CHRIS CHEN: Okay. Let's get started. This is Session II. I'm Chris Chen, and we have -- Actually Session II is broken up into two subsessions, one before lunch and one after on cellular function in vitro and both tissue engineering and imaging challenges around that.

I want to start off, and, just for the other speakers, I'll be using the timer just to give you guys a sense of when to start.

So I'd like to thank the organizers for giving me a chance to share with you some of our work. Mainly what I'm interested in is how cells form tissues, and this is relevant both in the context of development and also in the context of tissue engineering.

And one of the things that I was struck by is how different cells behave when they're taken out of the body. And one of the things that I think is very important in that disconnect between sort of what goes on when cells are in tissues and when they're out of the tissues is that they're in a completely different environment. And so one of the areas that I've spent most of my career studying is how cells basically pay attention to that environment.

One of the areas that I think is very important is the role of mechanical forces in this link between what cells do in different settings. And I'll show you some examples of how we use different approaches to study that.

Along the way, I'm going to also talk to you, just briefly mention some of the imaging challenges that I have in this work in the hopes of sort of helping to kind of lubricate discussions.

So mechanical forces are really important in the formation of different tissue forms and you can see this here in the developing gipsofila [phonetic], and you can also see this here in a nice video of the fish.

And what you'll notice is that during the process of development that you have all of these sort of complex tissue forms that begin to get organized and assemble, and those are all driven by forces, single cells that are contracting at either various points along the apex or basal side of the cells to cause a folding. And those forces are also important for cell migration and, ultimately, for the formation of various tissues.

It is also true that, at the cell level, that these forces drive [unintel.] of the organization. They drive the adhesions between cells and the adhesions between cells and matrix. And you can see this here in this movie. This is something I pulled from Mike Sheetz [phonetic], where you look at focal adhesions that are actually changing their structure in response to an agonist that cause cells to contract. And these changes in adhesion ultimately drive changes in cell signaling and function.

So the historical dogma for how these two events are coupled has mainly been driven around what we call a genetic model. And that is that there are gene programs that are turned on that basically act as master regulators that regulate differentiation, turn on forces through miacin [phonetic] expression and ultimately change the shapes that you need for morphogenesis.

But I would argue that it’s more likely that the forces themselves are the organizing principle that brings all of these different coordinated patterns together. So the forces, actually, are important in driving the genetic programs in differentiation, and, at the same time, are driving the changes in tissue morphology.

So I'll tell you a little bit about some of our model systems where we, I think, are starting to understand the process and also tell you a little bit about some of the imaging challenges that we've had.

So our work basically started looking at this model system mesenchymal stem cells, human mesenchymal stem cells.

Ah-oh. That was interesting.

Okay. So what we found was that these stem cells differentiate into different lineages depending on their adhesive and mechanical environment. And I'm showing here just an illustration of this.

We can pattern extracellular matrix onto surfaces, and we can seed cells onto those surfaces, so they attach on these pattern matrices.

What we found is that depending on the size of the spot that the cells are sitting on that they would change their fate.

So, for example, when cells -- these mesenchymal stem cells would sit on very small spots, they would

basically sit on the surface and remain spherical and undergo adipogenesis, the formation of fat.

When the cells were allowed to attach and spread and flatten on the surface by putting them on sort of larger patches of matrix, then they would undergo osteogenesis.

And it turns out that the mechanism by which this happens is that these changes in cell shape drive changes in cellular contractility. And these changes in cellular contractility actually feedback to trigger the differentiation signals.

This is also integrated in the context of changes in matrix stiffness. So you may know that when cells are plated on substrates with different mechanical properties, when the cells are sitting on soft surfaces, when the cells do contract, they can't really generate a stress between the cell and the matrix, and when they can't, then the cells don't feel a stress signal and then undergo adipogenesis. When the cells are sitting on stiff surfaces, then the reverse happens.

And this is quite interesting because we know, obviously, that different tissues in your body have

different mechanical properties, and we think that this acts as a checkpoint, if you will, for the cells to differentiate into the appropriate tissue types.

So here's an example of one of the experiments that we've done to study this process. What we do is to measure forces, we place cells on basically elastomeric substrates with vertical cantilevers that are on those surfaces.

