the matrix, reloaded

Dan and Agneta Simionescu found magic in the matrix, and it's helping them fashion a new way to heal.

by Neil Caudle

Dan and Aggie in college.

It was late, and snow flurries danced in the empty streets of Bucharest. Dan and Agneta had finally finished their work for the day—a liver-enzyme assay that had kept them in the lab until midnight. They were two Romanian undergraduates, studying biochemistry. They were falling in love.

More than three decades later, they remember that night when the world was alive with so many swirling possibilities. The world is still alive that way for them. As it is for their students. As it is for the surgeons who volunteer time to be part of their research. As it is for those who hear the story of their work.

This story is a romance. It begins with two students who fall in love and learn, together, how to mend a broken heart.

To get started, let's go back to Romania, during the early 1980s. The newlyweds were working side by side in a lab in Târgu Mures, in the heart of Transylvania. On the bench was a heart valve constructed from bovine pericardium, the sack-like tissue that covers the heart of a cow. Their boss, Radu Deac, a cardiovascular surgeon, had recruited Dan and Aggie Simionescu, offered them jobs and a lab and a house, because he believed they could help him save lives. Many of his patients needed heart valves, and Deac did not have enough valves to give them.

"He would send them home, and they would die," Dan recalls.

In those days, he says, heart-valve replacements were new on the market, and very expensive, and the Romanian government wasn't buying enough of them to meet the need. Deac had seen, in his travels, a new type of heart valve, designed by another Romanian, Marian Ionescu, that seemed to have promise but with room for improvement. "I know how to make these valves out of cow tissues," Deac told Dan and Aggie, "and you can help.'"

So the Simionescus, educated as biochemists, began to learn, on the job, the new skills of biomedical engineering. The work was so consuming that they could almost forget, during the long days and nights in the lab, the heartbreaking hardships of Romania in the 1980s.

Deac, stringent and exacting, continuously altered the design as the team tested and tailored each valve. "We had made for him, in the machine shop, a little device to test the valves," Dan recalls. "We would wait for him in the evenings, and after ten hours of surgery he would come into the lab, and he would mount the valve we had made during the day, and he would test it individually, by hand, to see how it functioned. After stringent quality control, maybe one in ten valves received the stamp of approval and were prepared for implantation."

Aggie still remembers very clearly her first glimpse of Deac's patients. "I went into the hospital to meet with him, and I saw some of the patients in their pajamas. I felt a bit unwell, because I was very young and I had never been in that part of the hospital before. I realized for the first time who we were working for, and that this was a huge responsibility."

Each day, the couple worked as though the patients watched them, waiting and hoping. Gradually, the tweaking and testing paid off. The heart valves were working; patients were going home to live their lives.

"It took us about five years to prepare those valves," Dan says, "and we made about a thousand of them. So our careers started by saving a thousand patients."

Learning from the failures

The Simionescus spent another five years helping Deac develop a method for repairing valves with bits of tissue snipped from the patient's own pericardium, the sack around the heart. Meanwhile, a few of the patients with artificial valves implanted before 1980—twenty or thirty of the one thousand, Dan says—returned to the hospital because their replacement valves were failing. The team tried to learn from these failures and improve the new valves, a line of research that became the basis of Dan's Ph.D. project.

