CHRIS CHEN: Okay, I think we're going to try and get started. This digital clock scares me. So it means we have to be on time. So our next speaker is Milica Radisic, and she'll tell you about her system.

DR. RADISIC: Thank you very much, Chris. So I'll tell you about our efforts to make healthy and diseased heart tissue in a chip. One of the main problems associated with making heart tissue in the lab is complexity in structure over many different length scales that then relates to function of the heart tissue. So if you want to have a contractile cardiac patch to be used to basically patch this part of myocardium that was damaged by myocardial infarction and in this particular image the patient had myocardial infarction that was so severe it made a hole in the person's heart such that.the arrow is going through the hole; we have to make a patch that is contractile at the centimeter scale. If you look at the millimeter scale and we look at the myocardial ventricle, in the heart the myofibers are aligned and parallel. They're elongated. But the orientation of the alignment changes as you go through the ventricle.

Then again if we look at the micrometer scale, if you look at individual cells that are responsible for contraction, we see that these cardiomyocytes are all rod shaped, and they have very well developed sarcomeres: these registers of sarcomere. As sarcomeres contract, the whole cell will contract.

And then on the nanometer scale, it’s the arrangement of sarcomeric proteins that enables the appropriate contraction of each unit of the cell. For us to get appropriate contractile function at the centimeter scale, we have to mimic structure over many different length scales. In engineering, we know this is a rather difficult problem. Not only that, but if you look into the heart, by volume it has very little extra cellular matrix. Most of the volume in the heart is occupied by cardiomyocytes. They make 90 percent of the volume of the heart, and they’re supported by very dense vascular network. This would be a cross section, and this would be a side view of the capillaries in the heart.’

About ten years ago, when I was training with Gordana and with Bob,we came up with this electrical stimulation approach where we would take cells, seed them into scaffolds. Thecells at that time were from neonatal rat hearts, and these resulting constructs we would cultivate between two parallel carbon electrodes connected to commercially available cardiac stimulator, and we would apply field stimulation pulses at about 1 Hertz that were super threshold so the tissue contracted with every pulse.

As a result of this, we were able to get tissue with significantly improved functional properties, for example, higher maximum capture rate, lower excitation threshold, and these improved functional properties were a result of improved structure.

So if you look at the cells in the stimulated construct, they're all elongated. They're all aligned in parallel like in the native heart. They have well developed sarcomeres, whereas the non-stimulated, the control tissues, had poorly developed sarcomeres and randomly alignedcells. These tissues, were about ten millimeters long; they were able to propagate electrical impulses which is one of the hallmarks of cardiac tissue, and we had significantly higher impulse propagation velocity in the stimulated group compared to the non-stimulated group.

We know there are many barriers to placing these constructs into humans, and they're related to safety of the cells that come from pluripotent stem cells. So in the meantime we thought that one of the ways we can enhance the field is by using these tissues as model systems for drug testing or to build healthy and diseased myocardium.

So why is making engineered heart tissue and using them as a model better than using heart slices. We see a part of the answer to this question in the slide. First, this is a slice from a rat heart that was kept in the lab, cultivated for about two weeks. And you notice at the end of two weeks, most of the cells are dead whereas in the engineered heart tissue we can control very precisely how many cells stay alive and we have methods of keeping them alive. We know how many cells we seeded. And not only that, there is a shortage of donors that we could get the hearts from – human donors that we could get heart slices from.

One of the first models that we used – that we looked at was a modelfor cell injection, and we did this with Peter Zandstra. The motivation for this was very large number of clinical studies that are currently underway and that are completed with injections of various progenitors of stem cells into hearts of patients.

Although when people do meta analysis they record significant improvements in functional properties – for example, improvement in ejection fraction, this improvement is very small. It’s significant but very small – about 3 percent. So we thought that availability of a model of a cardiac tissue ex vivo could enhance some of this effort by enabling us to study how cells proliferate and integrate in a cardiac environment.

