DR. FARSH GUILAK: Thank you very much, David. That's going to be a hard act to follow, and I don't have any fresh fruit in my talk – but I will next time.

[CHUCKLING.]

DR. GUILAK: And thank you to Gordana and Marcus for the invitation. It's been a wonderful conference. It's very enlightening to see so many different areas.

So, our lab works on all aspects of osteoarthritis. We're very interested in cartilage degeneration, repair, basic pathophysiology of joint degeneration. And today, I was going to try to focus – you know, I don't consider myself an imager, but I was going to try to focus on some of the areas where we use more imaging in the context of tissue engineering, which are areas of joint mechanics, cellular biomechanics and tissue engineering and stem cells – working at all these different scales and trying to understand cartilage function at these multiple scales – that of the whole body down to single cells and molecules.

Now, one of the problems we're always faced with is that cartilage has a very poor capacity to repair. If you have a defect in your joint, there's no blood vessels, no nerves, no lymph system. So, it doesn't repair, and it generally degenerates to an osteoarthritic condition.

Now, there's hundreds of papers each year coming out, trying to fix these cartilage defects; but, in fact, the real problem is osteoarthritis. And it's degeneration of the entire joint. And so there may be about 25,000 treatable cases of those defects every year. There's anywhere from 500,000 to a million cases of osteoarthritis that don't have a tissue engineering type approach for them. In fact, there's really no approach for repairing these types of joints, other than a joint replacement. So, you go straight from taking Motrin and maybe some nutriceuticals to having your joint lopped off and replaced with a metal-and-plastic prosthesis.

So, for many years, we've been working and trying to regenerate pieces of cartilage that we can use to try to repair – and many groups have worked in this area –

to try to take stem cells. We've mostly worked with adipose stem cells and now bone marrow meso- [unintelligible] stem cells and using, you know, the classic techniques of putting the cells in a gel or other scaffold. And some of the tests that we've run early on is to put them subcu, grow them in culture for weeks or even months. This is three months subcu. And sure enough, I think we can all show that we can grow a cartilaginous piece of tissue one way or another.

Now, what our problem has been over the years is that this little piece of cartilaginous tissue that has type 2 collagen and proteoglycan and all the right constituents has none of the right mechanical properties. And if you were to try to treat an osteoarthritic joint with this material that has basically the consistency of thick snot, it really doesn't work. And, you know, as early tissue engineers, we were very naïve when we went to this standard paradigm that we were going to have a scaffold. And as David just showed, we wanted it to completely disappear, and we started off with our hundred percent of our mechanical properties –

whatever they may be. For cartilage, it’s one of the most complex tissues, so we can talk about those mechanical properties for hours.

But as the scaffold degrades, our stem cells, our convercytes[?] make more so that the sum of the properties is a hundred percent of what we want, whether it’s in compression, tension, sheer viscoelasticity and so on.

So, every time we tried this, we got something closer to this, where – [chuckles] – our scaffold degraded. Our ECM made a little bit of nothing, and it was just one after another where, sure, you can fill a defect. It’s like filling a pothole in the road. When there’s a lot of road around it, it can support that defect-filling material. But when you have to repave the entire road, it doesn’t work.

And so we really retooled and said, “All right. What if we make a scaffold that has the mechanical properties of cartilage from time zero, and it degrades *very* slowly – one to two years – so that even if we use a stem cell or something that’s not highly

active, we give it a lot of time to make this extracellular matrix?

And so these mechanical properties of cartilage, as I mentioned, are very complex; and when we try to replicate the anisotropy, the viscoelasticity, tension compression nonlinearity, so cartilage intention is one to two orders of magnitude higher than it is in compression, we had a lot of trouble getting those types of properties with gels. So, hydrogels, agarose, alginate – [unintelligible] – and all these gels are wonderful for growing cartilage because they're so hydrated and have great diffusion properties, but they're extremely weak and have properties on the kilo[?] Pascale [phonetic] level of stuffiness, instead of mega Pascale.

