LORNA GIBSON: All right. So last time we were talking about tissue engineering scaffolds. And what we're going to talk about today still has to do with tissue engineering scaffolds, but we're going to look at it from a different perspective. So last time we were looking more at sort of a clinical perspective, and looking at those osteochondral scaffolds for repairing small defects in cartilage. And today what we're going to talk about are how cells-- how biological cells, interact with the scaffolds. And there's various kinds of interactions. So we're going to go through a bunch of these.

So the first one I'm going to talk about is degradation of the scaffolds. Then we'll talk about the cell attachment. Cell morphology-- so the shape of the pores in the scaffold can affect the way the biological cells-- what shape they have. Biological cells could also contract the scaffold and apply mechanical forces. So we're going to talk about that.

The stiffness of the scaffold and the pore size can affect the speed of cell migration. And the stiffness of the scaffold can affect the differentiation of cells, so from one cell type to another. So I thought today I'd talk about that.

This probably won't take the whole hour. The next topic is on energy absorption in foams. And so we'll probably start that towards the end of the lecture. OK.

So the idea here is that we're looking at how scaffolds are being used, really, to provide a 3D environment to characterize the behavior of cells. And in particular, how the cells interact with their environment. So let's write that down. So how the cell behavior is affected by the substrate it's on.

OK. So the first thing we're going to talk about is scaffold degradation. And if you think of the native extracellular matrix, the cells secrete enzymes which resorb that matrix and then they also deposit new matrix. So it was kind of like what we were talking about with the bone. The bone is always being resorbed and deposited. And if there's a balance between that those two, then the density of the bone stays the same. And if one of the rates gets out of whack, then you get osteoporosis and you lose bone mass.

So the idea is that in just the native extracellular matrix, the cells are producing enzymes that degrade the scaffold. And those enzymes are also going to degrade the tissue engineering scaffolds as well. And you want to be able to control the rate of degradation, versus the rate at which the native extracellular matrix gets deposited. Excuse me, sorry.

So you can kind of imagine if the tissue engineering scaffold did not resorb quickly enough, you'd have scaffold there. And the cells would be trying to put down their own extracellular matrix, and there wouldn't be a place to put it. And if it resorbs too quickly, then the cells don't have something to attach to. So there has to be a balance between the rate at which the enzymes are resorbing the tissue engineering scaffold, versus the rate at which the cells are depositing their own extracellular matrix.

So in the native extracellular matrix, the enzymes produced by the cells are resorbing the extracellular matrix. And then the cells are also synthesizing so they synthesize ECM to replace it.

So the cells are also going to degrade the tissue engineering scaffold that you put in. And the length of time that the scaffold is insoluble, or so that it remains in the body as a solid, is called the residence time.

And so then we require the scaffold degradation to occur over a time that balances with the new ECM synthesis. And so the scaffold residence time must be about equal to the time required to make new native extracellular matrix. So the degradation rate depends on the composition of the scaffold, on how much cross linking there is, and on the relative density. Obviously, the more scaffold there is, the longer it's going to take to degrade it.

And with synthetic polymers you can vary the molecular weight of the polymer. And sometimes if you have copolymers, one may degrade faster than the other. And you can control the balance of how much of each copolymer you have.

And for natural proteins, like collagen, you can control the amount of cross linking. So you can do the cross linking by various techniques. That's what's called physical methods. There's something called dehydrothermal treatment, where you heat the collagen up to 105 degrees C in a vacuum, in a dry environment. And that eliminates water and causes more cross linking. There's a UV treatment, ultraviolet light treatment, you can use.

And there's also chemical cross linkers you can use. So there's different chemical methods

you can also use to cross link the collagen.

OK. So the next thing I wanted to talk about was cell adhesion. And let's just wait a minute for people to catch up. Are we just about there? So this next slide shows a sort of schematic of how a cell would adhere to a substrate. So down at the bottom here, all these little squiggly lines are representing the extracellular matrix in the native tissue. Or you can think of it as a, say, a collagen scaffold.

But here we have the ECM. And this little blob here is our cell. This is the nucleus of the cell here, the little green blob in the middle. And the cell is attached to the ECM through something called focal adhesion points. And this schematic here is a blow up of that focal adhesion.

And at the focal adhesion there's proteins called integrins. And integrins pass across the cell membrane. So the idea is the integrins attach to ligands on the extracellular matrix. And then they also attached to the sub membrane plaque within the cell. And then that plaque attaches to the side of skeleton. Things like actin filaments within the cell. So this is what attaches the cell as a whole to the extracellular matrix, is these focal adhesion sites here.

And different kinds of cell behaviors-- obviously, things like cell attachment, but also things like cell migration, are affected by those focal adhesions there. So we have that the cells attach to the ECM at focal adhesions. And sometimes you see those referred to just as FA.