So when cells attach and spread, they attach on the tips and then when they contract, those tips deform, they deflect, and so you can imagine you can change the relative rigidity of the substrate just by changing the height of the pillars. They get softer.

And what we see is that when the cells are sitting on rigid surfaces, they undergo osteogenesis, and you can see this by various markers. And when the cells are on soft surfaces, then they undergo adipogenesis.

Now, interestingly, what we found is that when the cells are sitting on the softer materials, they stay more spherical. They don't spread out and flatten on the surface. And when the cells are sitting on stiffer surfaces, then they are able to spread.

We went ahead and started to try and understand how these things are linked. So if we look at, for example, traction force, which is the force that the cells are generating against the underlying substrate. What we see is that when the cells are sitting on different stiffness substrates, they basically generate the same amount of force, and what differentiates the cells is that the cells that are more spread generate more force.

The other thing that we find is that if we look at the focal adhesions, those spots that I talked to you about earlier, those focal adhesions assemble more efficiently when they're sitting on substrates that are stiffer, and what's driving that is that when they generate more contractility there's more stress at the surface and that stress is required for focal-adhesion assembly.

So this is just to show you how these different pieces of the puzzle are linked together.

Now, what I wanted to tell you is that in this set of data, it's actually a fairly complex set of data, in the sense that we have fluorescent images that we're sort of quantifying to look at focal adhesions. And traction-force data is complex because it's a vector-

based dataset. And all of this is sort of spatially organized. And so figuring out how to sort of compare and analyze and correlate these various pieces is something that we do in sort of a custom setting.

And one of the things that I was thinking about in this question-and-answer period earlier is that there's really no common platform to do this, even within our own lab. And so that's something that I'm very interested in.

And I want to give you a sense of how complex this dataset is. So here is a single cell recorded for about an hour or two, and you can see that what we're measuring are those forces that the cells are generating under every point on the surface.

So what I showed you earlier were correlations looking across thousands of cells in a single snapshot in time. But, obviously, cells are dynamic, and so you can imagine that what we really want to do is get a sense of the force changes over time, and not just sort of a single-scale or metric of force, but the entire sort of spatial distribution of the vector maps themselves. How to actually handle that data is something that I'm still thinking about.

So in addition to when we think about imaging, you guys often think about sort of scale or quantities, I'm also thinking about vector quantities.

So I mentioned that there's these changes in force that are correlated with increases in, say, oxygenic differentiation, and that's caused by changes in the adhesive environment.

One of the interesting things that we've observed is that if we watch these cells over time, it takes them about a week or two to differentiate. What we see is that there are changes in contractility that precede the developmental differentiation steps.

So, for example, if we take these mesenchymal stem cells and we hit them with factors that increase oxygenesis, about, say, half the cells will undergo oxygenesis and half of those cells won't.

And what we find is that the cells that will undergo oxygenesis at day seven, by the twelfth hour into the experiment, we can see that there's this increase -- dramatic increase in contractility in the cells, and that contractility predicts which cells within the population are going to undergo differentiation.

And, for us, that's very interesting because this not only tells us that there's a subpopulation, but it gives us a measure of which of the cells have that potential before they've actually taken the committed step forward.

So one of the things that I've been thinking about is the difficulty in getting this dataset was really in tracking these cells over days, right? So we're looking at cells where we want to track them on the microscope. The forces that we're measuring are acquired on a six-CX [phonetic] objective, and we want to look at millions of cells. And so figuring out how to do that is something that I'm still struggling with, and, again, I raise this just to sort of make you think a little bit about it.

The other thing is that oxygenesis, just as an example, is one of those differentiation programs where there's no single reporter that correlates to oxygenesis. There's an array of about 20 genes that need to come on at different times.

So the way that we ultimately decide or determine whether these cells have become bone cells is that we have to do real time at the back end, which is not live. It's not non-invasive. And so, even at the

end, you can’t then recollect your cells to do something else with them.

So it would be really great if we could find ways to functionally determine whether these cells are undergoing differentiation with other means.

So just getting back to my story, this is basically the control system that we’ve been looking at. So when cells attach on surfaces, the integrin receptors bind, the cells start to spread out, that spreading is dependent on the stiffness of the substrate. If the cells are able to spread, that provides a permissive signal that allows growth factors to trigger miacin-based contractility in the cells.