And so nonwoven, fibrous polymers have improved those properties. They're highly porous, but it's very hard to control anisotropy inhomogeneity and these other types of properties that we need.

Now, if we start going to structured materials, we start to get closer. And weaving, knitting – these

textile processes – can give you anisotropy, but only in plain, so you end up with a two-dimensional material like your shirt, which has good properties in two directions, but you can't make it thick without layering it. And then you have trouble with lamination and – and so one. And you're limited in pore[?] size.

So, what we did was turn to a technique for three-dimensional textile processing and making a composite material, so we have basically trying to mimic cartilage by having a woven, composite material where we could control the structure in 3-D. And to do this, one of my grad students, Frank Mutos [phonetic], built a three-dimensional weaving machine. So, instead of weaving one layer at a time, this one weaves 13 layers at a time using 600 resorbable fibers. And you can put in any fiber you want to, and so all these fibers get fed into about a one-millimeter layer of tissue which looks something like this. So, you can make all different structures. This is an orthogonal one where you can see the X, Y and Z fibers. And depending on how tight or loose you knit it, you can basically control the properties in

all different directions. You can control the porosity. This is PGA here. This is polycaprolactone. Again, changing all the fibers. There's too many variables for us to even mess with, so we use a standard orthogonal structure, and it's very porous, and the pores are aligned, as you can see.

So, we put cells in one end in a gel, and we can infuse it thoroughly through to the other side. And what we find is – this is work with Lisa Fried's lab at MIT. We can put cells in here, and these are bone marrow stem cells. And we get good results with adipose stem cells also, but we get very nice proteoglycan, type 2 collagen within these pores that then consolidates the structure. And the histology looks great, but the mechanical properties look exactly like[?] we want them to. And this is years of work distilled into one slide, where we tune the properties so that we want native cartilage properties over many weeks or longer in culture. And when we use this PCL, polycaprolactone with fibrin, we can get within that range of cartilage properties and maintain it over weeks at a time as the tissue is forming.

And then in tension, we can dial in what we want. This is a very stiff material in tension. This is 120 mega Pascals, and we could even get anisotropy. So, in the X versus Y direction, just by changing fiber, packing and diameter, we can get the properties that we want. So, now we can tune all these properties in a material, and so now we can try to cover the entire joint and replace the whole joint surface instead of a defect.

And the beauty of this three-dimensional material is that we can mold it and shape it so that it covers the whole joint, and it's no longer just a focal defect. And this is just proof of principle we can take an MR image, send it to a solid printer in about an hour and then mold it into the exact shape of a joint. And we focused on the hip because we, hopefully, won't need to do this complex process. We can just make it a hemispherical shape.

And when we grow this now – this is adipose stem cells. We can grow these constructs that are fairly large, 25 to 30 millimeters in diameter, in the shape

of the entire joint surface. And this is a structure showing the woven scaffold and cells that have been seeded on there, that grow into the pores.

So, the ultimate paradigm, if this works – and everything looks great in vitro – is that if you have osteoarthritis of the joint, before you would get a joint replacement, and to avoid getting a joint replacement for, hopefully, five to ten years, we can get stem cells from either liposuction or bone marrow, seed them onto a scaffold that’s the shape of the entire joint, remove all the osteoarthritic cartilage and then cap the cartilage surface completely on both sides with this regrown cartilage that has native mechanical properties.

And we’ve got some preliminary animal data that shows the scaffold, because it’s so porous, integrates really well into the bone so it’s held in place. And we’re now in the middle of doing a large-animal study where we remove the cartilage from the hip, replace it with a scaffold either naked like this one, or seeded with cells at the time of surgery, or pre-cultured, beforehand.

And so we're working on this now and trying to advance. At the same time as we go into these pre-clinical studies, we're also trying to look at different structures. And when you think about different tissues that you may be working on, they have very different structures and cartilage. But one of the advantages of three-dimensional textile processing 3-D weaving is you can make almost any structure once you set it up. The hard part is setting up the weaving machines to do this, but once it's set, you can reel off materials.

