

Rocky Tuan uses nanofibers, which mimic a cell's natural environment, as a structure to encourage and support the growth of adult stem cells into tissue like cartilage.

COURTESY R. TUAN

"LOOK AT IT FROM THE CELL'S POINT OF VIEW"

ROCKY TUAN PERSUADES JOINTS TO FIX THEMSELVES

BY JOE MIKSCH

Children are naturally inquisitive. "Mom. Mom. Mom. Why is the sky blue?" and all that sort of stuff.

Sung Chi Tuan was no different. As a tyke growing up in Hong Kong, he was full of questions. Often he'd take it upon himself to find the answers.

His father had a few wristwatches. On each was engraved the number of jewels contained in each timepiece. In watch parlance, a jewel is a little pivot used to reduce friction between all the moving parts that help keep time.

Young Master Tuan did not know this.

"So I would take the watches apart to find the jewels," Tuan says with a hint of decades-long disappointment tinting his words.

Although he found no multicarat diamonds or deep red rubies, Tuan admits that he did learn something about watches and, in retrospect, something about science: You don't get the right answers without asking the right questions, and, at times, desired outcomes can elude even the most prepared inquisitor.

But the most important lesson, Tuan says, is to keep asking questions.

Today, Tuan—now known as Rocky—is the founding director of the University of Pittsburgh School of Medicine's newly established Center for Cellular and Molecular Engineering in the Department of Orthopaedic Surgery. He is also professor and executive vice chair for orthopaedic research.

He and his wife, Cecilia Lo, both left positions at the National Institutes of Health (NIH)—he as chief of the Cartilage Biology and Orthopaedics Branch at the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), and she as principal investigator in the Laboratory of Developmental Biology at the National Heart, Lung, and Blood Institute—to come to Pitt.

Lo, a PhD, is the founding chair of the new Department of Developmental Biology in the School of Medicine.

She says that her husband's move from the NIH to Pitt was predicated on his desire to have a closer set of clinical collaborators. "The NIH is huge and has a lot of scientists, of course, but not as many tissue engineering personnel, and they don't have an orthopaedics department. He had collaborators at Georgetown and George Washington University, but in terms of having close contact with clinicians, that wasn't there."

Tuan's boss at NIAMS, director Stephen Katz, wasn't glad to see him go, of course, but believes that Pitt orthopaedics did pretty well for itself in recruiting Tuan. "Rocky is a tremendously enthusiastic scientist. He really has a 'can do' attitude," Katz says. "He is one of those rare individuals who makes the biology and engineering come together."

Tuan, as of fall 2009, was still getting situated in his brand-new digs at 450 Technology Drive, across the Monongahela River from the South Side. After making coffee—a process that led to a brief digression on the explosive potential of some kinds of nondairy creamer—and a pause to advise a couple of physical plant workers on where to hang a paper-towel dispenser, Tuan retired to his undecorated and mostly empty office, evidence of his recent arrival at Pitt. As construction workers banged about in the as-yet-unfinished building—the sound would be a companion throughout the conversation—Tuan put his 30-plus-year career in perspective.

After studying at Swarthmore College, Berea College, and Rockefeller University (where he earned his PhD in biochemistry and cell biology), Tuan has established himself as a premier tissue engineer cum stem cell biologist. He brings with him from the NIH a research portfolio with one major thrust: Use the body's own regenerative power—augmented with biomaterials, nanomaterials, and/or stem cells—to make musculoskeletal tissue exactly as the body would.

"The ultimate goal is cell-based therapy or cell-based tissue regeneration," Tuan says. "But in order to regenerate tissue, you need to understand the building blocks, the cells—they're the real tissue engineers. We're not; we're cheerleaders." He pauses and adds, "You've got to look at it from the cell's point of view."

Getting to a cell's eye view of things started at Rockefeller and required two catalysts: Aristotle and a chicken.

Tuan recalls arriving at Rockefeller not entirely sure of what he intended to study, a fact that made the program and him well suited for one another. As he describes it, Rockefeller wasn't a traditional pedagogical situation: "It was very unconventional. There were 400 faculty and only 20 students per class," during Tuan's time, the early 1970s. "There really weren't classes—we would go around to laboratories, and they'd challenge us to come up with something."

After a time, Tuan opted for Zanvil Cohn's lab. Cohn, a cell biologist and immunologist, who died in 1993, was expert in the function of T cells and macrophages, using his knowledge to investigate leprosy, tuberculosis, and AIDS. Yet, even after choosing the broad area in which he would apply himself, Tuan still wasn't sure of his scientific niche:

"So [Cohn] said, 'Why don't you disappear and come back and talk to me after a month?'"