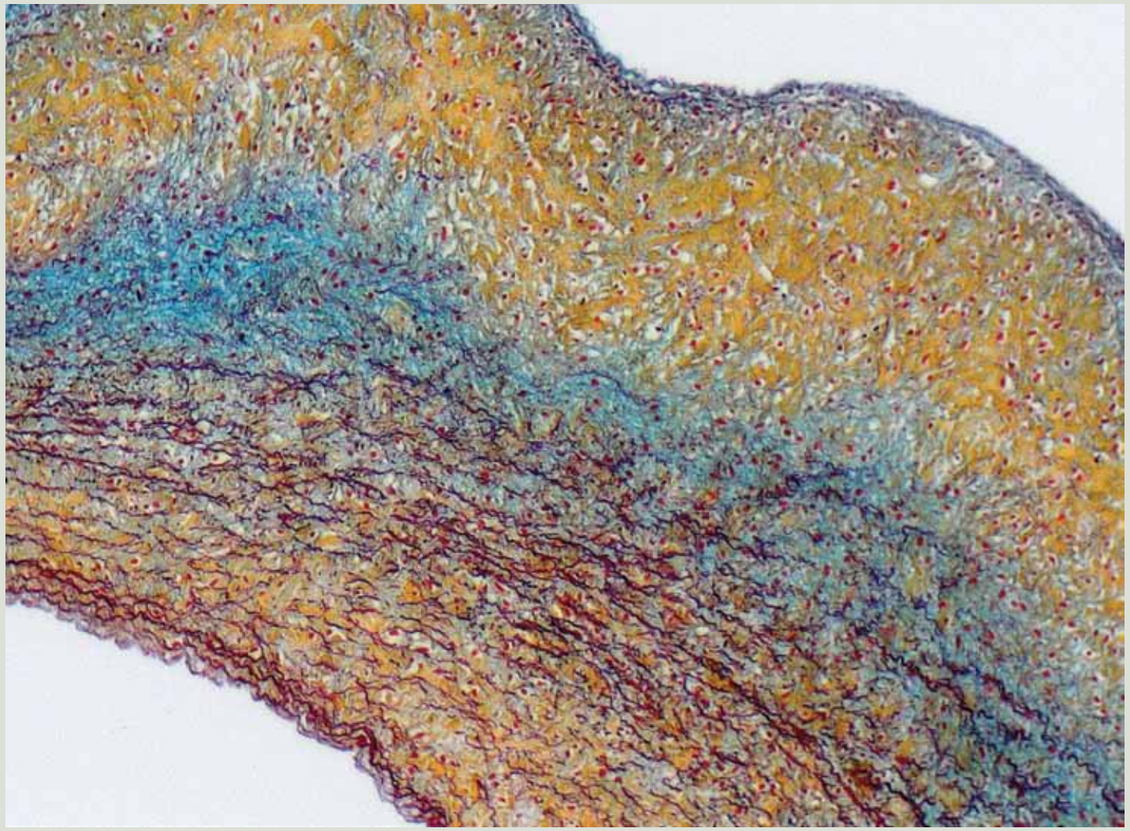
"By finding out how they failed, we realized that there were ways we could make them better," he says. "The heart valves failed because they started to calcify. You could find real stones that were built on them."

Before they calcified, the failing valves would begin to deteriorate and thin out. Aggie, conducting research for her Ph.D., discovered that certain kinds of enzymes were degrading the tissue.

The main trouble, the couple realized, was that the heart valves they'd been making relied on tissues with dead and dying cells—the best available option at the time. In those days, the team treated the tissues with a chemical that would prevent rejection by the human body. The chemical killed the cells, but the tissues remained strong and intact, working fine for years with very slow degradation. "Once the cells were killed," Dan says, "they became little points where calcium started to be deposited, and it grew there, like a crystal."

Radu Deac (right) with Dan and Aggie at a conference in 1997. In the 1980s, Deac hired them to help him make heart valves. They still work with surgeons, to stay focused on the needs of the patient and the realities of the clinic.

Simionescu lab



This section of heart-valve tissue has been stained in five colors to highlight various components. Purged of living cells, what's left is a matrix, a structure of mostly collagen fibers. The Simionescus populate the matrix with stem cells and grow new tissue.

Patterns in the flow

So these heart valves, which had saved so many lives, were not the final answer. By the time the Simionescus found their way to Clemson—Dan in 2001 and Aggie a few years later—they were ready to try something new. They brought with them several old habits worth keeping. For one thing, they would continue to work as a team, stronger together than apart. They would continue to collaborate with surgeons—people who, like Deac, understood what patients needed. And they would work with the patient in mind.

A true romance requires more than attraction and common interests. It is a daring adventure into the future, into big, ambitious dreams. In their new country, Dan and Aggie Simionescu began to pursue a big, ambitious dream.

"Imagine," Dan says, "that one day you could go to the hospital and have your own stem cells collected from you as a patient and that your own cells could be used to regenerate a new heart valve, new cartilage, or new tendon, and the surgeon could implant the new part in you. It would be yours, made of your own living cells. This is the future. And this is what we are doing."

The dream is on the verge of coming true. Which brings us to the science.

The heart from Snow Creek

Romance is not always a matter of moonlight and roses. Sometimes it requires the services of a slaughterhouse, where the heart of a pig goes on ice.

Each year, the people from Snow Creek Meat Processing in Seneca, South Carolina, take a field trip to campus, to see for themselves what goes on with the products they pack up and send to the lab. Take the pig heart, for instance. Dan will make use of its valve. His students will cleanse it with detergents, wash away its cells, and remove every trace of its pigness—proteins the human body would reject. What's left when the cleaning is finished will be an empty framework, neutral and inert—a well-ordered absence of life. Call it a matrix, a lattice, a scaffold. It is a weave of tough collagen fibers, a netting that holds life together, for any sort of animal, including us.

Nature has a frugal way of reusing a structure that works, handing it down from species to species over millions of years. When that happens, biologists say the structure is well conserved. The extracellular matrix and its collagen are well conserved. Humans have it, and so do the critters around us. In the matrix, at least, we are one.