And then as I mentioned, you have complete control of each of the fibers, so you can functionalize the fibers. You can use them to deliver drugs. One project we've been doing recently with Charlie Gerzbach [phonetic], who recently joined our faculty, is to site-specifically deliver viral constructs. We have lentivirus for TGF[?] Beta and BMP-2, where we want to form a cartilage-bone construct out of one set of stem cells. And what we've shown is that, sure enough, if you deliver TGF Beta, you get this nice proteoglycan and collagen 2 production from

mesenchymal stem cells, but the sites where you deliver BMP2, you get this nice mineralization, as shown by micro CT. And we can just attach the viruses very site-specifically to individual fibers as we weave this.

And in another study, working again with Lisa Fried, we showed that mechanical factors in a bioreactor can significantly improve the formation of cartilage. And I use this because I want to set up the next part of the study, which is trying to understand how mechanical factors – and you can see by the proteoglycan and collagen standing – that, in this flow-by reactor – and we see the same thing with mechanical loading – we get a great improvement of tissue formation.

So, one of our questions has been how do these cells respond to mechanical loading? What is the mechanism, and how can we try to duplicate that in vitro? And so what we've done is try now to understand how these loads get transmitted from the level of the whole body and understanding joint mechanics down to the level of the tissue to the single cell, even down to the cell

membrane, where they get transduced to a biological signal and work their way back up, either in the context of disease – of osteoarthritis, which is very mechanically related – or, as I'll show you, in the context of tissue engineering and bioreactors.

So, to start at the whole-body level, we've been working with one of our junior faculty members, Lou DiFreight [phonetic], who's developed a very nice method to combine MRI with dual fluoroscopy to measure cartilage and ligamentous and other soft tissue strains in real time, in vivo. And so what Lou does is he takes a standard MR image. It's a desk[?] sequence. [He] does a 3-D reconstruction of the cartilage and bone, then takes the patient through a dual fluoro system, where you get biplanar fluoro, basically stereogrammetry, and then aligns, using an iterative point technique, this MR image with the plainer fluoro images in real time.

So, from doing that, you can then track joint motions and calculate cartilage contact and ligament strains during in vivo actions. We can even do very high rates by putting external markers on the subject.

[Unintelligible]’s got something like 27 markers that we track with different cameras. We realign those markers using fluoro and MR, and what we can get are measurements of cartilage strains and ACL strains in vivo during fairly high-speed action, so that someone jumping off a two-foot table here, and we can, while they’re doing that, measure their ACL strains and look at their cartilage contact strains. So, this is very valuable.

We haven’t applied it yet to regenerative medicine tissue engineering approaches. We have applied it to ACL reconstruction, so I think this would be very valuable to do that.

What we’ve looked at, though, is in vivo cartilage deformations and strains, and what we find is that during these activities, we get relatively high strains of about 20 to 30 percent; but what we also see is that we s- -- we have diurnal strains in our cartilage. So, from morning to night, if we take an MR at eight in the morning, and one at four in the afternoon and compare them, we see significant volume changes in the cartilage and significant deformations.

And what we want to do is try to look at what those physiological levels of strain do to cells, and to study that, we've done a number of, now, moving down in scale from the joint level to the tissue level, we've taken pieces of cartilage and, using live confocal microscopy, looked at cell morphology, volume and activity while the tissue is being compressed. And what we find is that as we load the cartilage, the cells are deformed. They lose volume. They exude water, because the cartilage exudes water, and you have these osmotic changes. And what that does biologically is, if we look at calcium ion, it activates a number of signaling pathways. So, this is a dye for calcium imaging. And as you saw, when you compress it, the cells start to light up. And they're responding to something about the mechanical loading of the cartilage. And that's the big question we've had – is, what are they responding to?

