ANDRE TERZIC: So good afternoon. And on behalf of the Center for Regenerative Medicine, I would like to welcome you all to this CCaTS Grand Rounds. As many of you are aware, we have a very close interaction with CCaTS where we showcase regenerative medicine translational principles and their application on a regular basis as part of the CCaTS Grand Round series.

And today, I have a particular pleasure to introduce the speaker, Dr. Michael Yaszemski, who is the Krehbiel Endowed Professor of Orthopedic Surgery and Biomedical Engineering. And I'm particularly proud to introduce him because Dr. Yaszemski has been foremost one of the key champions of regenerative medicine. Not only the Mayo Clinic, but nationwide.

The journal *Nature* has showcased him just a year ago in underscoring his contribution to the field, and there are many. There are many. They go from promoting the concepts of regenerative medicine as they apply to the Wounded Warrior, an area that Dr. Yaszemski's particularly proud of. He has been very much involved with the Department of Defense at various levels, the Air Force office of the President of the United Uniform Services University, and many other roles that he has served in that capacity.

But he has brought to the Mayo Clinic something that we call AFIRM. AFIRM the-- on presidential decree, basically an effort, a national effort to promote regenerative medicine. And so we're very, very proud to have Dr. Yaszemski present today.

He will showcase a domain that is particularly critical for regenerative medicine, which is the musculoskeletal space. That's one of our key priorities. And so without further ado, I would like to introduce Dr. Yaszemski to tell us the newest in the bedside to bench and back as you see from the musculoskeletal tissue engineering space.

[APPLAUSE]

MICHAEL YASZEMSKI: Thanks, Dr. Terzic, for a very kind introduction. And welcome, everybody. Let me echo Dr. Terzic's comments about the Center for Regenerative Medicine. It's all around us, and as one of our past presidents, Brooks Edwards says, does Mayo know what Mayo knows? If you have an idea, there may be someone else here who shares that idea or a passion for it. Please just come to the center. Come to any of the folks associated with Center for Regenerative Medicine, and maybe we can help.

Now finally, we all have biases. You can see some biases in my title here. I'm an orthopedist, so musculoskeletal's what I'm going to talk about. But I'm hoping that the principles apply to whatever your particular type of regenerative medicine interest is.

You also see bedside to bench and back. Another bias of mine is that bench to bedside is an inappropriate and really not accurate way to present what we all do. I think everything starts with the identification of an unmet clinical need, some frustration that a clinician has in taking care of a patient, and there's just nothing to solve it. From that, we generate a research question that we work on in the lab, and then we've got to get it back to the patients. There's a couple of steps in that that I'll talk about at the end.

Now as way of disclosure, I have no disclosures relative to this talk. These are the things that are on my disclosure list. As an outline, we'll talk about clinical needs first for several musculoskeletal tissues, current treatment options to identify where the gaps lie, and then some novel regenerative strategies that exist or are on the horizon.

Begin with a clear description of the unmet clinical need, clearly articulate your research question based on that need, and seek constant interaction. This is an easy thing to do at Mayo. We're very collaborative. Teamwork's a big thing here. It takes everybody. You can't do this alone. There's no super woman, no super man that can run the whole gamut. You need all the different kind of colleagues, including regulatory and industrial colleagues, to get this back to be what your idea-- to turn your idea into a treatment for patients.

Now let's just talk about eras. In orthopedic surgery, there have been eras. There's been a resection era, a fusion era, a replacement era, which we're in right now, and a regeneration area, which is looming to eclipse the replacement era.

Here's a historical example. This is the first FDA-approved total hip arthroplasty done in these United States here at the Mayo Clinic in 1969 by our former chair, Mark Coventry. We've come from that because someone tried something new. John Charnley in England tried to get out of doing things like fusions and resections. If you had hip arthritis before John Charnley, one of the things you'd do is just get your hip cut out. The pain went away, but you had a floppy hip, and you gave up function for it.

Then in the area of fusion-- in the era, excuse me, of fusion-- if your hip hurt, you could get it fused. Again, the pain went away. It was very durable. But you lacked motion. You had a hip that was in one position and didn't bend.

Charnley did a total hip. He tried first to make the cup out of Teflon. So material science still looms with us today. Some of the materials today are on the nano scale. In his case, he chose Teflon, and in two years, they all wore out. Everyone was a failure.

He then chose polyethylene, and that's the cup we still most use today. So from about 1958 to today in 2015, the most common cup of a total hip arthroplasty is a polyethylene cup as made by Charnley.