And at the adhesion point the cell has integrins. And the integrins are transmembrane proteins, so they go across the membrane. And they bind to like ends on the ECM. And then the other end of the integrin is attached to the submembrane plaque within the cell. And then that connects to the cytoskeleton.

And then different kinds of cell behaviors-- so for example, things like adhesion, and proliferation, and migration. And that cell contraction, we're going to talk more about that in a minute. They all depend in part on this adhesion between the cells and the extracellular matrix.

And the biological activity depends on how many binding sites there are. So if you think of the extracellular matrix, it's got these ligands and it depends on the density of binding sites, how much interaction you can get. So things like how much cell attachment you can get, depends in part on just how many of these binding sites you've got for the cells to attach to.

And that density of the binding sites or the density of the ligands depends on the composition

of the scaffold. But also on the surface area per unit volume of the scaffold. So if you think of first, just the composition, if you have native proteins, like collagen, they have binding sites themselves. They have native binding sites. But if you think of synthetic polymers, like the resorbable sutured type of polymers that we talked about, they don't have binding sites and you have to coat the scaffold with some sort of adhesive protein.

And then the surface area per unit volume of the scaffold is related to the pore size and the relative density. Let's call the specific surface area, surface area per unit volume. And if you think of having some scaffold that's like an open celled foam, you can roughly calculate what the surface area per unit volume is.

So say each strut was a cylinder, then the surface area of each cylinder is going to be 2 pi rl. If each one has a radius r and a length l. Say we had n of them, that would be your surface area. And the volume of the whole scaffold, or one cell, would go as l cubed, the length of each strut cubed. So if we just forget about all the constants here. Forget about n. This just goes as r over l times 1 over l, and that goes as the relative density to the 1/2 power times 1 over the pore size. So the specific surface area depends on the relative density and on the pore size.

And if you have a tetrakaidecahedron cell, you can work out exactly what that relationship is. It's sort of a model. And that gives you the relationship there. And in this particular case, I think the relative density was 0.5%. And so it's a constant over the cell size.

So one of the things we did in my group was look at how cell attachment varied with this specific surface area. So we seeded cells onto scaffolds of different pore sizes. We kept the relative density constant and we changed the pore sizes.

Remember I said, when we make these scaffolds by freeze drying we can control the pore size, by controlling the freezing temperature. And we see that it's just a linear relationship between how many cells attach, or the percentage of the cells that were seeded that attach, and the specific surface area. In here we used MC 3T3 cells. It's sort of a standard cell one that you can get. So Fergal O'Brien was the post-doc in my group who did that.

So I'll just say we find cell attachment is proportional to the specific surface area. OK. So that's the cell attachment. So you can see how the scaffold design is going to affect how the cells attach. So there's some relationship between them there.

Another thing people have looked at is cell morphology. And so if you change, the sort of, orientation of the pores, how does that change the orientation of the cells? So this was a study done in another group. So here we have randomly oriented fibers that make up the scaffold. And here they're not perfectly oriented this way, but more or less. And then these are cells that have been seeded onto them, so that the green staining is the cells.

And you can see if the scaffold is random, the cells themselves line up with that fiber structure and become more or less random. And if the scaffold has fibers that are aligned, then the cells, they also line up and be aligned. So the morphology of the cells can be affected by the orientation of the scaffold pores.

Also the cell morphology can be affected by the stiffness of the cells. Or the stiffness of the substrate. So this is a substrate. Here this was a PEG-fibrinogen hydrogel. And they varied the cross linking of this hydrogel. So they got different modularly for the hydrogel. So these numbers here, are all the stiffness of the four different hydrogels. And you can see the cell morphology changes from being a spread out thing on the least stiff substrate, to being just a little spherical or circular blob on the most stiff substrate. So the cells respond to the substrate. And so how the cells behave, depends in part on their environment.

So I wanted to also talk about womb contraction. And talk about how cells contract scaffolds as well. So one of the things people have found when they look at say, skin and regeneration of skin-- so say you had somebody with a burn and the surgeons will clean the burnt out. And then what will happen as it heals, is scar tissue will form. And the scar tissue forms in conjunction with the wound contracting.

So cells will actually migrate into the wound bed and they'll pull the edges of the wound together to try to close the wound. And they won't close it completely, but they'll partially close it. And that's called wound contraction. And that is thought to be associated with the formation of scar tissue. So the cells can actually apply mechanical loads. And they can contract the wound.

And one of the things that Professor Yannas found was that if you use one of his collagen and gag scaffolds, you can inhibit that wound contraction. And if you can prevent the wound contraction from occurring, you also prevent the formation of the scar tissue. And that allows normal dermis to form. So you get normal skin.

So this photograph here is of somebody who had burns over their entire torso. And they put

this tissue injury scaffold on this part at the bottom, but not on that part at the top. And you can see these lines here are contracture lines from the scar formation. And you can see this skin down here is relatively normal.