Tuan holed up in the Rockefeller library—"It was open 24 hours; you could sleep there"—and just read.

"I read a lot," Tuan says. "Old stuff, new stuff, some Greek literature … whatever. In a sense, I was looking for a topic that related to improving quality of life but could be approached using modern technology."

Though he can't remember the title, Tuan says he came across a tome whose author argued that Aristotle was the world's first embryologist. Aristotle, the writer says, wrote a treatise on the egg. How, the grand old philosopher asked, could something with an oblate spheroid shape and hard shell give rise to something feathery, fleshy, bony, and beaky? The question, then, was not, "Which came first, the chicken or the egg?" but, "How the heck did an egg make a chicken?"

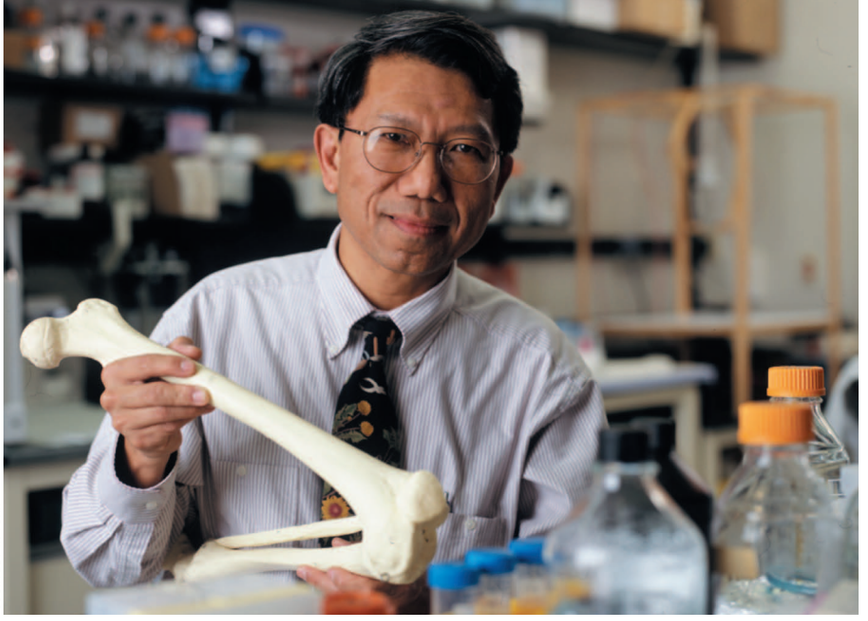
Aristotle didn't get all that far in answering the question, but he did note that all the matter that became a chicken had to be present in the egg. Tuan took it upon himself to move the ball forward; upon returning to Cohn's lab, he had decided to pursue how the calcium that makes up an eggshell becomes chicken bone.

"My boss said, 'What?,'" Tuan recalls. Cohn then thought of his med student days when he worked with eggs and viruses and told Tuan, "Sure, go ahead." (Apparently, chickens aren't the only things that spring forth from eggs—inspiration does, as well.)

In his resulting PhD thesis, Tuan established the manner in which calcium is transported from the shell to the embryo. He was the first to do so, which, naturally, was exciting and prompted a whole new series of questions centered on bone.

"I realized that the skeleton has to repair itself, that it's constantly undergoing turnover. Ossification is an active process. I asked myself, 'How is this happening and why?' I started thinking about stem cells, precursor cells, progenitor cells," Tuan says. "That's how I got into [orthopaedics]. I don't think I ever set out with any sort of business plan—year one, do this; year two, do that—I've just followed my nose pretty much.

"Remember early attempts at building airplanes? They tried to make them look like birds, but mechanically it didn't work. When the Wright Brothers built their airplane, it was totally different from the way a bird flies. It's got wings, but it's nothing like a bird."



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Tuan hopes to use biology and engineering to save joints, like the knee above, from the ravages of osteoarthritis. He also studied the skeletal development of chickens with an eye toward understanding how the process is biologically regulated.

"I've become very interested in repairing, regenerating, and engineering new tissues, particularly [those] related to the skeleton, and it all started with looking at how this calcium business works," he says.

Lo says her husband's tendency to yield easily to curiosity and grasp opportunity—as manifested in the Aristotle, egg, chicken, and calcium transport equation—was evident even when he was an undergrad. "He loves music, which he didn't have the opportunity to study when he was growing up," she says. "When he went to Berea College, he was in work study at the music library and listened to all kinds of music. He got his own musical education. His taking advantage of chances is a character trait."