So let's start with the bone first as one of the tissues to look at. What are current treatment options for bone defects? Bone defects can be several types. We'll classify them grossly into segmental defects-- i.e., a missing piece of bone-- or contained defects-- i.e., a hole in the bone. We have bone graft that you take from one part of your body, put in another part, autologous. Structural and trabecular allograft, graft from somebody else. Demineralized bone matrix, recombinant growth factors, and natural and synthetic matrices.

I'll give a couple examples from my own practice. This is a fellow where we did a total sacrectomy for chordoma, which is a malignant tumor that most commonly happens in the sacrum. This fellow's spine is not connected to his pelvis. In the past, these folks were just left to be, saying, well, they got rid of their cancer. It'll scar, and they'll do OK and have a good life. They generally don't have a good life, and we reconstruct them now.

So what do we do right now? This is a front and back view of what we do. We replace the sacrum with those two struts that you see down on the bottom, kind of tipped up like a tepee or a cathedral ceiling. That's in the front. And then in the back, we put a standard bone graft.

There isn't enough autograft bone to fill this up. We have to use something else. We typically use allograft. It doesn't always heal, and these people often have several operations to get them to a point where they can get around. We can do better than this, and the promise of doing better is in regenerative medicine.

Now what is a scaffold? I mean, we use this term a lot. A scaffold is, I think, best described as a substitute extracellular matrix, something that cells that are anchorage-dependent, which osteoblasts are, can attach to, can make their own extracellular matrix, and when that's done, the scaffold matrix will go away.

It's a temporary extracellular matrix awaiting the real extracellular matrix. It can serve as a delivery vehicle for osteoinductive factors, things that will encourage the cells to make bone matrix and provide temporary structural support to the reconstructed areas so the patient can start getting through rehab while you're waiting for new bone to grow.

Now the strategy of tissue engineering needs a scaffold, needs something for the cells to anchor to and make their matrix. Molecules to guide that function and cells to make the matrix. It's all about the cells. I think a central player in all of these is our cell biologists. Every one of our teams has one of our cell biologists on it to help us that don't understand cells to make the cells operate as optimally as they can.

Scaffolds can be many things. They can be polymers, they can be metals, they can be ceramics. So another one of our biases. I'm a chemical engineer. I was a polymer chemist in industry before, coming into medicine. We work on polymers. It's equally good to look at metals and ceramics.

Polymers, though, with our biases, I think, have a lot of properties that are good. All these things around the outside are controllable. We can dial them in for the application that we're working on. And that's one of the things that makes us like to do the polymer design for our uses.

Now I said there were two kinds of bone defects. Hence, there are two kinds of scaffolds to address them. A preformed scaffold is made for a segmental defect. It's appropriate for missing pieces of bone. We take a CT scan of the area, we bring it to the lab, and by one of the varieties of solid freeform fabrication, we make a piece for the surgeon to put in.

An injectable scaffold is something you can make flow through a trocar of some sort for a hole in the bone. And anytime we can do something without a large surgical approach, it's better than doing a large surgical approach. If

But the injectable scaffold brings with it other problems. It has to be fluid first so that you can inject it. It has to fill the arbitrary-shaped hole. Then it has to solidify and at the same time become porous so new bone can grow into it. And it has to carry with it the molecules to direct the cells, and oftentimes, to carry the cells too. The cells are susceptible to shear injury, and we have to work out the rheology of these things as we're putting them in to make the cells able to survive the trip.

Now let's look at examples. Here's typically an elderly woman. You see the humpback. This is osteoporosis. Now the best treatment for osteoporosis isn't what we're going to do. It's recognition and early treatment before it happens. So our endocrinologists are more and more having people recognize this issue and get treatment for it before it occurs.

But what would we do here? This is not a hole in a bone, really. It's not a missing piece of bone. It's just bone that isn't performing its structural role. But we can augment each of these bones once we see this fracture cascade start happening with something that is osteoinductive and will go away once new bone happens.

Here's a guy-- and by the way, I'm going to show a number of things from Iraq. I should have said it ahead of time. If somebody gets queasy, I'll say when one of these is coming up.

This is a guy I took care of in Iraq. This guy has a segmental bone defect. If you look at his hand on the right, he's got a good hand. He has one artery going to his hand. He is not missing just bone. He's missing cartilage, tendons, muscle, skin, bone, nerve.

So I'll bring up the concept of composite tissue regeneration. Very few folks involved in regenerative medicine of the musculoskeletal system are doing composite tissues right now, but it's essential that we learn how to do it. Some people are doing two at once, like a piece of bone with some cartilage on the end of it, a bone and a nerve near each other, a bone and some muscle.

But sooner or later, we do have to learn, probably again from a team perspective, from our developmental biologists who have very good models like Dr. [INAUDIBLE] zebrafish. We're looking at the axolotl to come here, the Mexican walking fish. Things that can regenerate themselves. We need to learn how this is done in nature and try our best to copy it.