And in fact, when people look at the histology of the skin the forms using these scaffolds, they find that it is pretty much the same as normal dermis. It doesn't have sweat glands and it doesn't have hair follicles. So you can't sweat from that skin and you don't grow hair. But apart from that, it's more or less normal dermis.

So this observation that if you can inhibit the womb contraction, you can prevent scar formation and you can get normal dermis to form. That's led to some interest in just seeing how is it that the cells do this contract l process. I think hitting the thing and my battery is dead.

So one of the things people have done, is they've just taken what's called, free floating scaffold. They've just taken little disks of scaffold and put it in a cell culture medium in a Petri dish. And they find that if you put, say fiberblast on it, the fiberblast will contract that scaffold. And people have measured how much the diameter of the scaffold changes. And so they've kind of measured this contraction just by-- it's almost like measuring a strain.

And what we wanted to do is we wanted to try to measure the forces that were involved. So we first developed something called a cell force monitor, and I'll show you that. And then we tried to calculate how much an individual cell could apply in terms of the force. So we used this scaffold here. This is the same collagen GAG scaffold I showed you before. And here's the cell force monitor. So that's just a schematic of holding a piece of the scaffold between two clamps. So here it is in elevation view.

And then I'll just build the whole thing up, so you can see how it works. So it's on a base plate. It's attached to a horizontal stage that's adjustable. Then there's a very thin beam here. So this is another adjustable stage here, and this very thin beam here. And that's attached to one end of this clamp. And here's the matrix. And this is attached to this other adjustable stage here. And then when we have a proximity sensor-- so what's going to happen is, this is fixed over here. The scaffold is going to contract with the cells applying these contract l forces.

This beam here is going to bend and the proximity sensor is going to tell us how much it's bent. So we can measure how much that's bent. If we know how much that's bent, and we calibrate the beam, we can figure out the force in the beam. OK. So we can figure out how

much is the total force that the cells are contracting with. And then this just is a little silicone well with some culture medium. So that's the whole setup there. Toby Fryman was a student who did that, who's married to Professor Van Vliet. And I have a very big soft spot for both of them. So anyway, that's the set up.

And the thing that Toby measured was the force, by measuring how much that beam deflected. And he measured the force over time. And he found that if he put say, a certain number of fiberblasts onto the scaffold, the force would increase and then reach an asymptotic point. And you could describe these curves by this equation here. Here's the asymptotic force. And it's a 1 minus exponential of minus time over a time constant tao.

And then this number here is the number of fiberblast that were attached at 22 hours. So he ran these tests for 22 hours. And when he was finished, he could count the number of cells that were attached in the scaffolds. So you would just wash off any cells that weren't attached and you can do accounting of how many cells are left.

And one of the things that he found was that if you plot that asymptotic force-- if you plot through this force over here, against the number of cells that were attached, you just get a linear relationship. And the slope of that is roughly the force per cell. And that's about one nano neutron.

Now this is a little deceptive because not all the cells are contracting. And not all the cells are lined up in one direction. So there are cells in different orientations. But just as an order of magnitude the cells are applying something like one minute per cell. So that's the effect of the cell number.

Another thing he did was he looked at what happens if you change the stiffness of that beam if. You make that beam in the device different stiffnesses, how do the cells react. And so the stiffness here are the stiffness of the system. So there's 0.7 newtons per meter up to ten, so it's a factor of a little over ten difference.

And you can see the displacement per cell changes. The stiffer the system is the less the cells can displace it. But if you then plot the force per cell, you find that the force per cell is about the same. So you develop about the same force. So that suggests the cells are capable of applying a certain amount of force, and not any more force. No larger force. So he did that.

Then we were interested in what was the mechanism of this. How were the cells applying this

force? Because I was kind of surprised to find out the cells even could apply forces. So we were interested in understanding the mechanism of this. And one of the things we knew that we didn't quite figure out how this all worked together was, we knew that the cells elongated.

If you just take a substrate, like even just a 2d substrate, and you put cells on it they'll be rounded to start out with. And over time, over a few hours, they'll spread. And that's pretty standard. Many types of cells will do that. So we knew the cells were starting off as rounded and they were spreading. So the cells are getting longer, but our whole scaffolds getting shorter. And so it wasn't obvious how was the cells going longer, but the scaffold's getting shorter.

And so the next thing we thought we would do is just watch the cells and see what they did. And so we measured the aspect ratio of the cells at different time points. And we did this by just impregnating the scaffold in the cells at different time points with a resin, and then using a stain, and then using digital image analysis.