For these studies we used embryonic stem cells, and they’re a good source because we can get many different stages from the same source. We can get undifferentiated cells or fully differentiated cardiomyocytes derived from embryonic stem cells. So in these studies we hypothesized that there is a committed cardiovasulcar progenitor, some were in between these two states that can give rise to cardiomyocytes and that can survive in cardiac environment very well.

This work was published two years ago in PNAS, and I will just summarize it in two slides. So this is what happens to contraction when we inject fibroblasts. The tissue is barely able to contract. This is engineered heart

tissue after injection of fibroblasts. When we inject mouse cardiomyocytes as a positive control, the tissue beats better. But when we inject ES-cell derived cardiovascular progenitors, we get much better beating.

And these improved functional properties were a result of the ability of injected cells to basically spread along the tissue. They didn't block the propagation. They actually – we've got very nice maps of impulse propagation throughout the tissue. And by new understanding we saw that the injected cells that were YFP-labeled were coupling with the host cardiomyocytes. And so not only by immunostaining but when we mapped calcium transients, we saw that basically injected cells were able to fire calcium transients at the same time and in synchrony with the host cells; this tells us that these cells were indeed coupled.

One of the advantages of using engineered heart tissue as a model is that we can digest the tissue and we can prove – we can count the cells and prove that the cells stayed there, and we determined exactly how many cells we had. And each of these time points as we looked, we saw that the injected ESC derived cardiac progenitors were present, and this correlated with the improvement in function such as lowering of excitation threshold.

More recently, we also used this engineered heart tissue as a model to study residual activity of undifferentiated cells. And so we found that when these undifferentiated cells are injected into the tissue or if

they’re present in the mixtures of cells that are injected into tissue, if we wait long enough for about four weeks, we can get evidence of formation of tissues from all three germ layers. It means that there’s ectoderm, mesoderm and endoderm, and this is consistent to studies published by Murry’s group in vivo where they saw formation of teratoma upon injection of undifferentiated cells in the heart.

I should also say at this point that there was – there is a controversy in the field what happens when you inject undifferentiated cells in the heart. Some people claim that if the dose is low enough, these cells will preferentially turn into cardiovascular lineages. In our in vitro system, basically no matter what the dose was, even with the lowest doses, we saw teratoma formation when we injected undifferentiated cells which is consistent with the reports from Murry’s lab.

Also more recently we were able to develop a model that enables us to mimic some aspect of diabetic myocardium. Diabetes is on the rise in the developed world,partly due to sedentary life style, and diabetic patients show dysfunction of cardiomyocytes that is independent of any other underlying heart disease. So we looked at the levels of glucose and insulin found in the blood of diabetic patients and also diabetic mice. We formulated a condition that had high glucose concentration and low insulin concentration and we called this hyperglycemic. And then we also had a group that was

hyperglycemic on insulin therapy and then something that would mimic a healthy control. By some molecular markers such as ratio of offbeat- myosin heavy chain to alpha-myosin heavy chain our engineered heart tissues were similar to the diabetic hearts. And when we placed these tissues on treatment with normally used diabetic drugs, we saw for example less cell death if you look at page 9 transcription in PID, and this is as expected.

So where are we going next with this? You noticed in the previous slides that the tissue was relatively big. It was about the size of a penny or a cent. And if these tissues are going to be really useful, they have to be smaller and we have to be able to do hydro put studies. So in summary, we saw that both rat and human engineered heart tissue can be grown in vitro using scaffolds and hydrogels and cells in electrical stimulation by bioreactors, and this technology enabled us to test ability of the different cell populations to survive in cardiac environment. With electrical stimulation we can mature human pluripotent stem cell cardiomyocytes.

In terms of the need for imaging, for us it would be something that can enable us to measure online in a high throughput way – not just in one tissue but in multiple different wells and hopefully going back to the same spot longitudinally. For monitoring at the same time the force of contraction, impulse propagation gene expression and viability in these cardiac micro tissues. And at the end, I

would just like to thank my collaborators and people in my lab. Those people whose names are shown in bold I actually showed you their work today and our funding sources. And I'll be happy to take your questions now.