Just a little bit about the polymers and one of the examples of how we use them. This is a polymer that was made in our lab a number of years ago by one of our fellows, Shanfeng Wang. And it's a copolymer. And with a copolymer, we can control the amount of each one.

Look at the gelation point, when it goes from-- remember I said, when you put it in, it's got to flow, and then it gets hard. The gelation point, we can control by controlling the composition, how this happens. Now one of the things we use in that control is, what are surgeons used to?

Really, the only polymer that the surgeon has hands-on experience with is polymethyl methacrylate, so-called bone cement. Surgeons are creatures of habit. Operations get designed and get perfected-- well, maybe not perfected, but advanced to a way that we all do them. And we get in a habit. We use polymethyl methacrylate. You get it, you mix it. In about a minute and a half to two minutes, it's cohesive. Before that, it's just going to drip everywhere.

After it's cohesive, you've got a short period of time in which you can inject it. Maybe up to, I don't know, about five minutes or so. After that, it's too hard to inject, but you can still mold it like Silly Putty. After about 12 minutes, you've got what you got. And if you don't like it, you're going to have to take it apart and do a revision operation at the time of your initial operation.

So we try to-- if we're going to try to introduce things to surgeons, and if we have the opportunity to say, look, it behaves mechanically, it behaves while it's getting solid just like bone cement, it's a little better than saying, look. We've got something new for you. Do something different. Because the operation may have been designed around something that gets cohesive in two minutes, and stops flowing at five, and gets hard at 12.

Now stereolithography, one of the solid freeform fabrication technologies that we can make things from. It's a three-dimensional structure, and we can control the pore size. Basically, it's a tessellation. We make a start, and it can expand in all directions to whatever external geometry we want.

Here's the machine we use. It's like a Zamboni machine, the ice machine where it resurfaces the ice. The top surface of here gets touched by the laser where it's supposed to be solid. It cross-links in that place. The elevator moves down. New monomer comes on top. It gets cross-linked, and we can build a three-dimensional structure from it.

But can that structure give us an internal architecture? This is the work of [INAUDIBLE] Lee, who was a graduate student here with us a number of years back. I would submit to you that it can be pretty precise. I asked [INAUDIBLE] to make something to show, how small could we make differences? And he went from half a millimeter to a millimeter, and can pretty much make the internal architecture good at each one of those size points.

In addition, Dr. Liu who's here with us worked as a co-director of the lab with me. Worked on putting microparticles within the walls of that three-dimensional structure so that we could design the diffusion path for bioactive molecules to come out of a microparticle to diffuse them through the wall and to diffuse into the pore. And by design of those microparticles, design of a wall size, we can make them come out at different times and have a controlled delivery of different microparticles, which we'll show in a moment.

Now other 3D technologies, just to say what they are, injection molding. We have a nerve program that we're doing together with Dr. Windebank and Dr. Wang. And Dr. Wang uses injection molding to make our nerve tubes.

Fused deposition modeling. Just kind of like, if you will, laying lines of toothpaste down, making a layer, turning the layer for the next one, making more lines of toothpaste. And you can make a 3D structure like laying different metal grids on each other.

Fiber electrospraying. This gets us into the nano scale. The stereolithography does also, but this, we can make fibers on a nano scale with precise architecture. Piezo or thermal inkjet printing, which is also used for bioprinting. So in bioprinting, the bubble that comes out and is deposited has cells in it. It's still a thermal or inkjet printer.

Now I talked about encapsulating things. We use fairly standard solvent extraction, lyophilization. And we can immobilize these. If you look on the lower right, those bumps kind of on the lunar landscape are microparticles within the wall of one of those pore scaffolds we showed. And we can design what comes out and at what rate it comes out as we feel it's needed.

This was done by [INAUDIBLE], who was a PhD student with us. And he found a way to slow down the use of rats by using radiolabeling. So rather than kill the rats at each time point, we could just do scintillation count at each time point.

But what you see, by putting those molecules in the wall and having the release be controlled, on the right side, this is bone morphogenic protein. The yellow line, you see that by two weeks, it's almost all gone. Orthopedists like to use this. We hesitate, however, because it's incredibly expensive. So to control the delivery and not waste so much might make it a little better for us to use.

And you'll see, if we put diffusion paths, the pink and the blue, it comes out a little slower. Because at the time we close someone's wound, the chance that cells are where we want the bone to grow is about zero. We have a hematoma there at that time. And while we're waiting for cells to migrate in and start to form the scar that we want to turn into bone, our very expensive protein is going out the drain tube. So this is another example of trying to say, what's the problem? And design something to address that problem.