So what we found was that the fiber of the fiberglass morphology looked like this. So the long thready things of the scaffold, and these little blobs here are the fiberblast of the cells. So here at time 0 you can see-- like I said, the cells are pretty rounded they're not very spread out. Here at eight hours you can see-- here's a cell that's gotten longer. Here's another one. This guy here is still rounded, it's not doing much.

22 hours, again, some of the cells are quite elongated. Some of them are still not that elongated. So they don't all become active. But one of the things we noticed, if you look at this image here, you can see these cells are attached at one end, and at the other end. But they're not attached in the middle. There's sort of a gap between the cell and the strut.

And this is another example here. Here's a cell here, and this is the collagen GAG strut that it's attached to. And you can see it's attached to the two ends, but not in the middle. And this starts to explain how it is that the cells are elongating but the scaffolds getting shorter. It's that the cells are just attached at two ends. And the cells are moving along a strut and they're attached to the two ends. And if you think of the cells attached through those focal adhesion points, they're applying tension to the cell. And the actin filaments in the cell are in tension. Obviously, filaments can't be in compression. They're only going to be in tension. And what happens is that puts the stress into compression. And if the struts in compression, at some point it's going to buckle. And you can see this strut here has basically buckled under that cell.

And so if the cells are getting longer, and they're buckling the struts, then that's going to shorten the struts and the whole scaffold is going to get shorter.

And so then Toby plotted the aspect ratio of the cell, so that is a measure of their elongation against the time. And again, he found one of these curves with the same kind of form as the curve for the forced development. And he found the time constant here for the change in the aspect ratio was about five hours. And for the development of the force it was about 5.7 hours. So the time constant for the elongation of the cells, more or less matches up with a time constant for developing the force.

So that's what that says. And that suggests there's a link between the elongation of the cell population and the macroscopic contraction of the population. So then we wanted to take it one step further. And we wanted to look at what the cells were doing live. Like as they were doing it.

So Toby devised this little schematic thing here. So he had just an optical microscope. He had a microscope slide with a fairly thick well in it, so that we could put culture medium in the well. We put a cell seeded matrix in here. And he had a heated stage here. And then he took little videos of what the cells were doing. And this required some patience because as you could see not all the cells did anything. Some of them just sat there and did nothing. So he would set this up for a day, and watch a cell, and it would do nothing. And then he would have to find another cell. But he did find some cells that were responsible for the contraction. And that was it was kind of neat.

So here's the scaffold again. All these little bits here are the scaffold. This is a strut of the scaffold. And this is a fiberblast parked on the scaffold. And this has a little video here. And you can see what's happening is the strut here is starting to buckle. And you can see these two sides here, those two things are coming closer together. So they originally were this piece here, and that piece there. And now they're at that point there.

And then if I let it go a little bit longer, it continues to do that process. And then the final thing--this kind of smushed up mess here is these two things having me brought completely together. And this strut here is some strut down over here. So you can see how the cells are elongating and causing contraction of the scaffold.

Here's a series of stills taken from another video that he did. So this sort of square thing is the scaffold. So b is the scaffold. And a, this little blob here, is the fiberblast. And you can see,

even from this image to this one, you can see that the fiberblast has spread a little. Do you see how it's kind of oozed out along the scaffold there. And eventually it attaches over here.

And you can see that it's buckled this strut underneath it. And here it's a little bit more deformed. It then grabs on down here somewhere and deforms it even more. So you can see that's more deformed. And then Toby put alcohol on the whole thing, which kills the cells and the cell let's go. And you can see you recover some of the deformation. You don't recover all of it, but you recover some of it.

This was another example. And this was kind of interesting. Here there was a scaffold junction where there were three struts that came together, a little bit like a strut. And there was a little cell right there. And you can see the cell elongates. You see how this elongated and its grabbing on up here somewhere. But the amount of force the cell was kind of pulling with must have been less than the-- or rather must been more than the force of the focal adhesion.

Because what happens was eventually the focal adhesion let go. And the cell kind of bounces back and ends up over here. So the cell was kind of snapped back on to the other focal adhesion over here. And here it's rounded again. And here it elongates again. And then this focal adhesion lets go and now it's moved back over to there. So these struts here are so stiff. They're much stiffer, I think, partly because they're triangulated. And it looks like they're just shorter and a lot thicker. The cell isn't being able to deform those. But it's elongating and then focal adhesion was letting go.

So this is a little schematic of what we thinks going on. So the cell starts out-- it's some elongation here. It's attached at that point. It's attached at that point there. And the cell is getting longer. And if you think about it as the cell's getting longer-- if you think about the Euler Buckling formula, the buckling load goes as 1 over l squared. So the longer the length of this piece of the strut of the scaffold underneath the cell is, the smaller the load it takes to actually cause it to buckle. So at some point it buckles like this.

And this is just a little force diagram. So the actin fibers are in tension and the matrix strut is in compression. Sometimes we saw some bending. So you could see if a cell was spanning between two struts, you could get the cell bending the struts as well. That was another possibility.

