DR. DAVID KAPLAN: All right. We're going to start the next session. My name's Dave Kaplan, so I'll give the next talk and try and keep the rest on schedule. Thank you.

So, what I'd like to talk about is a little bit different than some of the things we've heard about so far, and our interest really is in trying to marry the needs in regeneration – or, tissue regeneration with building devices that become integral to the repair itself. And the idea is simply to integrate optics and electronics into a repair mode, so that as you're implanting something related to a surgical intervention, for example, we can exploit some of the needs in optical imaging and electronics to really improve our ability to track the repair as well as to, hopefully, treat the repair at the same time, if there's a need to do that.

The ultimate sort of mandate here is everything we want to put into the body to do this has to disappear 100 percent. And so that's our guiding principle: how do we design devices to do this and not interfere with normal regeneration processes? So, the big

picture is here. And we heard a lot about this from the other speakers, so I'm not going to belabor the point; but the idea really is, can we exploit, for our needs, optical inputs as our imaging mode and get away from some of the limitations we've heard about, particularly this morning, from some of the speakers, where scattering, absorption limit depth profile and so on. So, how can we overcome some of that with using implantable devices instead of still probing from the outside in – moving away from that and trying to probe not only from the outside in, but use the inside to inform the outside further?

The same with electronics. We have lots of issues with electronic interfaces in vivo, whether they're biofal-[unintelligible], whether they're, you know, other types of mechanical complications; and we'd like to find ways to improve those outcomes so, again, we can integrate these ideas into the repair. So, our ultimate goal here is to really fully integrate both optical and electronic devices to improve our ability to image, diagnose and eventually treat whatever we're trying to repair.

So, today I just want to tell you where we are in the process of trying to move along this path, and it's going to be a long path, but I think we're making – *like* to think we're making some progress – at least today. So, the first half of talk, I'll speak more about the optic side. The second half, I'll just give you a little bit on the electronic side. And so our interest really is exploiting optical imaging for implantable medical diagnostics; and you already heard today optics would be a nice, you know, platform to use. They're safe in the visible range. They're low-cost, tunable, portable – all the nice things. And so how can we do that?

Challenge: you have a light source going into a tissue – it doesn't matter what the tissue is – and you're fighting against scattering, absorption and ultimately loss of resolution and depth. And so our approach is to think about designing materials and systems to help with that, and you can see some of our design criteria here. They have to be, number one, optically functional. So, we need optically clear, robust materials to start to think about even doing this. We need to make sure, again, they're fully

degradable; and, ultimately, we want to tune that degradation because in some cases, the repair may just a few hours to a few days. In other cases, we may need to last for weeks or months. And the last part is to turn that around. And if we can do all this reasonably well, can we activate that system to actually have a therapeutic benefit as a feedback loop. So, deliver a drug, or localize heating to kill an infection and these kinds of goals.

So, to do that, we go back to our toolkit in the lab that we use in terms of our materials, and we have four favorite proteins that we study very avidly in the group. For today, I'm going to really only talk about the silks, mainly because they form into very, very tough materials. They're relatively easy to process in all aqueous systems. And, hopefully, as I'll convince you, we can do the kinds of things I'm suggesting we'd like to be able to do in terms of optical and electronic platforms.

A couple of background slides on the silk to explain why we chose this. Number one, mechanical compliance. These are remarkable materials in terms of tension and

compression. In terms of toughness, they rival the best high-performance synthetic materials today. So, as a durable, implantable, manageable material, depending on format, we have lots of options to exploit.

Number two, I said we wanted to make sure we could control degradation and make sure when we're done, we're gone 100 percent. So, here's one example of where we can fine-tune that process. This is simply taking one of our techniques to control the processing of the silk protein into robust biomaterials, and you can see this is a vapor annealing process at different temperatures, from basically 4 degrees up to almost 100 degrees C. And what you see now as we expose those materials to proteolytic digestion, which is how this protein will degrade, it degrades extremely slowly here because we've locked in lots of crystallinity, and it degrades extremely fast here because we haven't at the cold temperatures. So, we can tune that very nicely.

You can see the end result of that from zero up to about 75 hours. You can see the pores just growing

and growing, because you have degradation along the walls as this material degrades.

So, we have a system that degrades. Does it degrade 100 percent? Absolutely. Here are the crystals that we grow of those proteins by AFM imaging as we erode it with proteases, and you can watch the crystals delaminate. Eventually, you get these little fibrils, and the fibril ends start to degrade away, and you're left with peptides and then amino acids. So, as a starting material for our goals, this gives us a lot of useful approaches.