Now Dr. Liu and I did this many years ago. We wanted to see if we can do a sequential controlled delivery. So we looked at, can we get vessels to come in? Because bone cells, unlike cartilage cells, need a blood supply to get their nutrients and get rid of their wastes. Cartilage cells, cartilage is aneural, alymphatic, and avascular. It gets its nutrients and gets rid of its wastes by diffusion. Bone cells are not so.

So we put, as you see over on the right, four things. An empty scaffold makes a certain amount of vessels. If we put VEGF in it to come out early, it makes more vessels. With BMP, there's no change. The two on the right are like the two on the left, because the BMP should have no effect on the vessels.

Now if you look at the bone, an empty scaffold or a scaffold with just VEGF makes no bone, as expected. If you deliver BMP alone, it makes some bone. But if you put VEGF first and get a vascular network, and then follow the delivery of BMP at a later time, you get a little more bone than you got just with the BMP alone. And so just one example of trying to do a sequential controlled delivery of the things you want in your regenerative field.

Now I'm going to give a clinical example where this might help. Masquelet technique is, for the trauma surgeons, if there are any in the audience, we know that segmental bone defects, one of the things that we do is put the metal fixation in to hold the proximal and distal fragments together. And where the hole this, we put polymethyl methacrylate, bone cement that we talked about.

Four weeks later, we do another big operation. Take the polymethyl methacrylate out. It was just there to make a space, so the space didn't fill in with scar. And now hopefully, there's been a vascularization around the periphery of it, and then we put bone graft inside. Two techniques.

So the question arose, could we do this better? Could we turn two surgical procedures into one? Now in the Armed Forces Institute of Regenerative Medicine, the AFIRM Dr. Terzic referred to, we're trying this right now. We're not doing it in people right away. We're doing it in goats right now.

But we're asking the question, instead of putting the bone cement in, the defect with the masquelet, can we put a degradable scaffold in? Can we deliver VEGF from that scaffold and see if we can get the whole volume with vessels in it? Not just the outside, because the volume was preserved by the bone cement, but it also prevented vessels from getting into the volume that we eventually want to be bone.

Then four weeks later, at the initial surgery, we would also harvest either adipose-derived stem cells or bone marrow stromal cells, expand and culture them ex vivo, and then put them in at the four-week point via percutaneous procedure. So what we're hoping to do with this animal study is to turn two major surgical procedures into one major surgical procedure, followed by an outpatient office procedure. We'll see how that goes. The animal studies are happening as we speak.

This is our bone cement. They say surgeons are creatures of habit. The white stuff is prepolymerized polymethyl methacrylate with some barium in it. That's why it's white, so you can see it on an X-ray. And it has the initiator to make the polymerization happen. The dark stuff is methyl methacrylate monomer, and the accelerator, which is a liquid. And you mix them together, and you get the two minutes, five minutes, 12 minutes that I talk about.

But it's all about the cells. The current bone grafting technique is such that if we want the cells to be there at the time we're taking care of the wound, that wound environment, be it traumatic or be it surgical, is not a real good environment for cells. So what we're trying to do is make a better wound environment and see if we can't have the cells come in in an office procedure later on.

The cells, we've been using either BMSCs or adipose cells. And with the help of our cell biologists, trying to get them to go down an osteoblastic path before we put them back in.

The growth factors. Dr. Dietz's lab uses platelet lysate. This seems to help the cells get, if you will, primed to go into the wound. Our orthopedic colleagues across the country also use platelet-rich plasma. It has pluses and minuses. And BMP seems to be the best biologically, but it's very, very expensive, which is a big negative.

OK, the transition points, I'll try to put a couple things in. WW Mayo was a surgeon in the Union Army in the Civil War. Moved around a lot with his family. Got sent to the Minnesota Territory in 1863. War ended in '65. He got out of the Army in '67.

So you might ask, why are we here with this really cold weather? Those of you who have military experience will know that when the husband, the wife, whoever the military person in the family is finally gets out of the military, the other spouse will say, I've been following you around every three years forever. We finally don't have to move. Kids are in school. We got a house. We're staying right here.

So if WW Mayo had been in Texas or Florida or someplace warm, maybe we'd be having this lecture down there now. And there he is with the two brothers.

Now let's talk about fabrication, solid freeform techniques. This is Piezo inkjet printing. And this is a commercial. We use this for new materials, because this is Styrofoam and a wax. So we can print any internal architecture with Styrofoam and wax.

And a number of years ago we had a visiting scientist, Ismail [INAUDIBLE]. He noted that the Styrofoam was soluble in acetone and the wax was not. And so when we got a new material, we can make this, put it in acetone. The Styrofoam goes away. It leaves us a wax skeleton where the pores should be. We can flow the monomers-- we have to have the correct rheology-- into that wax mold.