And so we think that the cell elongation was related to the contraction. The time constants for the two things were almost the same. And as the cell elongates there's a gap between the cell

and the matrix on the central portion. And then the cell is adhered at the periphery of the adhesion points. And then the tensile forces in these act. And filaments inside the cell induce compression in the strut, and that causes buckling.

And then Toby graduated. And then I got another student, Brendan. And Brendan saw what Toby did and he wanted to do a little more with that. Brandon was also involved that osteochondral project that I talked about last time. And Brendan this other thing as well for his project.

So he wanted to measure the force of an individual cell. So when we had that cell force monitor, that was the total force of all the cells in that one direction. But Brendan wanted to know if he could measure the force of a single cell. And now that we knew that the contractal process was related to buckling, We thought, well, we could just use Euler's formula. If we knew what the modulus of the solid was, and we knew what the dimensions of the struts were. So that would allow us to calculate the contractile force of a single fiberblast.

So I think I've shown you this thing here. So Brendan was the one who did these experiments. He cut a single strut out of the scaffold. And the single strut is about 100 microns long. He used a microscope to do this. He then glued it onto a glass slide and he used the atomic force microscope probe to bend the strut like a cantilever beam. And he measured this displacement here. And from that he could back out what the modulus of the solid was. He did these tests in the dry state. But we could extrapolate to the wet state from looking at the behavior of the whole scaffold. So he had a modulus for the wet scaffold solid.

And then this is our formula for Euler buckling here. So that's just the standard formula. I had a student from civil engineering, who looked at hydrostatic loading of a tetrakaidecahedral cell and he looked at buckling. If you had a tetrakaidecahedral cell and you load it in all three directions. He looked at the buckling. And he had calculated that the n constraint factor-- the n squared was point 0.34. So we have some idea of what that n squared value should be. Although it's somewhat of an estimate.

I had a UROP student who took Toby's images and measured the dimensions of the struts. So he measured the diameter and the thickness of the struts. And from that, we just plugged everything into the Euler formula. And we found that the average single cell force is somewhere between about 11 and 41 nano neutron. It was something like 26 nano neutrons. So it would make sense that it's more than the one nano neutron per cell because not all of

those cells were active and they weren't all going in the same direction. So Brendan Harley and Matt Wong did that part of the project.

OK. So that's the contraction. Are we good with contraction? So it's kind of interesting that cells will contract and we can measure some forces.

So the next type of interaction between the cells and the scaffolds that I wanted to talk about is cell migration. And these are some studies from the literature. These are two different studies. But the top one here, they've measured migration rate as a function of the cross linking treatment of a scaffold. And the decreasing stiffness goes this way. And so they're seeing that the speed of migration-- this is in millimeters per day. Cells don't move too quickly. They go millimeters per day.

But you can see that the migration speed, the speed at which the cells can move, depends on the stiffness of the scaffold that they're attached to. And in this study on the bottom here, what they did was they had just a flat 2d substrate. Just a flat polymer.

And what they did was they cross linked one part of the polymer more than the other part of polymer. So over here, this was the less cross linked. That was the soft part. And this was the more highly cross linked. This was the stiffer part. And they found that if they put a cell on the soft part it would migrate onto the stiff part. But if they put a cell on the stiff part, it would start going this way towards the soft part.

But when it got to the interface it would just spread out along the interface. And it wouldn't go into the soft part. So the cells were somehow sensing the stiffness of the substrate. And for some reason, I don't know what, but for some reason these particular cells seem to prefer being on the stiff substrate.

So this is just really showing that there's some interaction between the substrate stiffness and the way the cells are behaving and migrating. And then Brendan also wanted to study this. And he got some of the collagen GAG scaffold. He made some of the scaffold. And he stained that with a stain that made it turn red. So these lines here are all red struts in the scaffold. And then he put fiberblasts on to the scaffold and stained them green. So all these little blobs here that are green are the cells.

And then he used confocal microscopy. And the confocal microscopy allowed him to look at a certain volume of the scaffold. And he had some software that would track the centroid of each

cell as it moved through the scaffold. And so he had a thing he called spot tracking. So each of these little spheres here corresponds to a cell. And the white box is the volume of material that you could see in the scaffold.

And this color scale here really corresponds to time. So I've forgotten which round. I think blue is the original time 0, and then red is maybe five seconds, and yellow was 10 seconds. The different colors correspond to different times. So he could track the path of each cell and also what the position was at different time points. So he knew what the position was at different time points. And obviously from that, he could get the speed of the scaffold.

And he did these experiments on scaffolds of different stiffnesses, as well as, different pore size. And here you can see the cell speed. He's measuring it in microns per hour now. The cell speed increases at first and then decreases with the strut stiffness. So we don't know exactly why this is. But there is an effect between the stiffness of the scaffold and the migration speed.