The miacin contractility ultimately feeds back to help integrin assembly to form focal adhesions. And we think that that focal-adhesion assembly is critical in driving the commitment of these cells.

What I don’t have time to do is show you, obviously, lots of boring biochemical data that sort of shows how this works. So you can imagine that the way that force is regulated is through increased miacin activity. What drives that in these cells is this [unintel.] tPA pathway.

If we directly up or down regulate row activity without changing any of these parameters, then we can trigger the cells to differentiate as we want them to.

Now, the other interesting thing is that what I've shown you is a dataset looking at bone and fat, but what we see is that, depending on the [unintel.] cues and the adhesive cues, the mechanical cues, the cells can undergo different differentiation programs.

So we see, for example, in the presence of BMP, the cells will switch between bone and cartilage, and in the presence of TGF beta, between muscle and cartilage.

And the reason why I want to show that to you is simply to tell you that we think that these cells are not just looking at mechanical cues, but they're obviously combining sort of biochemical signaling and mechanical cues. And one of the questions that we're really interested in is how they combine these two sets of, in some ways, different sets of information to integrate their decisions.

The other point I want to make is that this link between cell shape and differentiation and mechanics

is not limited to just this one cell that we happened to look at, but seems to be a fairly general mechanism that is, perhaps, embedded within a lot of cell types.

How it's used is different in different cells. So, for example, in epithelial cells we find that this link drives a switch between epithelial and mesenchymal transition. So depending on the cells, the system is used in different ways.

One of the other things that I wanted to share with you is that we've been interested not only in forces when cells are sitting on two-dimensional surfaces, but what happens when we go into three dimensions.

And, obviously, the reason why that's important is if there really is this link between how the stresses are imparted on cells and how they then function, those stresses change dramatically when you're in a three-dimensional environment. The directions of the force vectors change as well as the magnitudes.

And also, just from a technical standpoint, we're interested in it because the only methods right now to measure cellular forces are 2-D systems.

So the way that we do this is that we embed cells within linear elastic materials. That turns out to be important just to solve the mechanical problem. So basic hydrogels.

And so, here, they're sitting in a polyethylene glycol based hydrogel that's degradable by MMPs, so cells can be polymerized within it and invade and spread within the environment.

What we do to measure those forces is we have to find, basically, the deformation fields within these structures. So you have a cell that's sitting within here, and what we do is we put in a high-density of fiduciary beads that we then track. The resolution of our force measurement is directly related to how many beads we can track. So, for us, high density is actually much more important.

Once we actually capture that dataset, then what we can do is we can poison the cell so that it doesn't contract anymore and the beads shift, and then we can look at the deformation fields as a result of that.

So once we have the deformation fields, what we do is we have to generate a finite-element model of this entire system for every cell in every geometry, and

then we have to solve the mechanical problem of understanding how the stresses ultimately drive these deformation fields. So this is a mathematical problem.

So for this conversion of this dataset into something that we can now model, we have to image the cell and then generate a finite-element grid of that cell and then solve this problem in sort of computational space and then come back and remap back onto the image dataset.

So one of the things that I find very important for this kind of work is that the image acquisition and analysis right now are not integrated in any way. So we use eight different programs, shifting from the image acquisition all the way down here and then back up. And each program, of course, the input dataset and the output datasets are totally incompatible. So my students then have to write MATLAB codes to then translate the outputs of one into an input dataset that the next program can read.

So, again, along the lines of sort of, Sure, that works fine for one problem, but how do we develop a common platform that would then be useful for many people to use.

I want to show you this dataset. This is a cell that is now starting to extend one of its processes down, and I want you to look at the time slices. The reason why this is done every 30 minutes is because that's the fastest that we can acquire without becoming phototoxic to the cells.

And so we're taking 200-nanometer slice resolution across this with a spinning disk. There are faster ways to do that now, but the light toxicity is actually quite high.

So I would like to find ways to make this faster. Why? Because during this process -- right? -- you can see as this thing extends it's sort of boring. If we take a single slice through this process and just watch it, it's actually very dynamic, dynamic enough that we would like to get three-second time slices, because the cell is actually extending and pulling back very, very fast during this process. So, right now, we can't do that.