When it cross-links, we now have what is commonly called a wax loss system. The cross-linked polymer will probably not melt till about 150, 200 degrees C. We raise the temperature above the melting temperature of wax, about 65 or 70. The wax goes away, and we've got a porous polymer with an interconnected structure.

This helps us when we've got a new polymer. We can reliably take any polymer that we can start with redox initiation, put it in here, and get a pore structure to work on. Here it is just in another.

Now we can also have porogens, leachable porogens. That porogen can also be a hydrogel that can carry cells and growth factors. We can make those things by injection molding.

But if you notice those pores, although those small pictures look good and say, look at the nice pore we've got, porogen leaching rarely, and in one guy's opinion, gets you a predictable, uniform, completely interconnected pore structure. But we, like everybody else in the field, use it.

Now other scaffolds. We first started this-- I mentioned we're doing nerve studies. But we thought, you know, gee. Nerves conduct electricity. Let's make a charged surface, and let's make a conducting scaffold that we can maybe put a potential difference across.

And Brett Runge did this when he was our postdoctoral fellow. Made a semi-interpenetrating network of one of our polymers, polycaprolactone fumarate and polypyrrole, which is one of the conducting polymers. There are a number of conducting polymers. Pyrrole is one of them.

And [INAUDIBLE], who worked with us for quite a while here and went to industry earlier this year, did a study on one of those charged surfaces. So on the left and on the right, those dorsal root ganglia are the same. They come from the same source. There's a squiggly neurite or two on the left. And when you put that same DRG on the same base polymer that has a plus one charge put into it, by putting-- at that time, we put lysine, which is plus one at neutral pH in the polymer backbone-- incredibly more neuritis, when the surface that the cell attaches to is charged.

So we got back to, it's all about the cells. And if we think about the cells and give them a better environment, hopefully they will generate more of what they are programmed to do.

Now we'll move over to another tissue, tendon and ligament. What are the clinical needs? Degenerative tendon tears, traumatic tendon and ligament injuries. Anybody, if you've pulled your quadriceps or Achilles tendon or tore your rotator cuff, know all about this.

And what are the issues, though? The junction doesn't heal well. The junction between the muscle and tendon doesn't heal well. The enthesis where the tendon enters into bone, it's hard to reproduce that, and it's never as strong as what it was before it got injured. And for ACL injuries, the intrasynovial environment in the knee, the tissue don't seem to heal as well in synovium as they do when there's not synovial fluid. Again, this is composite tissue regeneration. We're talking in one set of sentences about muscles, tendons, bones, and ligaments.

What are the current treatment options? Direct repair to bone like a rotator cuff? Direct repair to muscle? Some of you who might have tore your Achilles tendon, sometimes you get the cast with your toe pointed down. Sometimes you get the surgical repair. Or substitution. In the anterior cruciate ligament, you might get a bone-tendon-bone graft or a hamstring graft.

What about scaffolds? So these scaffolds are different. When we have bone, we've got scaffolds that have to resist shear and that have to resist compressive loads. This we have to have a scaffold that resist tensile loads. The cell is different. It's fibroblasts that'll make the collagen, and we need to deliver the signaling molecules as in any other place.

So what we thought, we thought we'd look at the enthesis. And so we made a scaffold that we make for bone regeneration, and then we tried to make tensile load-bearing scaffold. This is Brett Runge's work again with the copolymer of polycaprolactone fumarate and polymethyl methacrylate, 82% PCLF. He was able to get these to self-assemble into structures that have aligned members. These can bear tensile load.

Now here's the one I showed you before. It was the bone scaffold. These have regular holes throughout them. We make these by laser stereolithography. So the idea for this was to put some of the tensile load-bearing structures-- not the ones I showed you, we're using others-- into these, and see if we could get bone to grow and collagen to grow at the same time and in the same place.

Now the next slide is a blowup of one of those crosses at the junction of holes. That cross is the bone scaffold, and this particular stain is for collagen. So we have gotten now collagen growing, intimately applied to the bone scaffold where bone will hopefully grow. And hopefully, the tensile collagen will be embedded within the bone, and maybe we'll get a better fixation of the tendon-to-bone junction.

Now again, we want to see if the cells do their thing. And the cells on this tendon ligament scaffold grew pretty well. They attach, and they do their thing and make collagen. Here's what we'd like to have it outside. It's not like that today. And that will switch over to cartilage. We've got a little bit of time left.

