias that exist in this country, as described in a recent Proceedings of the National Academy of Sciences that I had the honor of editing.

These systems are often not addressed by our typical notions of diversity, inclusion, and equity. And I have described a new path forward that I have termed the IDEAL Path. IDEAL stands for inclusion, diversity, equity, anti-racism, and learning.

Anti-racism, according to Wikipedia, is a form of action against racism and the systemic racism and the oppression of marginalized groups. Being antiracist is based on conscious efforts and actions to provide equitable opportunities for all people on an individual and systemic level. People can act against racism by acknowledging personal privileges, confronting acts of racial discrimination, and working to change personal racial biases.

I'm proud to say that the American Institute of Chemical Engineers has adopted IDEAL; I hope other organizations, such as the American Chemical Society, do the same.

As I stated earlier, my autobiography was recently published, and it is entitled Success Is What You Leave Behind. One of the lessons I teach in the book is that "if you do good things, good things happen." And I think that regenerative engineering has been a good thing.

Our Regenerative Engineering Society is now a community of the American

Institute of Chemical Engineers, and I'm honored that the AIChE Foundation created and endowed the Cato T. Laurencin Regenerative Engineering Society Founder's Award.

I and the field have been recognized through election to academies across Europe, Africa, Asia, as well as all the National Academies in the US.

THE NEXT FRONTIER

We can create every individual tissue of the extremities. Our next goal is to use our convergence approach to regeneration to pursue the goal of limb regeneration.

We have announced at the University of Connecticut an ambitious goal of regenerating a limb. We call the initiative the Hartford Engineering a Limb project, and we have the goal of regenerating a limb by 2030.

We will do this with an ever-expanding and converging tool kit in regenerative engineering. And we hope to learn much along the way and create solutions for other problems that we see.



I'm excited about the power of polymeric chemistry and materials science in addressing grand challenges. People tell me this is going to be hard. But I tell my students, as Bob Marley stated, 'You never know how strong you are until being strong is your only choice.'

For instance, we know that in order to regenerate a limb, one must regenerate a joint. So much of our work has focused on repairing and regenerating joints.

We recently demonstrated a new class of stem cells—synthetic artificial stem cells (SASC), built upon polymer chemistry. We believe that the SASC may represent a new paradigm for regenerative engineering.

We know that stem cells can be a driver for regeneration through their differ-

entiation. I believe a leading role for stem cells is their secretion of biological factors that influence regeneration. So we asked: Can we channel these paracrine factors into a tailored and translatable regenerative technology?

We created the SASC and have used it in osteoarthritis treatment. First, we took a tailored compositional look at osteoarthritis treatment from the literature and picked four biological factors that have shown efficacy in treatment: insulin-like growth factor 1, transforming growth factor, human growth hormone, and fibroblast growth factor 18.

We used a double emulsion system to create microspheres containing these factors, each the size of a cell.

A coculture model was used to evaluate paracrine effects of SASC and adipose-derived stem cell treatment on chondrocytes.

We examined SOX9 gene expression after treatment. SOX9 is a master chondrogenic transcription factor. It is downregulated in an inflamed microenvironment. SASC was the only treatment to upregulate SOX9 after a 3-day treatment.

We then used a collagenase model for osteoarthritis and performed an induction in rats. And we found that both SASC and adipose-derived stem cells fostered the partial recovery of tibial moduli in rats treated for osteoarthritis.

And after 9 weeks of treatment, SASC- and adipose stem cell-treated joints were quantitatively similar to normal, healthy joints.

The polymer-based SASC has comparable anti-inflammatory and chondroprotective effects to adipose-derived stem cells and successfully attenuated osteoarthritis-mediated cartilage degeneration. We believe that SASC may represent a new dimension in stem cell therapy, providing the ability to tailor the paracrine response to a targeted tissue.

In closing, regenerative engineering is “the convergence of advanced materials science, stem cell science, physics, and developmental biology, and clinical translation toward the regeneration of complex tissues, organs, or organ systems.”

I want to thank my funders, and I am particularly thankful for the many decades of funding I have had from them.

I must particularly thank the NIH, which awarded me the NIH Director's Pioneer Award for the Hartford Engineering a Limb project.

I'm excited about the power of polymeric chemistry and materials science in addressing grand challenges.

People tell me this is going to be hard. But I tell my students, as Bob Marley stated, "You never know how strong you are until being strong is your only choice."

And let me close by once again giving thanks. To the extraordinary team at UConn working with me: the students, the staff, and the faculty.

Much of my work involves mentorship of Black students, and particularly Black men, who are endangered in America. I thank you for your commitment to excellence and the commitment I've seen by you to pay it forward.

I am happy to announce that the University of Connecticut launched a new institute, the Cato T. Laurencin Institute for Regenerative Engineering. Its pillars are overwhelming excellence in science; anti-racism and justice, sponsorship and mentorship; international, national and community action; transformative technologies for humanity; and entrepreneurship and economic value creation. We look forward to having further impact on the world.

And finally, I need to give sincere thanks to Dr. Lakshmi Nair, Dr. Yusuf Khan, and Dr. Kevin Lo. They were my students, and now they are my colleagues and friends. They have been with me collectively for almost 60 years and have made multiple moves with me in my career. I am grateful for their brilliance and their loyalty.

Let me once again thank the American Chemical Society for bestowing upon me the 2023 Priestley Medal. Thank you.