From an engineering standpoint, these cells are seeing all sorts of stress and strain and high[?] static[?] pressure and fluid flow, and as I mentioned, osmotic

pressure. They're losing volume because of the change.

So, what we've tried to do is try to distill these down individually. One thing we did early on was look at the effects of stretch. Is it just simply stretching the cells that's causing this response? And we took a micro pipette, pulled on the cell membrane probably 50 cells, three different graduate students, and this is the *one* cell that responded. [Chuckles.]

[CHUCKLING.]

DR. GUILAK: [Chuckles.] And it responded in what's typical of an injury response, but what we found is that these cells are not responsive to being stretched. However, they're clearly responsive to these loads and these other changes.

So, to try to understand that, we looked a little bit more carefully as to what happens to the cells when they're being loaded. And to do that, we took a much more careful look in the immediate vicinity of the

cell, what we call the “pericellular matrix” – which, again, using 3-D imaging now and confocal with collagen 6 labeling, we were able to show that this area is very rich in proteoglycans around the cell and, therefore, has a very high osmotic activity. And we wanted to know how stiff those properties are, so one imaging modality that hasn’t been talked about a lot has been force mapping using atomic force microscopy or scanning probe microscopy, so we can actually image the stuffiness[?] around a cell.

So, this hole is where the cell was. We map it by indenting a 40 by 40 grid, and what we find is that this region around the cell is extremely soft, about an order of magnitude less than the tissue itself. And with osteoarthritis, it’s extremely more degraded and much softer.

Now, what we find is that this region deforms. When you load the tissue, it deforms quite a bit. So, in fact, the region around the cells ends up – and I won’t show you all the numerous papers on finite-element modeling that we’ve done. What we show is that we have very high volumetric strains around each

cell; and these volumetric strains, when we put them into a theoretical triphasic model that takes into account solid phase, fluid phase and ionic phase, working with Mansur Hader at NC State, what we show is that there are these great, large osmotic changes around each chondrocyte [phonetic].

And so when we now look at an osmotic signal, as opposed to a stretch, what we find is that pretty much 100 percent of cells respond to these osmotic changes. So, chondrocytes are very sensitive to their osmolarity and not so much to their stretch.

So, the next question is, how do cells respond to osmolarity? And to understand this, we sort of have to think about our senses, and we all have learned about the prototypical five senses. Well, in fact, there's *way* more than five senses if we count them the same way. And we really think of the senses as these chemical senses, like taste, smell and so on and the solute senses. In fact, we have the ability to sense a number of physical senses. We think of touch as one sense, but touch, pain, itch – [unintelligible] – perception, texture, vibration all use different

sensors in the body; and hot, cold, noxious heat, noxious cold are also different sensors in the body. And, of course, osmolarity is one of our most tightly controlled sensors in the body. It's much more controlled than body temperature.

So, how do we sense these? And ten years ago, we didn't know a lot of this, and it's all been, in a fascinating way, uncovered in the last ten years by this class of ion channels called the "transient receptor potential," the TRP channels. And this is an ever-growing family of channels which are nonselective cation channels – mostly calcium channels that control all of these senses. And most of them are six-path[?] transmembrane channels with a pore loop. Four of these molecules form an active channel.

And so to understand these and put it context, most of these channels have some temperature sensitivity, and the one that everybody is familiar with is TRP V-1, transient receptor potential vanilloid 1, which is the chili pepper receptor. And so it's actually a noxious heat sensor. It happens to be activated by chili

peppers, and that's why capsaicin feels hot. It's turning on your heat sensor.

Interestingly enough, TRP M-8 is our cold sensor. So, cold is not just an absence of heat; it's an actual calcium channel, and you can guess what activates TRP M-8 chemically. It's menthol – right? And so TRP A-1 is a noxious cold pain sensor, and it's activated by wasabi. So, all these sensors are interestingly linked, so you look at them. Garlic, mustard oil, wasabi turn on your pain sensors. It's also the sensor in fruit flies that they sense DEET and Off with. It's called the "knockout fruit flies," the "painless fruit fly." So, anyway, just a brief overview of these channels.