Simionescu lab

Dr. Fred Nelson performs brain surgery on a rat at Clemson's Godley-Snell Research Center. With his help, the researchers are testing implants for replacing stroke-damaged brain tissue.

Surgeons help guide the research

Lots of research programs show promise, but not many are so promising that ten busy surgeons volunteer their time to contribute. That's the case with the tissue-regeneration studies led by Dan Simionescu.

"What Dan and his team are doing is incredible," says Dr. Chris Wright, a thoracic surgeon and chief of medical staff affairs for the Greenville Health System (GHS). "More than anyone I know, he has bridged that gap from basic science to application, and it's really going to pay off for patients. I'm convinced of that."

Wright began working with the research team about five years ago, after he attended a symposium where Simionescu presented his work on a vascular conduit. Simionescu introduced him to his student, Lee Sierad (see the story on page 20), and Wright decided to help Sierad develop and test regenerated heart valves. He serves on Sierad's thesis committee and guides the clinical aspects of the research. Wright enjoys working with the students, he says, not only because he can help them understand the clinical applications of their work but also because they teach him engineering.

"I really think that the technology and their approach will develop a heart valve different from anything we have now," Wright says, "because it will be dynamic and will grow with the individual. It's not an artificial valve; it is truly a replacement."

Wright's colleague at GHS, Dr. Fred Nelson, a neurosurgeon, works with Natasha Topoluk (see the story on page 19) to regenerate brain tissue damaged by stroke. On the Clemson campus, Nelson surgically implanted scaffolds seeded with stem cells into lab rats disabled by stroke, and Topoluk assisted. The team spent twenty-five hours in the animal lab over three days. The procedures were microsurgery and very exacting; there's not a lot of room to maneuver in the head of a lab rat. Nelson laughs. "Why they couldn't use a New York subway rat, I don't know."

After the procedures and a recovery period, "there was marked improvement on the functional scale," Nelson says about the rats that received the implants. "I think this is a promising possible treatment for stroke."

What motivates him to devote so much time to research? In addition to the potential to help human patients, he finds it rewarding to work with students and academic researchers, he says. "I think the science is fascinating, and I like the people."

"I really think that the technology and their approach will develop a heart valve different from anything we have now. It's not an artificial valve; it is truly a replacement."

–Dr. Chris Wright

Many of the projects mentioned in these pages are patented or patent-pending technologies available for licensing and are managed by the Clemson University Research Foundation (CURF). CURF is a nonprofit corporation that facilitates the transfer of Clemson University's intellectual property to the private sector for commercial development and societal benefit. For further information on these or other Clemson technologies, please visit www.clemson.edu/curf or email contactcurf@clemson.edu.

And so when the matrix is empty, our stem cells can move right in, like the next round of guests at a freshly cleaned hotel, without fear of rejection. *Mi casa, su casa,* the matrix says. Nothing lifelike remains there. There are no antigens to provoke an attack from the body. There are no dead cells for enzymes to degrade, no dead spots for calcification. And the stem cells make themselves at home.

Stem cells. The term may still carry baggage, for some. Not very long ago, people argued the ethics of using a particular kind of stem cells, those from human embryos. As it seemed at the time, embryonic stem cells were medical science's best hope for regenerating tissues and organs. But over the last decade or so, scientists have learned that several types of adult stem cells, which are not from embryos, are also "pluripotent." They can morph into multiple cell types and help generate many kinds of tissues.

Conveniently, a vast number of these pluripotent stem cells are stashed behind our bulging waistbands, in the fat below the surface of our skin. "Sometimes," Dan says, smiling, "it's good to have a little fat." (Even though he, by all appearances, is lacking.) One day, he says, our fat might save our lives—assuming we don't overdo it. (More about this later.)

If stem cells are actors waiting for their turn on stage, the fat below our skin is a cushy kind of green room. On cue, our stem cells come racing to the rescue, transforming themselves for the roles they are called on to play. For their research, Dan and Aggie can buy the stem cells they need from companies that extract them from fat removed during liposuction. But in the clinic, a surgeon would harvest a bit of the patient's own fat, and its stem cells, through a small incision.

"From a piece of fat the size of a walnut, we can get millions of stem cells," Dan says. "If you amplify them in the lab, you can get twenty million, a hundred million—enough to regenerate a small piece of tissue."

Dan and Aggie say that their collaborators, Jeff Gimble and Bruce Bunnel of Tulane University, have provided invaluable expertise on adult stem cells. "Every day we learn new things about the adult stem cells we find in our bodies," Dan says.