That spell as a musical autodidact led to Tuan performing with the Tanglewood Festival Chorus in Boston. "He has a nice baritone voice," Lo says. Will he do the same with the Pittsburgh Symphony's Mendelssohn Choir? "Maybe. He hasn't exactly had a lot of time as of late," she notes.

As Tuan gets his Pittsburgh lab up to speed, he's no longer particularly interested in chickens; he's more concerned with how to create cartilage, tendon, and ligament tissues to replace those ravaged by injury or age. When these tissues are injured or worn by overuse, the result is often osteoarthritis—a painful, chronic, and difficult-to-treat condition that affects some 27 million Americans, according to the NIAMS.

At present, osteoarthritis is managed through pain medication, physical therapy, and, in severe cases, surgery to repair the damaged tissue or replace joints. The problem with surgery is that the mended tissue—scar tissue, essentially—cannot bear the same weight as the native, healthy tissue. Further, it has a tendency to erode over time, necessitating follow-up procedures. Replacement joints, as sophisticated as they have become, don't hold up well over time.

Tuan says we can do better, and we can do so by mimicking nature.

Making man-made, or rather, "man-assisted" tissue in vitro from adult stem cells, particularly mesenchymal stem cells that are drawn from bone marrow, muscle, or fat, is the easy part—not that it's particularly easy. The real difficulty arises, Tuan says, in making something that looks like muscle, cartilage, or a spinal disc function like muscle, cartilage, or a spinal disc.

"The mechanical properties are lousy, but it's a beginning," Tuan told *Wired* magazine in 2008 of his earlier efforts.

To improve function and make the new tissue as good as the old, Tuan has decided to give these stem cells something of a kick-start. With knowledge about how cells differentiate during embryonic development—what growth factors play which roles at what times—Tuan is capable of turning mesenchymal stem cells into what he wants, or at least a fair simulacrum of it. But this is clearly not enough.

"This is a little different from the classical engineering approach of 'Oh, something's missing, I'll just build something that looks like it,'" he says. "In the body, it doesn't quite work that way. Remember early attempts at building airplanes? They tried to make them look like birds, but mechanically it didn't work. When the Wright Brothers built their airplane, it was totally different from the way a bird flies. It's got wings, but it's nothing like a bird."

Tuan's challenge, however, is a bit different from what Orville and Wilbur Wright faced as they, in effect, cheated their way around the avian model to achieve flight. Tuan has got to build tissue that not only looks like what it's intended to replace but also essentially *is* what it's intended to replace.

To ease the transition from stem cell to tissue, Tuan decided to build a template. In normal human development, embryonic stem cells, as they differentiate, assemble themselves into heart, bone, muscle, cartilage, etc. This is easier for them because, as Tuan says, embryonic stem cells are much more adept at becoming whatever they need to become. Adult stem cells are less malleable, but still open to influence and less likely to proliferate out of control.

"An embryonic stem cell is like a kindergartner—it doesn't know very much, but it can become anybody as long as it's taught the right things," Tuan says. "Adult stem cells are more like high school graduates. They've already learned a few things but have developed some bad habits. Still, though, you can influence them by putting them with the right friends."

Their best friend, in Tuan's metaphor, is a scaffold matrix. Without a space-filling guide the mesenchymal stem cells can glom onto, there's a high risk that they'll be swept away before they can become tissue. "Unless you can make cells happy," Tuan adds, "you're not going to have the right kind of tissue in the end. What do cells like? Well, they live in a matrix that they make themselves." Logic then dictated to Tuan that he try to make a matrix as close in appearance, structure, and function to the original matrix as he could.

In high-resolution microscopy, an "original matrix" appears as a bunch of nanometer-

scale fibers. Tuan takes a liquid polymer and, using a technique borrowed from the textile industry, spins the stuff rapidly in the presence of a strong electrical field. As the polymer attempts to diffuse the charge, it forms into—you guessed it—a bunch of nanometer-scale fibers.

"The cells love this!" Tuan says. "They think they're at home. It's assisted development. Otherwise it might take them a while to put all that stuff [the matrix] together. Now that we gave them the stuff, they can decorate it with their own molecules."

The scaffold can also be treated with other molecules that help attract mesenchymal stem cells and induce them into turning into what Tuan wants them to become. "We can attach those [biologically active] reactive groups to the fibers according to whatever we want," Tuan says. "What we have done now is load these fibers with very small molecules, and we can add hormones or growth factors. Let's say you have osteoarthritis; you can add drugs to treat it. The goal is to use these fibers for structural tissue regeneration and for therapeutic applications."