The other thing that we're very interested in is you can imagine that if there is this link between cells and their cell-matrix adhesions, their structure and ultimately their function, then, when cells make

contact with neighboring cells, then, obviously, that completely changes the mechanical environment again. So what happens in multicellular systems?

I want to just show you here these are endothelial cells that have been cultured in monolayers and what happens when endothelial cells and most cell types come into close contact then they form sort of a tight junction-based monolayer.

When they do that, all cells are contractal [phonetic]. Then, when they contract, their forces don’t just transmit down to the underlying substrate, but they also pull on their neighboring cells. When they pull on their neighboring cells, you can sort of imagine there’s only a limited amount of miacin that’s in the cell. Some of that miacin is now -- the energy is transmitted to neighboring cells instead of to underlying substrate.

When that happens, the cell’s focal adhesions, which are, as I told you before, force sensitive that are in the middle of these structures disappear, because they don’t generate stress on those substrate interfaces anymore.

On the other hand, the adhesions that are at the edge here become incredibly large, and that's because the cell is experiencing not only forces from itself, but all of its neighboring cells -- a tug of war, right?

What that does to these cells is it triggers proliferation. So cells at the edges of the monolayers will proliferate, while cells in the interior won't. And that is all driven by a force-driven process.

And you can see here, we made these donut-shaped monolayers, and you can see on the interior edge, even though this is a free edge, it doesn't experience the same kinds of forces, and those cells on the inside don't proliferate to fill the hole, but the cells on the outer edge do.

And so this is a way in which forces pattern cell behaviors, again, not just at the single-cell level, but at the multicellular structural level.

This is just sort of a higher-resolution image of these types of structures, and what you see is that, if we just look at this, is you can look at the cell boundaries, but what you're notice immediately is that, in this microtubule stain, all of the

microtubule organizing centers are facing away from the nucleus to the free edge.

And if you look at these structures, they're all oriented. So all of the cells are pointing outwards, away from their neighbors. And the cells that are maybe two layers in now are randomly organized.

So the reason why I want to bring that up is that, again, the mechanics of being oriented is different from being non-oriented. The cells' mechanical forces that are generated at a leading edge are very different than a rear edge.

So, again, if you think about multicellular systems, then we're not just talking about sort of generic, force-generating muscles that are contracting uniformly all around them. They're oriented, and that orientation will change polarity. It will change the direction in which they migrate. It will change how they export various proteins.

Now, when we think about multicellular systems in 3-D, now things get a little bit more complicated, because not only do they generate forces for driving, say, proliferative responses, but those forces remodel the

matrix, and you'll hear about a number of talks that talk about this later.

So I just want to show you a couple of illustrations of this. This is a collagen gel that we've put into a sort of a microwell [phonetic] where we have pillars. And you can see that these cells will contract the gel into kind of a tight ball, normally, but because these anchors sort of capture the gel on the edges, then the collagen gets aligned, and, again, you'll see some examples of sort of systems that are a little bit like this.

What we've been doing with this is looking at a couple of different things. So one is -- Here's one that has floor posts, and by doing this, when the cells contract this gel, they generate non-uniform stresses within this 3-D structure. The stresses are very high along these parts of the pillars and then they sort of become lower everywhere else, and we can form these gradients of stress as a model system to see how cells respond to the stresses.

And you can see, for example, here that fribronectin expressions, tinacin [phonetic], et cetera, increases in these areas of high stress, but not in areas of low

stress. Cells are remodeling as a result of those stress fields.

And not only that, we could see here we've put in a fibronectin frat [phonetic] with Viola Vogelstein [phonetic], where we can see that when frat goes down that means that the fibronectin fibrils are being stretched, and we can see that stretch in those regions.

The last thing I want to show is that we've been putting some cardiomyocytes into these devices, so that we can measure forces when they contract, and the reason why I wanted to show this to you is primarily for the time component.

So you can see here we're imaging calcium, and you can sort of see the calcium waves [phonetic] when these cells start to synchronize. And one of the issues is being able to record these sorts of datasets fast enough to capture the contractility events.

What I would like to be able to do is what Ralph showed, which is that we'd like to be able to take sort of molecular-level resolution, subcellular resolution of the structure within these constructs during the force -- the motion of the constructs.