[APPLAUSE]

DR. CHEN: All right, I'm going to start. So this last part that you showed with the artery and the vein, that was quite interesting. So would it work if you put, I don't know, two arteries next to each other, or is there something special about that?

DR. RADISIC: I don't think there is anything special about an artery and a vein. We've done this with arteries and veins from mice, rats and from humans. But we've also done it with cardiac explants. So there is nothing special about the choice of the vessel so far. We know that it's easier to harvest arteries and veins than tissues. We also noticed that if you use cardiac explants, we can also get oriented and connected microvascular tube. So we get higher density. So perhaps from the density standpoint, it is not advantageous to use artery and vein, but it's advantageous from the standpoint of manipulation.

MS: How fast does the heart beat?

DR. RADISIC: Which heart?

MS: The pseudo heart.

DR. RADISIC: The pseudo heart. Okay, so I had some maximum capillary rates. So for rat heart, we were able to get it to like 400 beats per minute, and with human

cardiomyocytes we capture at let’s say three to four Hz. We can try to stimulate faster, but it won’t capture it. It won’t respond. So that would be 180 to 240 beats per minute.

MS: Very nice. Can you comment on the stability of your microvascular networks? I’m wondering if pericytes can migrate out and stabilize them?

DR. RADISIC: So we had – we did stain for some microvascular pericytes, and you know, and NG2 and also smooth muscle actin, and you do get some of these tubules that have pericytes around them. But I wouldn’t say that all of them do. So there is still room for improvement in that respect.

MS: So Sam McClain from Wash. U. Do these – can you show that these contract against a load? That would obviously be the most important thing for them to do rather than simply to wiggle around in the cell culture dish. If they don’t, can you train them to do that like a weight lifter or something?

DR. RADISIC: Uh, I’m pretty sure we can train them. So with electrical stimulation, as long as you have contraction, it is important to stimulate them above thresholds so that you have contraction. But I should also say that we used some more recently, and this is a work in progress also. That’s why I’m not showing it. Some of the approaches that Chris described where the posts can actually deflect and we can measure force of contraction.

So we do measure force of contraction. But I can also tell you it's very small. It's a lot less than what I would like it to be.

MS: It's not like in vivo?

DR. RADISIC: Yeah, yeah. That's why I'm not showing the data yet until we improve it. Yeah. With human, it's very small, I should say. With rat cells, people have sown decent forces of contraction.

MS: I wonder if you could comment on the future when you put these patches into people or maybe into a rat first, how do you expect them to integrate? How do you expect these myocytes to be provided with oxygen and nutrients? Do you think that there will be vasculature? What do you think, will it survive indefinitely, or is this something that you want to stay alive for a week?

DR. RADISIC: Okay. So in terms of integration, I should say first that in 2006 there was an outstanding paper published in Nature of Medicine by a group from Germany around Zimmerman and Eschenhagen, and they showed that if you have a rat patch and you implant it into a rat heart that the cells do integrate, and the measures were not just histology. They also measured impulse propagation, calcium transients and force of contraction. It was a very thorough study. They showed that these patches can integrate.

I doubt you can study functional integration at that level between human cardiomyocytes and rat cardiomyocytes

because the physiology of the two cell types is very different. So if you are trying to evaluate integration, I'm not sure you will get it, if you put human cells into rat hearts. So I think it's important to match species for these studies.

In terms of vascularization, we know there are several high impact papers in PNAS that show that if you had even some kind of premature vasculature, the graft has better chance of surviving in the heart. So there is a consensus right now in the field that you probably need all three cell types in these tissues where the main working cell type of cardiomyocytes you need some primitive vasculature and some residual mesenchymal cell types such as a fibroblast for grafts to survive. And then there are also other papers that show that the more mature the vasculature is at the time you put it in, the better chance it has to survive. So that's why we are optimistic that this last approach as I showed you in the last slide will work when we go in vivo.

DR. CHEN: Thank you for the second time. Why don't we move in.

[APPLAUSE]