DR. SHAY SOKER: Well, thank you.

[INFORMAL COMMENTS.]

I'll spend about half of the presentation talking about some new approaches for bioengineering of whole organs, but then I'll move into using imaging to look at bioengineered tissues. And I'm presenting work that was done by many people, both in my group and other groups at the Institute for Regenerative Medicine.

This is where we started, actually, in Boston, in Tony Atala's lab. And Tony is a urologist, and he faced with a problem that some kids had faced – kids that were born with spina bifida. And these kids, due to damage to the nerves, have a nonfunctional bladder. Depending where the damage is on the nerve, some of them get neurogenic bladder. And the problem with a neurogenic bladder is that it's not compliant. It's not innervated, so it's not cycling. And these kids need is expansion of the volume of the bladder.

And normally, what they would do is take a piece of the bowel and make a pouch and then – increasing the volume of the bladder. But this is, as you can understand, not the optimal treatment. Bowel is not bladder, and bladder is not bowel, and that puts these kids into multiple risks.

So, in the late ’90s, Tony and the group set to bioengineer a bladder using the part – and you guys have probably all seen – taking a biopsy, growing the cells, putting it on a scaffold, first testing to see if you can actually form a tissue; and then essentially moving into the preclinical trials, where we took biopsies from dogs, made a balloon-shape[d] scaffold. In this case, this is polyglycolic acid, PGA; seeded it with the urothelial and muscle cells on the inside and the outside, and then implanting it in dogs. And that was published in ’99.

This work actually led to a small clinical trial that has actually grown since then. This is one of the girls. This is probably ten years ago, and a paper was published in ’06, but the paper was published only after we had a good five years’ follow-up on the first

patients. And basically, what you see here is the bladder of this girl before the augmentation and after the augmentation. And I hope you can appreciate that there's a threefold increase in volume. So, using bioengineering technique, this girl now can have her own bladder tissue used to expand her volume.

She doesn't see this. She doesn't have x-ray eyes, but what she feels is – what she felt are sort of the spikes –pressure inside the bladder as soon as she was drinking a sip of water. And after the surgery, you can see that it sort of plateaued, and then this very sort of slow increase in pressure, and it doesn't cause leaking. So, this project was then moved to a company, Tengion [phonetic], and Tengion is now producing some new bladders for kids.

I want to move to a different project in a lab – bioengineer blood vessels. Bob Tranquillo talked about it, and I will show you an operation which is not much different. There's multiple needs for bioengineered vessels[?]for vascular disease, [to] create those bypasses. But like many others, we focus on vascular

access and AVFand, Elazer Edelman gave some preview on AvFs.

The problem is that the patency is 70 to 85 percent at three months, but then it drops after six months. There's a need to create those vascular access for dialysis. This is our version of blood vessel engineering. We use electrospinning technology to create those tubular structures. They have very nice fibers. We seed them with endothelial cells on the inside – and smooth muscle– cells on the outside, and then we put them through a bioreactor phase. I won't take you through the functions of the bioreactor; but we can control both the flow rate and the pressure. And, through a computerized pump, we can control even pulsation.

What we can do is we can take this blood vessel and run it through different protocols – arterial, venous, or the AVF. And then we would ramp up the flow rates over time for about seven days, and then we would start pulsation – pulsatile[?] pressure.

What we see then is that this is immediately after seeding. And as you expect, as you expose the endothelial layer to fluid flow, the endothelial layer gets confluent and flattened. More so, what Yaz[?] has done in my lab – he was looking at the effect of different flow rates, as well as sheer stress and cyclic pressure on the endothelium. What he was able to see is the change in the endothelium. You cannot see it based on the histology, but if you do electron microscopy, you can see a change in the endothelium, depending on the cycle that he was using. And as we expected, there's an increased expression of endothelial cells – [unintelligible] – under the cyclic pressure protocol.

So, all of that to suggest that you can precondition the cells by using different mechanical stimulations, and it results in both anatomy as well as biochemical changes.