The second part is we need a material that's very versatile. In some cases, you may want to make fibers. In other cases, films and so on, depending on the optical device or the electronic interface. And so these proteins we've learned to engineer over the years to do just that, and I think that gives us a nice template to work from, which I'll be talking about.

So, now into the optics a little bit. One of the things we discovered a number of years ago [is] if you

take the native silkworm cocoon – so, that's what comes from nature and goes into textile manufacturing eventually. That's what a textile silk fiber looks like. It's pretty messy. It's not highly purified, because you don't need to purify it in the textile world. In the lab, we've learned how to clean those fibers up so that they're pristine, and each cocoon is a single fiber about a thousand meters long.

And they turned out – when you clean them up very nicely and couple a light source to the end of those fibers, in fact, they were fiber optic cables naturally. In other words – so, these proteins, as they come from nature, by the nature of the organized crystalline structure, in fact, are like pipes. And we can do this in wavy, straight – doesn't matter. And if you make films, you can actually make the films that look optically clear through the entire spectrum of the visible range.

So, in fact, these are as good as glass and the best plastic today in terms of optical devices. And so as a protein, we now open up a window into fully degradable systems that we can start to exploit.

We can take that a step further. Instead of taking what Nature gives us – ‘cause that’s going to be limited in terms of size and shape, et cetera – we can reprocess these proteins. And one example here is we take a fiber optic, and we dip it in a solution of the protein after we’ve reprocessed it, and we pull out a new fiber. And, in fact, this new fiber, as you can see when you go to the end, is still able to conduct that light as a fiber optic cable.

So, this opens up lots of opportunities in terms of building fibers with surface, functional, features for diagnostics – all implantable, all degradable – that you could do readouts of what’s going on in vivo as you implant these or use these during a surgical repair. That’s one example. You can extend this and make really complex features, which we’ve done in collaboration with Jen Lewis at UIUC, so there’s really no limit in terms of the way you could integrate these complex optical devices into tissue repairs in many modes.

So, the other part I just mentioned is the fact that you can also make these same proteins into film, and if you make these films in different sizes and shapes and cure them the right way. They're optically clear as well. And, again, you could see the transparency through the entire visible spectrum.

And this really came to bear when we were starting our work on tissue-engineered human cornea, and the idea here is to generate single films of patterned silk so we can guide the corneal fiberglass. These lay out their new extracellular matrix. We can stack these layers together, form a pseudo-corneal replacement tissue, and you can see the optical features here. And following these by all the optical techniques you've heard at the meeting, you can actually start to watch ECM deposition in the different layers and the helo-[unintelligible] fashion, and these are now into animal studies to see about remodeling and integration.

But that led to lots of interesting observations, at least to us. One was in a different program, we were able to take these films and use something called

“nano imprinting.” So. think of just stamping on the film little nano indents along the surface of these optically clear films. You could do this with temperature. In fact, the silk is stable to above 200 degrees C. That gives you lots of windows to operate in. Or, you could do this at room temperature just by putting a drop of water on the surface. So, everything’s in water. Very simple. You stamp, and outcomes a – a hologram simply based on defraction optics on the surface indents on this film.

And this is amazingly robust, so the feature sizes we can get to – and this is by either nano imprinting or casting, but by nano imprinting, you can see the sort of repetitive, high-fidelity feature sizes you can place on the surface of these film. Large arrays of these, and this is just done on a lab bench, and these feature sizes now can get down to tens of nanometers with high fidelity, depending on the specific goal of a particular project.

And so, again, you can make self-standing holograms just by taking the films of the silk protein, stabilizing it and then imprinting on the surface.

And you can see it’s a fairly robust and simple process to carry this out today.

In doing so, you can start to add new functions, and one of these relates to structural color, which I’m sure is very familiar to many in the audience here. And simply by changing the spacing and the size of these little nano indents we place on the surface of these optically clear films, we can change the pattern and, therefore, the colors that evolved from those films. And this gives us the ability to design in different modes of optical color into the system. And turns out to be essentially a diagnostic system, where you can track what’s going on in the surrounding environment.

So, for example, here’s the film. Here’re some of those patches with little nano indents, where we changed the spacing. You can see the different colors that emanate from the film. If we now place that film in water, we’re changing the local defraction around the film. We now shift those colors. And so simply going from air to water, you can see a color shift. And now, if we simply dope that solution around the

film with different amounts of glucose, as an example, you now shift the wavelength peak that you see coming from that film, and you can build a standard curve. And, therefore, these would be essentially self-standing diagnostic systems that you could think about implanting as well for the same reasons we've been talking about.