Tuan recently completed a trial of the scaffolding involving injured pigs. In the trial, researchers sutured cell-laden scaffolds over damaged cartilage in the pigs' knees. Within six months, new cartilage had formed. What's most encouraging to Tuan is that the new growth not only looked like what it replaced but had mechanical properties similar to the native cartilage. Tests on goats are on the horizon and will probably be completed within two years. After that, Tuan says—human clinical trials.

As this work progresses, Tuan is also looking to learn a bit about regeneration from soldiers with blast injuries that necessitate amputation. The muscle at the end of the injured limb—where a prosthesis would attach—often becomes bone as it heals. This unwanted transformation can occur in the time it takes for a soldier to be transported from the battlefield, to a U.S. military hospital in Germany, to Walter Reed Army Medical Center in Washington, D.C.

It turns out that the wound site attracts stem cells at up to 4,000 times the density found in bone marrow and much, much higher than found in normal muscle tissue. This overzealous attempt at healing is likely what causes bone to form, but Tuan has yet to sort out why the stem cells make this error in differentiation.

Some good, though, has come out of this problem. These extra stem cells, Tuan found, also have the ability to induce nerve growth. He suggests that perhaps these cells can be harnessed to induce peripheral nerve repair. "We're not too far along on this," Tuan says, "but it's a work in progress for sure."

Tuan's not alone in Pitt orthopaedics when it comes to regenerating tissue, particularly cartilage. His colleague Constance Chu mines a similar vein. Chu, the Albert Ferguson Associate Professor of Orthopaedic Surgery and director of the Cartilage Restoration Center at Pitt, is preparing to test her work on horses.

An MD, Chu recently received a $1.7 million Grand Opportunities grant from the NIH for her project "Multicenter Cartilage Repair Preclinical Trial in Horses." She's collaborating with investigators from Cornell University, Colorado State University, and the University of California at San Diego.

Chu also works with bioactive scaffolding and bone marrow stem cells, but her Grand Opportunities grant takes another approach: "What I'm doing I call 'back-table tissue engineering.' Basically, we're aspirating bone marrow from horses and concentrating stem cells and clotting factors by centrifuge in the operating room." This material can lead to tissue regeneration once implanted in the knee.

While Chu admires, and shares, Tuan's approach, she thinks lab-engineered cartilage may take longer to reach the clinic than her "back-table" work. "With gene therapy or nanotechnology or synthetic polymers, there are major hurdles regarding proving safety and efficacy," she says.

If the horse trial goes well, Chu plans to pursue clinical trials in humans within two years.

Tuan sees a lot of promise in Chu's work. In the meantime, he's going to keep busy with his scaffolds. "When you're dealing with tissues whose main function is to bear weight, I think it's good to have a scaffold that can take care of that aspect of it," he says.

Questions remain, of course. Tuan thinks it's vital to develop a deeper understanding of how cells communicate with one another while they construct new cartilage or tendon as well as how a cell's environment in the body influences its function.

"This goes to a very fundamental question about the niche of a cell. This is a very hot topic right now in the stem cell field. 'Niche' is just a fancy French word for microenvironment," he adds, digressing to say, "Although 'environment' is of French origin as well … the French know everything!"

Becoming serious, he continues, "The niche is defined as the environment that maintains the stem cell in a stem cell state while allowing it to differentiate upon induction. We need to know what all the environments the cell deals with are and tease them apart to understand what cells do when stretched or subjected to the pressures that go along with being in a ligament or tendon."

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Freddie Fu, David Silver Professor and chair of the Department of Orthopaedic Surgery at Pitt is confident his new recruit will find the answers he seeks. "I've been following his career for 20 or 25 years now, even before he was at the NIH, and I think he's leading the world in this biological approach to the problem," Fu says. "It's a solvable problem, and I think he's going to find a solution."

Tuan says he's glad to be at Pitt to pursue this stage of his work.

"For the kind of work I am interested in, Pittsburgh is a powerhouse," Tuan says. "I don't have to come in here and build anything, which is actually nice. Here, science is truly multidisciplinary, and I think it's a lot more fun when you have different kinds of people thinking about these problems. It's great to be in a place with that kind of interaction."

Well, Tuan concedes (as construction workers drill and hammer nearby), he *does* have to build something—cartilage, muscle, tendon. After all, that's why he uprooted his lab and came to the banks of the Monongahela River. ■