So, the next step was when Luke Neff[?] came into the lab as a vascular surgery fellow. He took those small pieces that we engineered, and he did a[n] AVF[?] fistula graft in the neck of the sheep. The point

here is that you can actually needle-stick it three times a week for about a month, and it recovers, unlike the synthetic counterpart made from Dacron. They don’t heal. They seal, but they don’t heal, and we think that because we have cells on the blood vessel. The bioengineered blood vessel has much better potential to heal.

I told you about two example[s] of bioengineering of small tissues. The bladder – and we’ve done urinary sphincter and blood vessels. We are working on other tissues, as you might expect. We’ve published two years ago about the work on urethra that has gone into patient. We’re doing pre-clinical studies on cornea, on skin and bone and cartilage. But we treat all of those as simple tissues.

So, what about more complex tissues? And when you start thinking about more complex tissues, you face several challenges. You need a good biomaterial. You have to make sure that you have good vascularity[?], and if it’s a large-volume organ, you have to develop a way to get the cells in.

What’s currently available for most of the tissue engineering beginners are some sort of very poor selection of some synthetic materialThere’s a lot of work done on the chemistry of those scaffold[s], but you can formulate them as a certain structure, and you have a limit to that. You can actually use natural materials, such as bone matrix, or collagen; but they’re also limited. And if now we look at liver, to fabricate such a very complex vascular structure, vascular network, or a complex structure of the liver, we’re still not there.

About almost six, seven years ago, I startedthinking, along with others, about using the native tissue as a scaffold. What’s good in native tissue? First, this is the authentic structure, where the cells are, so it would have the structure to support; and also, it would contain bioactive molecules specific to that tissue. Usually, it’s very hard to put them in the right place and at the right moment. So, this is a method that have been now used by many laboratories. We call this method “decellularuzation.” You take a tissue, you run a detergent through it, and then you’re left with the extracellular matrix (ECM), and

then you have the scaffold. And then you can put the new cells.

In our case, give credit to Doris Taylor, who published the first paper using this technology for bioengineering of whole[?] heart in rats. And what she was able to see, using SDS as detergent, that she can completely clear the organ, leaving behind ECM and then reseed it with neonatal-derived cardiomyocites.

We began our work on liver. We can take liver from multiple animals. Our detergent selection is not as harsh as SDS[?]. We use a triton[?] base, and, at least in liver, within two hours we can clarify the entire liver, leaving behind all the vascular structure. This is the portal[?] triad[?] at the high magnification we can even see the collagen fibers.

What we also leave behind is authentic extracellular matrix, sort of the – the – the big three: collagen, laminin[?] and – fibronectin. And they are not only there, but they’re in the right place as similar to the fresh tissue. We quantified it also by – Westerm Blot [unintelligible].

So, one important aspect that is close to my heart is the vascularity. The minute we saw the ECM, we could actually see even under light microscopy that the vascular structure had remained. We didn't know if it's – patent [unintelligible] – so we took some – dextran beads [unintelligible] – and infused it, and you can see that they remained within the capillaries, so the capillaries are intact. And, actually, it was – that picture was picked up on the cover of "Herpetology." We did other studies using a contrast agent with similar results.

So, would it withstand transplantation? We took the unseeded– [unintelligible] – scaffold, connected it on one end to the portal circulation – this is the native liver – and the other end to the vena cava, and this is the clamp. And what happens when you remove the clamp is that the scaffold filled up with blood, and no leaking, and the blood remained flowing for about half an hour and then clotted.

So, what about cells? We use the same system that we use to decellularize – the organ in order to

recellularize it. In this case, we use endothelial cells – the endothelial cells were GFP-positive. And what I want to point out is that we can access different areas of the liver. For example, if we infuse from the vena cave – this is retrograde – we can deposit the cells in the center of the hexagons, but if we infuse[?] them from the portal veinwe can get them at the periphery, and here you can see the electron microscope showing that –three[?] endothelial cells covering the capillary. We actually put some blood in there to see that they can keep the red blood cells in.

So, what about the parenchymal cells? We actually went to Lola Ried[?]from UNC, Chapel Hill, who's been a collaborator for several years. She had developed liver progenitor cells. I won't go too much through that. Just to show you that when you take those progenitor cells they are capable of making both the hepatocytes and the bile duct, basically, when you switch from growth media into differentiation media, these cell switch from secreting alpha fetal[?] protein to albumin.