We're, of course, interested in the osmotic sensor, which is TRP V-4. And this controls your thirst in your body, so in collaboration with GlaxcoSmithKline, we did a screen on all these different tissues and, sure enough, in cartilage this channel is highly, highly expressed; also in kidney, where you see osmotic changes.

Now, running through a number of studies, to make a long story short, this channel is present in chondrocytes. It's highly active. We developed these imaging methods to look inside the tissues. This is live chondrocytes inside a piece of cartilage as we activate this channel, and what we see is the agonist propagates over the cells. The calcium wave goes through all of them and goes up. And by using a number of chemical inhibitors and by actually knocking out the channels – this is now a mouse where we've been able to knock out the channel, image chondrocytes for calcium inside the cartilage. What we show is that, in a control mouse, as you change the osmolarity, the cells respond. In the knockout, they're insensitive to osmolarity, insensitive to responding to mechanical load. In fact, these mice get arthritis. So, at six months, you start to see these degenerative changes, and by 12 months their cartilage is gone.

So, what does that mean? Well, we need to control this channel now. We think we understand that some of the mechanical transduction – maybe all of it – is occurring through this channel, so we wanted to screen

a number of compounds. And this is a fairly expensive thing to do, so we needed one of these instruments. And unlike the really elegant, homemade devices that we've seen in all the talks earlier, we bought this off of eBay – [chuckles] – for $3,000.

[CHUCKLING.]

DR. GUILAK: It's a $300,000 machine, and we got lucky. And it was probably one of the most exciting days in the lab, when we were bidding on this. But we got it, and we were able to screen a number of compounds. And, again, working with GSK, who unfortunately has shut down their program in this area, so we ended up doing some of the screening ourselves – we found these compounds that actually are very selective activators and inhibitors of this channel.

So, now that we have this, and we can turn on and off this channel, it opens up the possibility for what we call "artificial mechano-transduction." So, now, instead of loading your cartilage, loading your joints, you can tell those cells when to turn on and off; and this becomes very valuable. So, for example,

if your joint is not being loaded – it's immobilized – and you need to tell it that it's being loaded and artificially exercise it, we can turn on the channel. And in other cases where you have altered[?] loading, and you want to shut it off, we can do that. And this is important for post-trauma, obesity – a whole class of mutations in this TRP V-4 channel that cause skeletal dysplasias [that] we need to now control the channel for.

And in the last couple of slides, I'll show you our application for tissue engineering, which as I mentioned, a bioreactor can be very valuable. When we have these complex shapes, it's harder to mechanically load them. So, what we'd like to do is artificially load them. And one of the real seminal studies in understanding chondrocyte response to loading came from Rob Mock in Gerard – [unintelligible] – and Clark[e?] Hung's[?] group. He showed that if you put chondrocytes in[?] agarose, mechanically loaded them, you could get a significant increase in proteoglycan deposition, mechanical properties and so on simply by mechanically loading them.

And so what we tried to do is replicate this experiment. We took chondrocytes, put them in agarose gel. This is a DIC image, where we can texture-correlate and get the strains in there while we load it. And what we showed was that – we grow them first for two weeks. Then we load them, and sure enough, we get about a twofold increase in proteoglycan deposition. Now, is this channel responsible? When we turn off the channel, sure enough, we completely block this mechanical response. So, we think this channel is what's controlling the response of chondrocytes to loading. And then the converse test is if we take an activator – now, instead of mechanically loading, if we just pulse the chondrocytes with this activator, what we see is that at low doses, we get a very slight increase; but at higher doses, which are still nontoxic, we can simulate the response of mechanical loading – just with a chemical activator. So, that's work that's in progress.

I'll conclude there, and I realize when Gordon[?] asked me to talk about imaging, I said, "I'm not an imager," but a lot of these slides have the word

“imaging” either in “MRI” or in “Imaging Plate Reader” or so on. And imaging function takes all forms. We can look at biomechanical function in the whole body, or done at the level of the membrane. And I see many more applications of this to tissue engineering, regenerative medicine.