Clinical needs. Degenerative joint disease, post-traumatic cartilage degeneration, and other conditions that kids have like osteochondritis dissecans where a little piece of cartilage. So this is the difference between generalized arthritis that you get as you age and focal cartilage defects in an otherwise normal joint.

Now what are the clinical issues? Total joint arthroplasty's pretty good. So if I am an old guy with a bad knee and I'm in your clinic, and you tell me, I've got the best thing for arthritis. It has worked in every animal model. You're going to be the first person I put it into.

And I'm thinking, hmm. Total knee arthroplasty, take the surgeon about an hour, I'll be going home the next morning, it should last 15 to 20 years. I ain't trying that new technique. And neither would I suggest that to one of my patients. I don't think there's equipoise in that.

Now you fast forward and go to a child with an osteochondritis dissecans, a focal cartilage defect with an otherwise normal joint, or you go to a young person who's had a sports injury or a car injury and gets an injury to their cartilage, that joint is going to go bad. There's no question about it.

All the treatments that we have available today-- OATS, microfracture, autologous chondrocyte implantation, mosaicplasty-- they all do something. They make cartilage. It delays things for a while. But none of them is perfect, and none of them makes the joint normal at the point of the injury like it's normal anywhere else. That person will get their arthritis delayed, but it's going to happen at a much earlier time in their life than it currently does.

So now you tell me, I've done this in animals. It works great in animals. It makes new cartilage. It hooks up with the old cartilage, et cetera. You know, I might try that, because the alternative isn't great.

So the other thing to learn from this, at least for us, is that when we're trying to get into a first human use, there's got to be equipoise. It's probably better to design the first human use to be for a problem for which there really isn't a good answer. Maybe there's a so-so answer like we have for the young person with a cartilage injury, but it's probably not wise to break in and try to supplant something that works real well most of the time.

So what are those treatment options? I just mentioned them. Here are some cartoons about the different ones, mosaicplasty and OATS and periosteal chondrogenesis. And again, it's all about the cells. In the periosteum, the cambium layer of the periosteum has the cells in it. That's where the healing cells come from in a fracture repair, and that's we're hoping to try to-- one of the places we're hoping to try to get them from in a cartilage repair.

And we just had a thesis defense. One of our PhD students, Michelle Casper defended her thesis yesterday on cartilage regeneration by periosteal chondrogenesis.

Now again, the scaffold can be anything. We made a different polymer for this. Polyethylene glycol fumarate is the polymer we used. It can be made into a hydrogel. That's why we chose it. But there are many ways to get the needs you design in a scaffold. This is just one of them that we used.

Let's switch to skeletal muscle engineering. What are the clinical needs? Volumetric muscle loss or atrophic muscle that maybe had a nerve injury for a while. Got reinnervated, but some of the muscle was dead already.

The clinical issues, just like for the tendon, the musculotendinous junction is an issue in muscle regeneration, just like it was in tendon regeneration and innervation. The new muscle has to have innervation to it, or it's not going to work.

Now I've got another Iraq picture coming up, for those of you who don't want to see it. This is, again, composite tissue regeneration. You need a tendon and a muscle in a nerve all together.

Here's a guy. His body is up to the left of the picture. That's his knee on the right. My hands are holding the retractors, holding the skin open. And the fellow who's assisting me is putting a sucker through the entrance wound. This guy got a high-velocity gunshot wound to his thigh, and the entrance wound is just a little hole. But you can see the metal tip of the sucker inside right there.

The blowout is huge. This guy's going to do OK, but we had to cut a lot of dead muscle away. And he's going to be missing muscle. He's going to have weak adductors pulling his hips toward the midline. It's going to be weak. And he's going to have weak knee extensors. His quadriceps, a lot of them are missing.

This is volumetric muscle loss, and there's a great need for this in not only military trauma, but civilian trauma. And we can do it, same thing. Cells, scaffold, and signaling molecules.

There are two ways that people in this field are doing it. They either do it with the person as the bioreactor and put it all in the person, or they use a bioreactor ex vivo and start getting the three-dimensional tissue to grow a little bit, and then they put that three-dimensional tissue in.

Among the things that are happening in musculoskeletal regenerative medicine, this is very early. And people are trying both right now. It's not clear how it's going to turn out.

Now I'm sorry I didn't prep this. This is another guy I took care of. This guy got burned over about 80% of his body in Iraq. And we have to do-- you know, the skin becomes this tight, nonpliable entity that you're basically shrink-wrapped in. You've got to cut it or everything underneath is going to die. We have to cut him across the chest, because the chest wall will not be able to move. Then he won't be able to breathe.

And typically, in past times, people with that high of skin loss are just going to die. You can't salvage them. But in the AFIRM, one of the fellows, Steve Boyce at the University of Cincinnati, made an engineered skin substitute. From the 20% remaining skin, he makes these polymeric membranes, grows to cells on them, the cells grow collagen, and then a little bit at a time, he covers the body.