And another thing he did was he looked at how the cell speed varies with the pore size. And as the pore size gets smaller, the speed goes up. And we're not entirely sure why that is. But I think that might be related to this binding site thing too. As the pore size goes down, the number of binding sites is going to go up. And if you think of the cells migrating by having these adhesion sites, and the adhesion sites are just at the ends of the cells, and the cells kind of putting out a little extension, and then looking for somewhere else it can bind.

The more binding sites there are, the faster it's going to find a binding site. And the faster, I think, it's going to move on. So I think that the cell speed depends on pore size, at least in part because of the increase in the binding sites with smaller pore sizes. So pore size and the migration.

And then the last thing I wanted to talk about was cell differentiation. And this is a study study by Engler. And one of the things he found was he put mesenchymal stem cells on 2d substrates. Just flat 2d substrates of different stiffnesses. And again, he could control the stiffness by cross linking.

And what he's showing up here in the first bit is that he's looking at the stiffness of tissues of different kinds. So here's brain type tissue. Something like one kilo pascal. Muscle might be something like 10 kilo pascal. And collagenous bone-- this is sort of the osteoid that is the precursor of bone, not the bone itself. Is about 100 kilo pascals.

And what he did was he put these mesenchymal stem cells-- so here's his cell onto his substrate. And he varied the stiffness of the substrate. And then he looked at the shape of the cells. So here's the least stiff substrate, so between point 1 and 1 kilo pascals. And here's 4 hours, 24 hours, 96 hours. And these cells formed long processes extending beyond the cell body. And they looked kind of like neurons. So they he called those neuron like. Then there's an intermediate stiffness of substrate here. And these cells became even more elongated. And became something like a muscle cell, myoblast like.

And then cells that were put onto a substrate that was between about 25 and 40 kilo pascals, they developed a shape that was something like an osteoblast, like a bone cell. So one of the things he was looking at here, was how the stiffness of the substrate affected how a stem cell might differentiate into different cell types.

And another thing that he did was he looked at different cell markers. And he found that the cells were expressing markers that were corresponding to the types of tissue. So I couldn't tell you the names of all these things and what they are. But I think the red here is expressing more of a particular marker. And I think these wounds were related to nerve tissue. These wounds here, were related more to muscle tissue. And these wounds here were related more to bone tissue. So the things the cells were expressing also seemed to correspond to the different types of tissue that they were corresponding to.

So I'm just going to end this part by going through a little summary here. So what I've tried to show you today is different types of cell behavior that are affected by the scaffold. And they're affected by things like the number of binding sites, by the pore size, by the stiffness of the scaffold.

So we started with a cell attachment. We saw that the cell attachment increases linearly with a specific surface area. We saw that the cell morphology depends on the orientation of the pores. And that kind of makes sense, they got to line up with the pores. We talked about the contraction behaviors. So the cells bind at the periphery, the cells elongate, and that causes this buckling. And you can calculate the buckling forces. It's around 10 to 40 nano neutrons. We looked at the cell migration speed. That increases with the stiffness of 1D fibers. And we looked at cell migration in the collagen gag scaffolds. So that depends on the stiffness of the pore size. And then there was this final study on the cell differentiation.

So I wasn't going to write any notes on this because the slides I think pretty much explain it. So

I was just going to put the slides on the website at the end after today's lecture. So are we good with how cells and the scaffolds of the environments interact? Because I think it's not so obvious that this actual mechanical environment makes a difference.

People think of the chemical, the biochemical environment. That obviously affects the cells. But people don't think at first that something like the sort of structure of the pores, the pore size, or the orientation of the pores, or the mechanical properties are going to affect how the cells behave. But in fact, they do.

So that's it. And this is all various people who worked with me on these projects. So it was a lot of fun. OK. So hang on a sec here. What's this all about? I'm going to get rid of that. Go away. Here we go.

OK. So are we good with cells and substrates? Yeah? OK. So let's just take a little moment and I'll rub the board off. And then we can start the next bit.

OK.

OK. So that's the end of the medical material stuff. So we talked about the bone. We talked about the tissue engineering scaffolds. And then we talked about the cell scaffold interactions. So now we're going to go back to more engineering topics. And the next thing I wanted to talk about was energy absorption in foams.

So foams are very widely used for energy absorption applications, things like bicycle helmets, different kinds of helmets. You buy a new computer, it comes in foam packaging. And the reason foams are used so much is they're extremely good at absorbing energy from impact.

And in fact, they're better than the solid that they're made from. So let's just look at this curve here for a minute. So here's a stress strain curve in compression for the foam. And the material that it's made from would have the stiffness something like this. It would be much, much stiffer than the foam.