When we take those cells, and you infuse them in the liver, the first thing that we find was very exciting for us is that the cells can actually know where to go.

This is staining[?] for endothelial cells and -- brown endothelial cells. They're going to the capillaries; whereas, the hepatocytes are in the parenchyma. We could show that the cells are becoming functional, staining for bile duct epithelia cells. And this is a composite staining for endothelial and hepatocytes.

So, these organoids, as we call them, are active. They can secrete urea and albumin in vitro.

We have just recently applied the same technology into kidney. As you can see here, a kidney takes much longer to decellularize. It's a lot thicker. We have to use SDS[?] in here, but we can clean the kidney from the cells, leaving all the structures behind. The vasculature is intact; and, interestingly, we can see that if we infuse fluid and measure the pressure in the native and the decellularized kidney.

We took those kidneys, implanted them in pigs. These were pig kidneys. This is the implantation. We actually knew that a couple of days after implantation, the kidney was clotted. There was no blood flow in it, but we actually left it for 30 days, just to see what remains afterward. And I can just point to the bottom here, that upon histology, we can see, as expected, many inflammatory cells in the cortex region, but also in the – these are some clots inside the kidney. Nevertheless, the structure remained, and now the challenge is to make sure that it's not going to – that we would recellularize it with –– endothelial cells so it won't get clotted.

So, we're continuing using the same technology. We just published a paper together with a colleague in the U.K. on bioengineering of intestine, and we're ready to publish a paper on making a pancreas.

So, that's the summary for the first part –scaffolds are needed to provide the right architecture in profusion – The ECM is a good substrate to work with for scaffolds. And I think this is a statement that every tissue engineer would make.

But we still have some challenges, and we still cannot tell when is the organ mature enough. When can you start applying pressure to a bioengineered tissue? if you start too early, it’s not good; and you probably don’t want to wait too late.

Let me give you some examples to what I’m saying. these are some of the dog studies of the bioengineered bladder. Here’s the complete cystectomy. the whole bladder was removed; and so there was residual fluid here after one month, but nothing there.

Here’s the polymer[?] only. Some fluid one month and very little after 11 months. And I can tell you of the histology here, it’s all fibrosis. This is – the tissue-engineered new bladder stays the same.

This is a compliance test, you infuse fluid into the bladder, and then you measure the pressure inside the bladder. these are the three groups that I show[ed] you. So, let me overlay this line here. Here’s something very interesting that we found. This is the new bladder, starts and stays around 100, and then at

three months goes down and then goes up. And then, actually, it ends up 120 because now the bladder is larger and is softer because of the patch that we put.

But look at the polymer only. This is PGA polymer. Starts very low, goes *up* three months and then down. So, the three months is – was very intriguing. Well, guess what? The three months is the time when the PGA starts to disintegrate, and that explains why up to three months it was holding the urine, but then it sort of broke up and started disappearing. And that's where the tissue replaced the ECM.

Another example. This is the study that we've done on the preconditioning of the vessel. And what you can see here [is] we exposed it to blood, and we can see that only under these conditions we see no adhesion of blood particles.

The question is, is it ready? And – because we don't have the of proper thermometer, we don't really *know* if it's ready. So, the solution would be imaging. Yesterday, we heard a lot about luminescence; fluorescent –– microscopy. Today, we heard about

micro-CT and so on. There's[?] multiple imaging modalities; however, many of them are not applicable to in vivo, real-time imaging.

We used fluorescently labeled[?] cells that we can construct, or bioengineer tissue and then follow this tissue over time. This is a muscle tissue, and what we can see here [is] that when we switch the muscle from growth into differentiation, we get fibers. When we add endothelial cells and pericytes, we can actually get even better muscles there.

We can take those muscles, implant them subcutaneously, and we can still follow the cells. And then when we look at the tissue very closely, what we can see is that our endothelial cells that were labeled "red" are still there. We see a clump of autofluorescing red blood cells. So, you can engineer tissue using fluorescently labeled cells and then you can follow them *in vivo*. But all of that was done using destructive methods, so we had to look for a better method.