The magic in the matrix

But tissue generation is not as simple as dosing an injury with stem cells. If a wound is massive, stem cells cannot find the remnants of structure they need to begin the repair. They float around and die. If a disease is too virulent, it overwhelms the stem cells, and they cannot thrive. The matrix, Dan says, gives stem cells a place to hole up and get ready to grow new tissue.

Which brings us to a bona fide breakthrough, a discovery that has attracted not only the attention of scientists and engineers but the passionate, personal investment of students and busy surgeons and colleagues. The Simionescus have shown that human stem cells, extracted from the fat beneath our skin, can multiply and populate a matrix, transform themselves, and begin to grow replacement parts biologically the same as our original equipment.

Somehow—and the exact how of this so far remains a mystery—the stem cells read the matrix and learn what to be. Perhaps they detect some kind of chemical signal, or perhaps they are reading the structure itself, but they get the message. Whatever destiny the matrix ordains, the cells make a lifelong commitment. They are transformed. They set off a chain of events that lead to

Today, Dan and Agneta Simionescu have branched out, running separate labs.

But they still use the word "we" when they talk about their work. How do they manage it—working so closely together, after all of these years? Aggie smiles, considers the question for a moment, and laughs. "I don't really know how we do it," she says, "but we do."

"We always talk," she adds, "and the work is exciting for us both. When we go to conferences and take notes, very often we write down the same things, because the same things are interesting for us. I think we complete each other."

Patrick Wright

new, living tissue. The heart valve they form doesn't just *look* like a heart valve—it *is* a human heart valve.

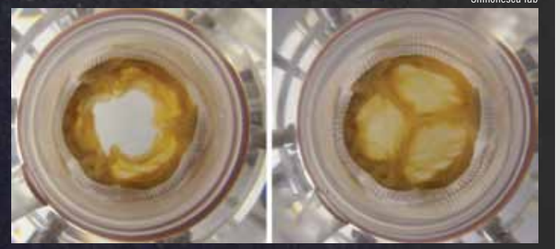
"If you looked at one of these valves in a patient," Dan says, "the only difference you'd probably see is the sutures the surgeon would use to implant it."

In concept, all of this seems simple. But each kind of tissue is different, requiring its own specialized method for inserting stem cells into the matrix. "The process is called seeding," Dan says, "and it takes a little bit of imagination and trial and error to learn how to put the seeds where they should be. If we do that right, in cell-culture conditions, where it's warm and humid and the nutrients are there, the stem cells change into the right type of cells. It's like the matrix tells them, 'You should become this type of cell.'"

So far, each type of tissue the lab has studied responds to this method. "Every month or so we have a new example," Dan says. One example is an intervertebral disc, the padding between two vertebrae. "We took the discs from pigs, removed all the cells, and we put in human, fat-derived stem cells, and they became intervertebral disc cells in the lab," Dan says. "This may help surgeons treat back pain."

Simionescu lab

Simionescu lab



Above: Two heart-valve bioreactors, designed and built at Clemson, work in tandem. **Below:** A porcine aortic heart valve open and closed in the bioreactor.

Putting the parts through their paces

The lab also makes arteries, cartilage, ligaments, skin, heart valves, and new tissue for stroke-damaged brains. Students develop and test these parts using equipment they build themselves—bioreactors that simulate conditions in the body.

"We have developed a bioreactor for each type of tissue," Dan says. He credits Lee Sierad, a Ph.D. candidate in Dan's lab, for advancing this "extremely challenging" part of the work (see the sidebar on Lee Sierad, page 20).

"So we have a heart-valve bioreactor, for example, which allows you to take a heart valve and seed it with cells, and then subject it to the kinds of things that would happen if it were implanted in a heart," Dan says. "Before too long—in two or three weeks—the tissue matures, the cells change into what we want. We think that this is the way we can prepare a living tissue replacement, ready for implantation."

The bioreactor can also give the growing replacement part some fitness training. "We've learned that some tissues need mechanical stimuli to mature and to grow, to start to regenerate," Dan says. "We can make a better implant by taking the cell-seeded scaffold—an artery, for example—and pulsating it mechanically to make it ready for implant. We call it conditioning. It's like any athletic conditioning." Mechanical stimulation may also help teach the stem cells how to differentiate, to turn into a particular type of tissue, he says.

Through all these steps, surgeons from the Greenville Health System track progress, advise students, and set goals for the work. (See the sidebar, "Surgeons help guide research," on page 16). There are ten of them, at the moment, and they are all volunteers who, as Deac did, see promise in this kind of research. Sometimes the surgeons come to campus to meet with the team or assist with implants in the animals used for testing. Other times, they work with the team in a lab at Patewood, officially the Clemson University Biomedical Engineering Innovation Campus, a joint venture with the Greenville Health System.