One thing I think is interesting is that we can take two relatively standard methods, like standard MR and dual fluoroscopy, and come up with a new method for imaging function that isn’t there before; and that controlling and understanding mechanotransduction could have significant implications – not just in disease, but also replicating bioreactor function. And as I mentioned, for these very complex shapes where it’s going to be very hard to have bioreactors that load everything evenly and so on, maybe we can just cut to the chase and use chemical means to replicate those transduction methods.

So, I’ll conclude there, thank my lab and a number of people who’ve worked on these multiple, different projects; and, of course, funding, almost all from NIH and the Arthritis Foundation. So, thank you for that

– and a number of collaborators over the years. Thank you.

[APPLAUSE.]

Q: Yeah, my first question is what is your model or hypothesis about how force is transducing a signal?

DR. GUILAK: So, what we think is happening is that, as you compress cartilage, you squeeze water out. You have this dense proteoglycan matrix, particularly right around the cell. Those proteoglycans get compressed closer together. They attract more counter ions, and you have an increase in osmolarity and a change – a fluctuation in osmolarity. This channel is osmotically sensitive. And we don’t know exactly how that happens, but it looks like the channel has charge on it, so it opens and closes due to changes in osmolarity.

Q: Can I -- can –

DR. GUILAK: And that’s direct transduction.

Q: -- can I offer another hypothesis?

DR. GUILAK: Sure.

Q: So, I checked. This channel is kind of interesting, because you see it being linked with polycystic kidney disease, and it’s actually – it looks like it’s present in the primary cilium.

DR. GUILAK: Yes.

Q: And so if it is in the primary cilium, that could be a force[?] sensor. And so if it’s sitting there as a primary cilium, if that twitches, that could actually transduce a signal locally.

DR. GUILAK: Yeah, that’s a great hypothesis, but we’ve looked at that, and it turns out this channel, TRP V-4, is expressed in the primary cilium and chondrocytes. If you knock out the cilium, they lose their osmotic sensitivity. So, we don’t know exactly why. And the channel is everywhere, but there’s something about –

Q: But that’s a good thing – isn’t that – relative to the hypothesis?

DR. GUILAK: -- yeah. It’s – well, it supports – it’s not a mechanical sensor, like, if you – it’s not, like, physically tilting the cilium.

Q: Yeah, they[?] can – right.

DR. GUILAK: It’s an osmotic change in the cilium that activates this channel. And we don’t know – I didn’t have time to go into all the ciliary data, but we’ve done a few knockouts and a few chemical disruptions of the cilia; and it looks, probably for reasons to do with the cytoskeleton that’s inside the cilium –

Q: Sure.

DR. GUILAK: -- links to the channel and controls it.

Q: Right. It doesn’t necessarily have to be mechanical, but –

DR. GUILAK: Yeah.

Q: -- as a sensing sensor.

DR. GUILAK: Yeah, so it is. It does look like it's ciliary-driven, so –

Q: Okay.

Q: I have a question. So, in your textile composite systems, what's the optimal way to interface to the underlying – [unintelligible]? You kind of mentioned it was working, but it wasn't clear if you – [unintelligible] – suture, glue. [Unintelligible] –

DR. GUILAK: Yeah. So, what we do is we create a bleeding bed and fix it around the edges, and in the hip it turns out the center of the hip actually – on the acetabular side, there's actually a big hole in the cartilage where there's ligament. So, we can screw it in place from the center and then around the edges. And because it's this really looped fabric, and we have a bleeding bed of bone, as soon as the bone heals, it sort of grabs on to those loops and holds it in place. So, we have to somewhat immobilize

for a couple of weeks, and then it seems to integrate into the bone and just hold those loops in place.

MODERATOR: Any other questions for Farsh? Now, let’s thank him again, please.

[APPLAUSE.]