Together with a group at Virginia Tech, we're now developing a new imaging technology. Some of the speakers actually alluded to the depth of imaging. Standard fluorescent microscopy can actually look only at the surface, and then you can go from control[?] – [unintelligible] – photon. You can go in, but can you actually exceed the five millimeter or the one centimeter? And the idea here to actually cross[?] an animal skin, if you want to image the blood vessel.

So, the idea here is to decouple the source of the light – of the laser light for activation and the detector. So, we do that by embedding sort of micro imaging channels inside the scaffold. And this is an example of how this is done. So, we put labeled cells here. This is the scaffold. We deliver the laser through a fiber optic, and then we can collect the data. And what you can see here is the sort of the scan. And as soon as you get to excitable material, then you can record some fluorescence.

You can do that over time. Here again – and while this is playing, what I want to tell you is that this picture was done on the other side of the scaffold, so

basically if you looked with a fluorescent microscope, you won't be able to see anything. And this picture was taking with a regular fluorescent microscope, and you can see the nice sort of alignment both in the static, as well as you've seen before, in the dynamic pictures of the two methods.

This is the control camera looking directly at the specimen. This is looking through the scaffold, and you can see that, by reconstruction, you can get very good approximate – spatial distribution of the cells. And it doesn't really matter the depth, because now you can put a pigskin under the scaffold, and you essentially get the same picture.

So, what is it good for? One is, as I said before, we need to know when the vessel is ready.

So, let me just state the obvious. Why do we need imaging for bioengineered tissue – bioengineered organs? – scaffold degradation, biomechanics and for translation to the clinic.

But this wouldn’t have been done without the hard work of all these people. The liver-kidney team, Tony Atala and James Yoo have been there from the beginning. Our imaging team together with Virginia Tech –– team and our funding. Thank you.

[APPLAUSE.]

MODERATOR: Okay. Questions for Shay.

Q: Yeah, nice talk. I have a question. So, in your comparison, you were comparing basically sort of two, different models. One is sort of your typical bioengineered material versus your decellularized material, and part of your conclusion was that the structure of the material is going to be really important for the function. But it seems to me there’s really two variables, and they’re not separated. One is the structure, but the other is the material that remains, because from my understanding as a cell biologist, if you treat this tissue with detergent, you’re going to have proteoglycans and all these other materials that are going to be remaining, which would not be ever really present in your

biosynthetic material. So, how do you separate structure from material? And in that sense, if you were to make your material using the same material as in the pigskin, or, alternatively, in the decellularized material, treat it with prot- -- [crosstalk] – well, not only detergent, but you've got all these proteoglycans that are there – right? So you have not only protein, but a lot of other things. So, how do you separate these two issues? You've got two variables here.

DR. SOKER: Right, it's a great question. I think the way the biomaterial field moves is to try and mimic the – the native tissue. The decellularized tissue is actually the exact native tissue,

Q: Sure.

DR. SOKER: -- right now, they're still different. Right now, the biomaterial, as good as we tried to make it, is missing many biochemical, proteoglycans, growth factors and so on. It may mimic the structure, but not the biochemistry.

So, I think you're absolutely right that the chemistry of the scaffold and the structure of the scaffold – together make the tissue, and the cells actually responds to both. for example, there's another dimension, and that's biomechanics. So, you have to use biomechanics in order to make the right tissue. So, we understand that they're separate and using different systems, we at least can compare and see how they differ, how can a blood vessel made from a decellularized blood vessel versus a blood vessel made from an electrospun?] scaffold?

Q: I see, but proteoglycans, in particular – is that being addressed

DR. SOKER: We're trying to bind some proteoglycans to the electrospun[?] material. I can tell you that binding a single proteoglycan is, a graduate student thesis. And there're many of those. I'm not talking about many of graduate students; many of the proteoglycans.

DR. SOKER: -- it just takes time, but I think that we're sitting in a good place where we can actually compare the two systems.

Q: Okay. Thank you.

MODERATOR: Okay. I think we should thank the speaker again, please.

[APPLAUSE.]

MODERATOR: And our last speaker this morning will be Matthias Nahrendorf talking about "Optical and Fusion Imaging."