The surgeons are essential to the research, Dan says. "Biomedical research has to come from the clinic. It cannot be the other way around. We go and talk to the surgeons, and they tell us about their biggest challenges. If you ask a vascular surgeon, for example, he says, 'Well, the obese, diabetic patient has no arteries. All of them are calcified; they're gone. Can you give us a product, because there is nothing on the market?' So we go to the lab, and we get started."

More than a dose of green tea

I said earlier that our fat could someday save our lives, assuming we don't overdo it. When we lard ourselves with too much fat, we are asking for a world of hurt, especially from diabetes. Obesity and diabetes are the twin scourges of our era, an epidemic growing worse. Uncontrolled diabetes lays waste to the body, calcifying and destroying blood vessels and arteries, killing tissue, ending lives.

As Dan's lab assembles and tests new tissues, Aggie concentrates on the formidable problem of how to regenerate tissues that can repair what diabetes has wrecked. It would do little good to implant a new artery in a diabetic patient, if a toxic soup of fats and sugars and cross-linked proteins quickly attacked the new tissue and turned it to stone. We hear a great deal about the

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Natasha Topoluk in the lab: cautious optimism from early results.

Repairing a stroke-damaged brain

So far, stroke is catastrophic. It torches a part of the brain, kills the tissue, and leaves nothing but a gap, an empty hole. The hole does not heal; the tissue does not regenerate. If we're lucky, brain cells may wire around the hole—neurons connecting new pathways. Sometimes, patients can regain some function. Too often, they can't.

As of today, there is only one FDA-approved clinical treatment, an enzyme known as tissue plasminogen activator (TPA). It's useful for only one type of stroke, and very few patients qualify. For the vast majority of stroke victims, a hole in the brain is for keeps. And so far, all attempts to fill that hole have failed.

Natasha Topoluk would like to change that, and she's off to a good start. Topoluk, a Ph.D. student working with Dan Simionescu, got her first taste of research as an undergraduate working with Agneta Simionescu, helping James Chow with diabetes studies in Aggie's lab (see the sidebar, page 21). Later, Topoluk set up shop next door, in Dan's lab, for her graduate work.

Now, with a brand-new master's degree in bioengineering from Clemson, and a Ph.D. in the game plan for 2016, Topoluk and her mentors may succeed where a great many others have failed. Last summer, she and the neurosurgeon who advises her, Dr. Alfred Nelson (see the sidebar, page 16), implanted what Topoluk calls constructs—matrix scaffolds seeded with stem cells—into the brains of three rats with strokes so severe they could not walk.

When I ask her what happened, she takes her time to get the wording right. Speaking with the confidence of a seasoned pro, she says, "We saw almost complete recovery in our animals less than four weeks after implantation."

All three rats, rendered helpless by stroke, started to walk little by little. In less than a month, they regained some of their motor functions and some reflexes. The rats in the control group—which did not receive the construct—did not. So far as we know, no one else has done this experiment. Research groups elsewhere have been injecting stem cells into stroke-damaged brains, but most of the cells do not engraft and eventually die, never yielding neural tissue. Other researchers are using gels to try and hold the cells in place. But gels are not the stem cells' native habitat; the matrix is. So Topoluk and Dan Simionescu are convinced that a matrix seeded with stem cells is a better way to regenerate tissue, even in the brain.

"With the matrix, you can probably keep a cell population in place," Topoluk says. "Then it just becomes a question of how to manipulate that cell population to take on neural-cell characteristics. I'm making it sound simple, and it's not simple, but that's the idea."

The results so far represent an auspicious beginning, but they are not sufficient, if the goal is to help human patients. The research team, Dan Simionescu says, "is cautiously optimistic," but no one is drawing any firm conclusions before the next rounds of animal studies. Topoluk wants to know exactly what happened in the brains of those rats. Did the stem cells in fact transform themselves into neurons, or recruit new cells into the matrix? Did the regenerated neurons connect with the brain cells around them, restoring the pathways demolished by stroke?

To answer those questions, Topoluk is analyzing the brain tissues, studying the implant areas for proteins expressed by neural cells, which would indicate that stem cells had indeed served a regenerative function in the rats' brains. In the next phase, she will run more experiments, with greater numbers of rats, for statistical significance and to see if she gets the same results. Nelson has agreed to continue to help her.

"We really lucked out with Dr. Nelson," Topoluk says. "He did the implants, he's on my thesis committee, and he even takes the time to review our grant applications. He is *so* enthusiastic and just genuinely interested in what we do."

This spring, Topoluk began an internship with Nelson's department in the Greenville Health System, learning how neuroscience works from the clinician's point of view. Other Clemson students with similar internships have actually scrubbed in to observe surgery in the operating room, and Topoluk hopes to do that too.