And this fellow stayed alive. 81% total body burn, and he's going to do OK. So much like-- muscle was very early in musculoskeletal regenerative medicine. Skin is very well advanced. This is available now for people.

Now another Mayo thing. Those of you who are in biomedical engineering know about Earl Wood. In World War II, something new happened that wasn't due to enemy action. It wasn't a kind of injury. It was due to the onset of jet airplanes. Germany beat us, a couple of months ahead of us with the Messerschmitt 262.

But pilots started dying without getting shot out of the sky. And of course, G-induced loss of consciousness we know now is the reason, but people didn't know that back then. Mayo agreed to work on it. And this is an example of how something that you solve when you have to because a war's going on helps people later in general.

Earl Wood and his team. And some of his team is still here, by the way. Erik Ritman just retired. Was on Earl Wood's team. Not in 1940 when this happened. And Barry Gilbert, who's still working. I don't see Dr. Gilbert. I don't see if he's here. But he and Dr. Ritman were on the Wood team later on.

But three things came out of that investigation. Number one was the G-suit, which, fighter pilots today-- today's G-suits essentially operate on the same principle as the ones that were made here in 1940. The bailout bottle, which you see on the right, if you jump out of your airplane high enough in the atmosphere, you can asphyxiate on the way down because there's not enough oxygen. Bailout bottles today are just like they were back then. And the final thing is the pilot's face mask which, again, with small modifications, is the same as the BLB mask that was developed here.

And Earl Wood-- there he is as a young man-- built the first human centrifuge in this country to study this. And he was frequently the subject. And if you see on the left, he's tipped back. The idea for treating G-LOC, G-induced Loss Of Consciousness, was to decrease the distance between your heart and your head so the heart could pump blood under more and more pressure. And today, fighter planes today have the pilot tipped back just like this.

Also you get to see him passing out here. From the left to the right, he's taking more and more gs. You see him pretty alert on the left, and he goes to unconscious in the middle. And over on the right's got that just waking up, what just happened look.

And he died a number of years ago at age 97. And he addressed us shortly before he died and said, I have probably passed out from loss of oxygen in my brain more than anyone in history, and I made it to 97 with my faculties intact, so maybe there's hope.

[LAUGHTER]

All right. We're going to switch now to translation. We talked about the unmet clinical need to drive the research, preclinical and clinical. We talked about clinical equipoise to do the first in-human use. There's got to be a good balance in patients for trying something unknown for what they might get out of it.

But then there are these other things, the regulatory issues. The FDA, the IRB. And then there's conflict of interest. For example, with the conflict of interest, if I develop something, I can't be the surgeon who consents patients. I can't be the surgeon who puts it in, because I mean, I've got a vested interest. I might make some money if it works. And that will cloud your judgement.

And if you're getting to do these things toward clinical use, I mean, you've been involved since the unmet clinical need. You've done all the research. Now you say, I want to close the loop and finish. But don't do that. As much as you think you won't, your judgment will be clouded if you have an interest. Pick a trusted colleague to lead the study once it goes into human use.

Now the regulatory issues. Is it an IDE? That is, an Investigational Device Exemption at the FDA? Do you have a device? Is it an IND, an Investigational New Drug application? That goes for both drugs and biologics.

Do you have Good Laboratory Practice, Good Manufacturing Practice to put it together? Because you have to do that. And these are the things that go into GLP and GMP. The reason it's good to know this is can you do it yourself, or do you have to get a company? Because if you have to get a company, then you have to convince some CEO that she or he should expend money to develop your product. You also give up control.

So one of the things we're doing here that Dr. Terzic has led through the Center for Regenerative Medicine is we are gradually getting GLP, GMP expertise. Some of it's already here. For example, the virus and vector lab is up and running, started by Dr. Steve Russell. And now he's passed on the leadership of that.

We have brand new biomaterials and biomolecules to make implants and to make drugs and immunoglobulins, and any other kind of small molecule. And there's the Human Cell Therapy Lab, led by Dr. [INAUDIBLE] and Dr. Dietz. So we pretty much have the makings-- some very well-developed, some just new-- of doing GMP for anything.

The idea there is then that you as the inventor, with appropriate attention to conflict of interest details and issues, you can do the first in-human study here and have complete control. And not worry about some CEO or some person that he or she has put in charge is going to be very quick to say when there are adverse events, as you will be if you are running it.

Please consider that, and when you're ready, please talk to any of us in the Center for Regenerative Medicine. These capabilities are developing and-- are developed and developing very rapidly. And hopefully, more of us will work with the FDA to get the first approval to do in-human use here before we pass it off to someone we don't know.