And if you think about how much energy you can absorb, the energy you can absorb is just the area under the stress/strain curve. That's the energy you can absorb in a given volume of foam. And so when you're thinking about these energy absorption problems, it's not just that you need to absorb a certain energy. You need to absorb it without exceeding a certain peak

stress.

So whatever it is you're trying to protect, at some point it's going to break. This is what you want to avoid. You want to avoid it breaking. So you don't want to have a stress bigger than the stress that's going to break whatever it is, your computer, or your head, or whatever.

So say you have a given peak stress that you can tolerate here. And we've normalized things by the solid modules. But just say that's a peak stress here. The foam is going to absorb this amount of energy up here, this whole little shaded region. And the solid is going to absorb that little, teeny weeny bit in there.

So what you want to do is absorb the energy without exceeding a certain peak stress. And the foam is always going to be better than the solid that it's made from. There's a couple other things that make the foams good because they're more or less isotropic, maybe not perfectly. But roughly, they have the same properties in all directions.

Sometimes you don't know what direction the impact's going to come from. And so if you've got the same properties in all directions or roughly the same, that's a good thing. You also want the protective thing to be light. If you're paying for shipping for your computer or whatever, the fact that the packaging is light makes the shipping easier. If you have a helmet for your head, you don't want some big heavy thing. You want something fairly light.

And foams are cheap. So the fact that they're roughly isotropic, they're light, they're cheap, this all helps as well. But from a mechanical point of view, foams are very good at absorbing energy. And so what we're going to do in the next-- the rest of this lecture and on Wednesday-- we're going to see how we can convert these stress/strain curves into what are called energy absorption diagrams.

We're going to look at some energy absorption diagrams that we just measure from the stress/strain curves. And we're going to look at how we can predict the energy absorption diagrams as well. OK. So the main idea here is that the impact protection has to absorb the energy from the impact but without exceeding a certain peak stress.

So the direction of loading may not be predictable. And foams are good because they're roughly Isotropic. And they would have the same energy absorption capacity from any direction. And foams are also light and cheap.

We can say for a given peak stress the foam is always going to absorb more energy than the solid it's made from. So other things that make foams good are that they have a capacity to undergo large deformations. And they do that at roughly constant stress.

So that if you look at the stress strain curve for the foam, you're going to be able to absorb all this energy under here. And these strains that the foam might go to might be 0.08 to 0.09, so huge strains on an engineering scale. And then this is your energy-- would absorb is that area under the stress/strain curve.

So I wanted to say something about strain rates too. So typically we're going to be talking about problems of impact. And in impact, the strain rates are typically on the order of 10 to 100 per second, something like that. We're not going to talk about things like blast. If you have a blast loading, then you have to take inertial effects into account. And blasts involves strain rates, which are 1,000 to 10,000 per second, much, much higher.

So we're going to talk about strain rates that are about 10 to 100 per second, maybe a bit more than that. And for instance, you can roughly estimate what one of these impact rates would be. So you had something that you dropped from a height of 1 meter. Then the velocity on impact is just if you just equate the potential energy with a kinetic energy. The velocity and impact is just the square root of 2gh.

So g's the gravity acceleration. And h is the height. So that's the square root of 2 plus 9.81 meters per second times 1 meter. And that comes out to 4.4 meters per second. And say you had some foam packaging that was 100 millimeters thick. Then you could say roughly that the strain rate would be approximately equal to that velocity over the thickness, so 4.4 per second over 0.1 meters. That' would be 44 per second.

So it's somewhere in that range. Obviously, the thickness could be a little bit smaller, it could be bigger. But it's in that ballpark. And if you do tests on servo controlled instrons or you do a drop hammer test, you can get strain rates in that ballpark. OK. So we're talking about impact and not blast.

OK. So most of the energy that's absorbed is really absorbed in that stress plateau. So if you think of the stress/strain curve, most of the area under the stress/strain curve comes from the area from underneath the stress plateau. So the mechanisms of absorbing the energy are going to be mechanisms that are associated with a plateau stress. So for elastomeric foams, we've got elastic buckling of the cells.

And one of the advantages or disadvantages-- depending on what you want-- of this is that the deformation is recoverable and you got to have rebounds. So if you have an object and you drop it onto elastomeric foam, it's going to bounce around like that. So the elastic deformation is going to be recovered, and you're going to get rebound.

If you have a foam that has a plastic yield point or is brittle, then the deformation is going to be largely from dissipating plastic work or work of fracture. And in that case, there's no rebound. But once you've loaded it, you've crushed the thing, and you've permanently deformed it, and you can't use it again. So sometimes if you ride your bicycle like I do, if you have a helmet, you should wear your bicycle helmet.

If you have a problem, if you have an accident, and your helmet get smooshed, that's it. You have to throw your helmet away. You can't use it again. And this is why.