"If we can see firsthand what the clinical setting is actually like, then we can better target our protocols and our approaches to get there one day," she says, "so that what we make is actually useful to doctors and surgeons."

Research with laboratory animals, for Topoluk's studies as well as others in the Simionescus' labs, was conducted in collaboration with the Godley-Snell Research Center, headed by John Parrish, university veterinarian. The center includes two surgery rooms and complete facilities for housing and treating laboratory animals.

Craig Mahaffey

James Chow (left) and Lee Sierad (right) work on the vascular bioreactor for testing artery scaffolds, using the Patewood facility.

Putting new parts to the test

Lee Sierad likes to cook, so if it takes a while to launch the business he's planning, he could always find a job in a restaurant somewhere. In high school, he worked his way up from dishwasher to line cook, so he knows his way around the kitchen. But the business he'd really like to start would design and build bioreactors, devices that could simulate conditions in the body and help bioengineers regenerate and test replacement parts. In a way, a bioreactor is a kind of test kitchen for tissues designed to help people heal.

Sierad, who is in his final year of work on a Ph.D. in bioengineering, built his first bioreactor as part of his master's project, working with Dan Simionescu in the Biocompatibility and Tissue Regeneration Laboratories.

"It's a system to pump fluid through a heart valve, the way it works in the body," Sierad says. With its chambers, pumps, and valves, the bioreactor can simulate blood flow, and the pressures and rhythms of a beating heart.

But Sierad isn't just testing new heart valves; he makes them. With guidance from Dr. Chris Wright, a cardiac surgeon with the Greenville Health System, Sierad tailors heart valves taken from pigs, removes their cells, and mounts a cell-free, scaffold-like matrix in the bioreactor, where he can seed the matrix with stem cells and kick-start their transformation into new tissue.

"Dr. Wright gives me a lot of input on the end requirements of what our replacement valve should look like," Sierad says. "That's extremely valuable, because we're researchers, and we don't have much idea what goes on in an operating room."

The heart valves have performed like champs in the bioreactor, and the team is ready for tests in large animals. Sierad and Simionescu, collaborating with surgeons in Romania, are implanting the heart valves in sheep. Large-animal studies of this kind are a necessary step before trials in human patients.

"That's a big hurdle, and we've made good progress on it," Sierad says.

Meanwhile, Sierad is concentrating on another part of the circulatory system, the aorta, a heavy-duty artery that distributes oxygen-rich blood to the body. Because it is so strong and thick, the aorta presents special problems for tissue regeneration. For one thing, it's tricky to remove all the pig cells hidden deep inside its layers.

"Aortic tissue has fifty to a hundred layers of elastin," Sierad says, "and those layers are so tightly woven together that they prevent the solution from reaching the structure." Working with Laine Shaw, a senior in bioengineering, Sierad has developed a specialized system to target different portions of the aortic group and perfuse fluid through them to remove the cells.

"Laine has done a tremendous job developing the device," Sierad says. "That's a huge advantage for us, to be able to build the devices and understand the mechanical engineering—fluid dynamics, pressure, and all the other aspects—it's a lot of engineering."

This kind of technical problem solving appeals to him, but so does the potential to help people heal. The culture of the lab and collaborations with surgeons such as Chris Wright keep everybody thinking about the patients.

"If we are able to do this then people who are on the organ-transplant list, who are just waiting, will have another option for extending their lives," Sierad says. "That's the whole reason I got into bioengineering in the first place, rather than say, aerospace engineering. I could really make a difference in people's lives, rather than just make their flight smoother."

"We're trying to make tangible, off-the-shelf products."
–James Chow

Defending implants from diabetes

It is difficult enough to engineer a matrix populated with stem cells, and use it to replace a body part. It is difficult enough to design that matrix to fade slowly away as the patient's own tissues and cells take over and make the part their own. But if the patient has diabetes, the degree of difficulty goes way, way up.

"We can barely make this work in a healthy, normal patient," says James Chow, a Ph.D. student in Agneta Simionescu's lab. "In a patient with diabetes it would fail catastrophically."

Chow, who plans to finish his Ph.D. in May, has been working with Aggie Simionescu for years, ever since he took a course from her in bioengineering. In the simplest terms, his goal has been to develop matrix-based constructs that can resist the onslaught of diabetes, to help patients survive.

To understand what's at stake, consider what diabetes does. It attacks tissues and cells with inflammation and oxidation, crosslinking proteins and disrupting the functions of cells. Chaos ensues. "The cells lose their identity and their function," Chow says.