These are fixed samples, obviously. I would love to do this live, and we just can't acquire fast enough. Because these are spontaneously beating, we don't have a way to synchronize our imaging to the beats as was shown earlier. So, again, just sort of examples of things that I think would really help the field.

So one of the things that I want to just conclude with is just showing you that I really believe that cellular forces, [unintel.] architecture, cell structure, adhesion signaling, are all tightly coupled in a way that allows us to link tissue form and tissue function, and it's that link that, to me, makes it so important that we have good tools to really characterize, quantify, measure those structures, because if the structure is form, and form is function, then we need ways to do that.

So I'm out of time. So I think we have maybe a couple of minutes for questions. [Applause].

I'd ask myself a question, but -- [laughter]. Go ahead.

MALE SPEAKER: So I have a question about the relationship between the cell shape and force. It

seems that the tension in the cell is always correlated with the cell shape. And I'm wondering if there's some way with your PDMA, MS microstructures to be able to force the cells into a defined shape and then manipulate the tension that they have on the substrate by the elasticity of the substrate and try to sort out whether the differentiation of cells is more to do with cell shape or more to do with the ability of that cell to apply force to its surroundings.

CHRIS CHEN: So we have done that experiment. I just didn't have time to sort of explain it. It's both. And so you can override the effect of cell shape by fixing it to a specific shape, and you can say, Increase contractility, drive osteogenesis or decrease contractility and drive adipogenesis.

You can also take a cell and put it into different shapes and drive these same functions without changing contractility. And so the cell is using both in some ways, and it isn't entirely a linear process.

MALE SPEAKER: Thanks.

FEMALE SPEAKER: So when you say that contractility precedes osteogenesis, do you know that if in your

cell population every cell has the same propensity to respond to the forces? And another way, can like the -- Is it -- Like pure stochastic processes, there are some subpopulations which are more responsive.

CHRIS CHEN: Yes, that's a great question. So the only dataset that we have that answers that question is this dataset where we followed longitudinally these populations and looked at their contractility.

If you look at the dataset, you'll see that there's a subpopulation that increases their contractility more. Those are the ones that all undergo osteogenesis.

Within that population, there's a spread of contractility. Within the other population that didn't increase contractility, there's also a spread.

What we haven't yet been able to do is sort them. So, right now, we're developing reporters that are force responsive, so that we can take these cultures and then sort the population that responded from the population that didn't, before they actually differentiate.

FEMALE SPEAKER: Could be a marker.

CHRIS CHEN: Right. Yes. And then ask if this is a stochastic process or not. So I don't know.

Oh, okay. One more.

MALE SPEAKER: Chris, yes, great presentation. So, as you've shown, and we use extensively, cells exhibit contact guidance in these aligned three-dimensional gels. So how do you take your block diagram, all these interactions that you've been parsing out, and explain how cells sense aligned fibers?

CHRIS CHEN: They do sense aligned fibers, but I don't know how. If I can sort of simplify that, when we look at micropatterns' surfaces that have lines on them, cells also align, right? So it isn't necessarily the topography per se, but we do see that typography has a larger effect on alignment than just where the adhesions are.

If we look at it dynamically, very fast, what you find is that when a cell pulls on a -- contracts isotropically on an aligned fiber, you can imagine there are adhesions that are pointed perpendicular to the fiber, right?

What we see is that those adhesions don't grow, and they'll break because they can't grow. The stress gets too high. The ones that are aligned, grow. And so then if you imagine you cycle this a few times, then only the adhesions that are in alignment, which happen to be the ones where the actin is aligned, are the ones that survive. And so, over time, this all becomes progressively more aligned. So I think that that's what's going on.

MALE SPEAKER: As opposed to a differential, mechanical tension that the cells experience, if they're pulling with the fibers versus against the fibers or a differential porosity?

CHRIS CHEN: Yes, sorry, I was answering in the context of our micropatterns, which are stiff, right?

MALE SPEAKER: Ah, okay.

CHRIS CHIN: Given that observation, I guess I would predict that, on the collagen fibers, that if the adhesion and the forces pointed perpendicularly, it can't generate the stress that's needed for the adhesion to grow. So perhaps a different mechanism, but it's still the same system.

MALE SPEAKER: Thank you.

CHRIS CHIN: Yes.