[INAUDIBLE], even if it doesn't get smooshed, if you hit your head at all, [INAUDIBLE].

Exactly.

[INAUDIBLE]

Yeah. You need a new helmet. Yeah. Go ahead. Talk about helmets because I'm on a helmet conversion thing. Yes. You've got to wear your helmet.

And you should change it every now and then. Anything else you'd like to add about bicycle helmet safety? No, absolutely. You've got to wear your helmet. So I know several people who would have had their head smooshed had they not been wearing their helmet. So you have to wear your helmet.

Let's see. OK. If you think about natural cellular materials, things like wood, they often have cell walls that are fiber compensates. And you can dissipate energy by mechanisms related to the fiber nature, so by things like fiber pull out fracture.

And then you can also have open cell foams with fluids. You can have fluid within the cells. And if the cells are open cells, the fluid effect is really only going to be important if the cells are extremely small or the fluid is particularly viscous, or the strain rates are very high.

So in most cases, the fluid effects aren't important in open cell foams. But, for example, you could try to make an open cell foam that had more energy absorption by putting a fluid into it. So you could put glycerin into the fluid, and that would increase how much energy it would absorb. Or, you can put this honey into it. That would make it more energy absorption.

And enclosed cell foams, you may have an effect of the gas within the cells. But it's really only going to be significant if you have elastimeric foams where the cell faces don't rupture. The cell faces rupture, then the gas is just going to flow out of them, and that's not going to do much.

So the next step is I want to go from having the stress/strain curve that we've become very familiar with, and make something with that that is a little easier to see graphically that shows how much energy we can absorb. Remember, what I said what we're really interested in is absorbing a certain amount of energy without exceeding a certain peak stress. So what I'm going to do is plot another plot that's based on that.

It's going to be the energy absorbed. So w is going to be energy absorbed per unit volume. And I'm going to plot that against the peak stress.

OK. So we're going to look at three different regimes here. We're going to look at what happens in the linear elastic part, what happens in the stress plateau, and then what happens in the densification part. So let's think about the elastic regime first.

And if I moved up-- say I moved up to some point right there where the little x is on the stress/strain curve. Then the amount of energy I absorbed would just be equal to this little bit here. And if I moved up, and then the peak stress would be this peak stress there. We'll call that sigma p1 and w1. And if I moved up over here, I'd be at w2. And that would be sigma p2, right?

And if I know the modulus, I know what that relationship is. And I get a relationship. And these are going to be-- I'm going to do this on log scales here. There's going to be log, and that's going to be log. I'm going to get in that linear elastic regime.

The energy is going to go as the peak stress squared over 2 times the modulus of the foam. Remember, energy is a half stress times strain. And I can say strain is sigma p over e. So it's 1/2 sigma p squared over e. So on my log1 plot here, this is just going to be a straight line like

that.

And then I'm going to get to this value here. I'm going to get to my collapse stress here. So let's call that single star. And at that point, the more I go along here, every point I go along, like that, I'm going to absorb more and more energy. But the stress isn't going to go up at all. So then this thing here is going to go like that because I'm absorbing more and more energy. But the stress just stays the same.

So this is good news if we want to absorb energy. And then once I get to the densification point, then it's going to do the opposite thing. As I go along here, at each increment I'm not absorbing that much more energy. But the stress is going up.

So at some point it turns and starts to look like that. So this part here corresponds to linear elasticity. This bit here corresponds to the stress plateau. And this bit here corresponds to densification.

And the point where I would like to be is right here, because here I'm going to absorb the most energy possible through the peak stress. So you can think of that as sort of an optimal point. And I'm going to refer to that as a shoulder because it's the shoulder between where the curve bends over again.

So I've only got a couple minutes left. But let me just show you one thing and then we'll talk about this more next time. So I've just done this for one relative density. But if you look at the screen, you can imagine I would have stress/strain curves for lots of different relative densities. And let's say these are all at the same temperature and all at the same strain rate.

And I could draw a curve that looks like that for each stress/strain curve. And if I did that, I'd get a family of them. So this is our energy absorbed here. I've normalized it by dividing by the solid modulus. This is our peak stress here. And I've normalized that by dividing by a solid modulus.

And I've got a sort of family of these things, right? They all have the same shape. But they shift depending on the relative density. And then the thing that makes life good is that these shoulder points you can connect with a line. And you can mark off the relative density for those shoulder points on each line.

And then the last step you can do is you can just plot these lines. And you can repeat this for different strain rates. So this would be a family of these guys here. There's a family of those

lines at different strain rates. And then you would join up the points that correspond to each relative density.

So you can make a drawing that looks like this that summarizes the most energy you can absorb for a certain peak stress for foams of different relative densities tested at different strain rates. You could do it for different temperatures if you wanted to. So next time, we'll talk about that. But I'm going to stop there for today. OK? Are we good?