He finds evidence that this onslaught may also involve a Maillard reaction, the same chemical process that browns meat in a frying pan. In a diabetic body, sugars react with amino acids, crosslinking in a way that stiffens the tissues. Blood vessels are especially vulnerable, so a classic symptom of diabetes is circulation failure that damages or kills tissue. Today, the ravages of diabetes are so widespread that demand is huge for replacement veins, arteries, and other components of the circulatory system.

But against a monster like diabetes, a vulnerable new implant would stand very little chance. Chow and Aggie Simionescu think they may have found a silver bullet. It's an antioxidant known as PGG (pentagalloyl glucose), a natural polyphenol similar to the antioxidant compounds in green tea. At Aggie's suggestion, Chow found ways to introduce PGG into the extracellular matrix the team uses to engineer a construct. PGG, he found, could attach itself to the matrix and hang out there long enough to protect the scaffold from attack while the wound healed and tissue regenerated. After several months, PGG detaches itself and gets out of the way.

"We've shown that PGG inhibits harsh inflammation," Chow says. "It's like this perfect antioxidant that can slow down or fight the reactive oxygen species that damage tissue."

He has tested this process by preparing constructs with and without PGG and implanting them under the skin of laboratory rats. In the implants treated with PGG, the matrix survived, and PGG-treated constructs populated with stem cells developed normally.

It's the combination of PGG and stem cells that shows the most promise, Chow says. The stem cells, he explains, not only help form new tissue; they also help integrate the implant into the body by modulating the immune response and promoting anti-inflammatory agents that enable the growth of new tissue.

For all of this work, Chow says, he depends on collaborating surgeons and clinicians, especially those from the Greenville Health System, who keep the work grounded in the real-world practicalities of patients and treatments. He works closely with Dr. John Bruch, an endocrinologist, and with Dr. Christopher Wright, a cardiovascular surgeon, and several other clinicians contribute, as well.

"We're trying to make tangible, off-the-shelf products," Chow says. "This is what we call translational medicine, not just science for science's sake."

Chow plans a career in industry, developing medical devices, and he says his experience running a project in the lab has prepared him well. It's an entrepreneurial endeavor, with many of the complications of running a business.

"You learn to manage a project through all of its cycles, meeting goals, training the students, writing grants, and presenting your work," he says. "Aggie and the clinicians are there to advise me, but it's truly my own project."

Craig Mahaffey

James Chow and Aggie Simionescu examine a heart valve.

About that fear of rejection...

The Simionescus are not the first to show that the human body can readily accept an implant prepared with a matrix, and Dan says that millions of people are living proof. Two examples of common treatments: injections of bovine collagen in cosmetic surgery and implanted pig matrix for skin regeneration.

dangers of oxidation, these days, and find ways to pack antioxidants into our diets. Diabetes unleashes a storm of oxidation, and a dose of green tea is not enough quell the storm.

Even so, antioxidants might have value in tissue generation, Aggie thought. She and her students, including James Chow who expects to finish his Ph.D. this spring, began to study an antioxidant known as PGG (pentagalloyl glucose), a natural polyphenol used in herbal remedies for various diseases, including diabetes (see sidebar on James Chow on page 21). The team began treating the extracellular matrix with PGG and implanting the matrix under the skin of rats with diabetes. The matrix survived. Better yet, when Chow populated the treated matrix with stem cells, the tissue developed normally. The implications are enormous: It might indeed be possible to implant replacement parts that could repair and resist the ravages of diabetes.

The success was not due to PGG treatment alone, Aggie says. "The stem cells have a very good effect, an anti-inflammatory effect. In tissue engineering, you want a little bit of inflammation, because you want stem cells to come in and start remodeling your tissue, but you don't want this to happen too quickly."

Ideally, regenerated tissue would not rely forever on the matrix used to build the implant. Instead, it would begin to regenerate its own matrix, replacing or extending the implanted one. (The Simionescus often use the word *scaffold* instead of *matrix,* to suggest the analogy of building a house: After the house is built, the scaffold can come down.) Aggie's team has found that a recently discovered type of cell assists in matrix regeneration: the type II macrophage.

"For a very long time," Aggie says, "we thought there was only one type of macrophage, but now we know there are two types." Type I actually increases inflammation, because its role is to degrade tissue and clean away debris. But type II macrophages help with healing and regeneration, and they seem to be attracted by stem cells. "We believe that these stem cells send signals to these good macrophages and start regeneration," Aggie says.

But in the case of diabetes, stem cells and their allies aren't sufficient on their own. They need the safe haven of a matrix treated to withstand the onslaught of calcification. So in tests with laboratory animals, the implants that fared best were those with a PGG-treated matrix populated with stem cells.