I think that at least personally, once I'm convinced that it's safe, if I've made it, if I'm convinced that it's safe, I'm not interested in scale-up, et cetera, then I'd be happy to give it to a company. But I do want to have control personally until the safety has been established.

Pre-submission meeting with the FDA. Who attends? Get your team together. Decide who's going to talk to the FDA. Is it a pre-market application, which takes a whole lot more than a 510k? That is the substantial equivalence pathway.

Now commercialization. Commercialization and translation are two different things. They're distinct. They may overlap, but both have to occur to get a new treatment available to patients population-wide.

For commercialization, you need a company partner, unless you are going to be a company. Now you know through Mayo Clinic Ventures that there's an employee entrepreneurship program. If you want to take a leave of absence and start the company and take it further, that's an option now. Otherwise, you've got to convince a CEO that it's worth taking on.

If you work with that company, what's your relationship with them? Those of you who've done this are well versed in it. Those of you who haven't, it's critical that you know whether you have a consultation agreement with the company or a know-how agreement. Basically, the difference between the two is that in a know-how agreement, the generation of new intellectual property is anticipated. And the rules for how that will be split between Mayo, the Mayo investigator, and the company are clearly laid out in advance before you say anything and give away your ideas.

Consultation. If you have consultation, they're going to pay you for time. And if the next greatest thing in the world comes out of that, and you say, well, where's Mayo's piece? Where's my piece? There is none. They paid you for your time, and that's it. You have to have a know-how agreement to have royalty income flow to Mayo Clinic.

And what's the inventor's level and nature of involvement? I just told you mine. I want to be involved until I'm sure it's safe. Then I'm not interested anymore. A company can scale it up and market it and distribute it. But that's different for everybody, and those different levels are available to all of us. So those of you who want to do it, please talk to Mayo Clinic Ventures and understand what the possibilities are. You'll probably find one that fits you.

Now funding issues. Like Sister Generose says, "no money, no mission." Where are you going to get it? If you're going to start your own company, you've got to for commercialization. The value will depend on whether you get funding or not. And value's are a hard thing to define.

You have to get some-- the person who's going to give the money has to have their definition of value hold. You can get it from industry by licensing your patents to industry. You can get it from investors. You probably have to give them equity if you're starting a company. And you can get it from venture capital firms.

They want a piece of the-- you're not going to get something for nothing. You have to give up something that's part of the value of what you're doing to have other people. Other people may not be interested like you in solving the clinical problem. It's purely a money-- it's a money proposition to them. But to you, you have to be aware of and know how to deal with the different varieties of money handling in these situations to make it come out safe for your patient.

Now the first round of bedside to bench and back cycle is complete when your treatment has received clearance by the FDA, is manufactured, distributed, and sold by a company, and has received a payment decision by the Centers for Medicare and Medicaid. But the cycle-- that's just the first role of the cycle. I got the five-minute warning. Thank you.

It goes round and round, because rarely is the first thing the best thing. There's always chance to improve it as you use it. The physicians who are prescribing it, whatever it is, a drug, a device, a biologic. The patients who are being treated with it. You have to follow up, and you've got to find out if you can make it better. You never have to stop. It's round and round and round.

But the doctor-patient relationship. Remember, this is the difference in the basis. Industry is not our enemy. Industry is essential. If I'm doing tomorrow's spine operation, I can't go home tonight and go in my workshop and build the implants that I'm going to make. An industry has to be involved.

But there are differences. We the physicians are bound by the doctor-patient relationship over everything else. The industry is bound over everything else by a fiducial responsibility to their shareholders. There is a junction of those two spaces where we can be involved and work together, and we should do so. There are also out of bounds there that we can-- there are lines we cannot cross. So the more you get to do this, recognize the out-of-bound lines, and make the plans that you have to work with industry, such that you're going to stay out of those areas.

The physician will ultimately make the decision. In our society, the doctor-patient relationship is still the cornerstone, and a physician and a patient will make the decision as to what the treatment's going to be. It won't be made by the company or the institution, as it is in some other countries. Your treatment will only get used if the physician taking care of that patient thinks your treatment is the best thing for her or his patient.

And so it leads to culture change. It's only the standard of care for its intended use when the physicians who care for patients with the condition eventually reach a consensus, either in the literature or at professional meetings, that what you designed is the best treatment for their patients. Then it's the standard of care.

Acknowledgments of the funding sources. These are the funding sources. The team-- I get to stand up here and present everything. The work all happened by the team, and I thank all of you who I see in the audience today. And thank you all for listening. I'll take any questions now.

[APPLAUSE]