All of this makes Aggie hopeful that patients with diabetes may eventually have the replacement parts they need. And this, in the end, is her goal. Ever since that evening when she first walked the floor of a hospital ward, she has remembered the point of it all: the patients.

The Valley of Death

No romance can run its course without facing a peril, a nemesis to fight. The Simionescus have never had it easy—in Romania, in finding their way through a new country and a new culture, or even in science, which is always a struggle with setbacks and complications. But they have collaborators who can help them over the technical hurdles—experts in stem cells or the extracellular matrix, surgeons and engineers, for instance. The peril they dread most, at this stage, is the Valley of Death.

They do not mean Death Valley, the football stadium. They mean a chasm that yawns between success in the lab and success in the clinic. As they watch their projects march forward, yielding heart valves and intervertebral discs and tendons and arteries and so much more, they know they are nearing the edge. They will come to a halt at the Valley of Death.

Here is how it works, as Dan explains it: "If you look at the timeline for a product going into a patient, it's split in two parts. The first part is what you've heard about, the research and work in the lab. The second part is clinical trials, testing in patients. In between the two parts is the Valley of Death. And it's scary. Why? That's where you're supposed to do the large-animal testing. The first part can be covered by federal funds. But before you can go into clinical trials, you have to test the products in large animals—pigs, dogs, or sheep, for example. The FDA requires that you do this to prove safety, efficacy, and feasibility. But federal agencies rarely provide grant money for large animals; it's very difficult to get, and the work is very expensive. And usually companies will only fund clinical studies, when the product is ready for patients. We can do work with rats and mice, implant the tissues under the skin and detect if it's antigenic, if there's a reaction. We do this all the time. But then we get stuck. We need a hundred thousand dollars just to run one experiment with ten heart valves in ten sheep. Where do we get that?"

These days, Dan and Aggie are looking for ways to build a bridge across the Valley of Death, working every angle they can find. They spend much of their time writing grants, fighting odds in a time when federal funding is iffy and scant. In passing, Dan even wonders aloud whether people who love football might also like to help repair the injuries it inflicts upon tendons, ligaments, and cartilage.

The most promising prospect so far, ironically enough, has come from where the Simionescus began—Romania. "We applied for a grant, and we got a million euros ($1.5 million dollars) from the Romanian government to test our technology over there," Dan says. "We are working with an amazing group of surgeons, veterinarians, and biologists who will help us implant heart valves in sheep."

Last year, six of these scientists came to Clemson for several weeks and studied the technology.

If those implant studies turn out well, the team could possibly win approval for compassionate implantations—heart valves for human patients who would die without them. And if that works out, maybe, just maybe, American companies would see the potential, would invest in more large-animal studies to help bridge the Valley of Death.

Into the quest of their lives

The Simionescus want this success for the surgeons and their patients, certainly, but they also want it for their students. They want their students to see their hard work cross the valley, to reach the clinic and begin saving lives.

When they arrived at Clemson, the Simionescus were first and foremost researchers and problem-solvers, but now they are teachers as well. When Aggie describes her first experience in the classroom, it sounds very much like her first visit to the hospital ward. Once again, she confronted a daunting new responsibility, one that would change her life.

"Until I came here, I very rarely taught," she says. "At Clemson, the bioengineering students had to take a tissue-engineering course, so Dr. LaBerge [Martine LaBerge, professor and chair of bioengineering] told me, 'You are doing tissue engineering; do you want to teach it?' So I said sure. But then once I began to prepare the course, I was very nervous at the beginning. It was a big responsibility."

Tissue engineering is a complex field that incorporates multiple disciplines— chemistry, biochemistry, biology, engineering, physics, and more. Students take classes in these subjects, and Aggie helps them pull the pieces together and apply what they've learned. But as she teaches, she listens. Students bring energy, enthusiasm, imagination, and ideas.

"I always listen to the students," Aggie says. "I take that part very seriously. They are *so smart*, and they have great ideas. I just love the students."

In both Simionescu labs, undergraduate students from several departments work side by side with the graduate students, participate in group meetings, and appear as coauthors in publications. "This way we educate the next generation of scientists," Dan says. "Our student alumni are now doctors, professors, nurses, lawyers, and entrepreneurs, and more."

And so this story, which began with the first little flurries of romance, ends with another kind of love: a passion for leading young women and men, as Radu Deac did for Dan and Aggie, into the great, swirling quest of their lives.

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Graduate students in the Biocompatibility and Tissue Regeneration Laboratory include Laura McCallum, Allison Kennamer, Chris Deborde, Natasha Topoluk, Jason Schulte, George Fercana, Michael Jaeggli, James Chow, and Lee Sierad.

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An international team

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