And, obviously, this is not sensitive enough for a glucose sensor; but you can go away from what we call "periodic arrays" and move towards aperiodic arrays, and now you can really crank that sensitivity way down so you can really detect levels of glucose at the level that would be meaningful from a diagnostic standpoint.

And one other short note on this. You can, in fact, even take something like hemoglobin, place it in these silk films, stabilize it for months and months and months. And simply by placing this hemoglobin-doped silk into a suitable spectrometer, you can track the shifts in the wavelengths of the hemoglobin, based on the oxygenation-deoxygenation. You can cycle that, and essentially this becomes a self-standing

spectrometer at the same time. Again, further ideas as we've heard about in the meeting so far, where you could track oxygen levels very nicely in tissues, depending on what you're doing.

So, that leads to sort of another realm of the optical features, and this first starts with micro molding technologies, which many of you may be familiar with based primarily today on PDMS and standard ways to mold elastomers as surface molds to then cast whatever-you-may want-to-build devices. We essentially have replaced that technology now with the sill aqueous process protein. So, here's our solution: a silk protein. You can dope it with virtually anything you want. It's water- and room-temperature as a process, so you can add functional enzymes. You can add antibodies. You can add vaccines, all of which we've done with success. And now you simply pour that onto whatever mold you've created as your template. The silk dries. You cure it, as we've talked about, to control degradation lifetime. You peel it off, and now you have a device that's self-standing, again, that has all the features that we're talking about.

And so one example of a device for optical implants that we're working on is shown up here. Think of these as a standard stop sign as you come at night, and your headlights are reflected back at your eyes so you see it. This is what you really see here, a reflector made of pure silk. In essence, these are micro prism arrays that are molded or cast-molded in this process right here, so it's a very simple process. And keep in mind at this point you could also add therapeutics very easily into this device, as I'll show you.

And a simple way to show the value of this is if we take a slab of gelatin placed over this "mirror," if you will, you could see the reflected light is very strong compared to the – in the absence of placing this mirror or reflector underneath that slab of gelatin. So, we enhance signal recovery by shining a light on this device simply by changing the surface morphology and building in these micro prisms.

And this is what it looks like to us. They're quite beautiful structures, ad this gives you a little more

detail; but nonetheless, you get significant signal enhancement by using these kinds of optical devices to improve the reflection of the light back to the receiver.

So, here's what we've done. We've implanted these in vivo now, so these are the silk reflectors here. You see the plain film, so a film without the prism arrays there. Here's the optical output. And if we have the micro prism arrays, you see a significant enhancement – about threefold. This gets to the point can we go deeper or better for diagnostics in vivo without having to dissect through the skin? This suggests a path forward to try and do that.

You can enhance this further in different ways to add function. So, here are the same micro prism arrays. Now we've simply, in the process, added gold nano particles; and gold nano particles are – [unintelligible] – relatively innocuous in the body. And then we can implant these devices again into the animals and look at the effects in terms of the light that is reflected back to the receiver. And you see we don't change that effect, which is a good thing,

and that means the gold doesn’t disrupt the benefits that we saw here. But now we can go in with a laser and target and heat locally that region of the reflector because of the presence of the gold nanoparticles.

So, for example, you have a surgical intervention. You’re trying to track, repair a mode[?], and let’s say an infection starts. This would be a very nice way to locally heat and perhaps dampen that infection ’til the regeneration mode takes over more effectively.

And these are just some more images. We can do this with sutures. You can see the suture here. We can do this with the micro prism arrays, as shown here as well.

And then the last part of the optics side is to say if we take these micro prism arrays and dope them with therapeutics – in this case, we used doxorubicin as a model example – you can track by proteolytic digestion here are the original micro prisms. As we digest them with the enzyme, they start to dampen in terms of

their features. And as you do that, you'll notice we can also correlate the drug release with the loss of reflectivity of those devices. So, we can start to look from the outside in, if you will; and as we change reflection and what we can sort of track either in vitro or in vivo, we can tell ourselves, "Well, this also relates to what drug is left or not and when it might be time to think about secondary strategies."

I also point out just as another point of stabilization – because here it's 60 degrees C, and you see the do-[unintelligible] is very stable under these conditions. In the silk, it doesn't degrade at high temperature; whereas, in solution, it clearly goes away in terms of its function because it's turned dark, et cetera. So, there's lots of values here – not only as a matrix for optical enhancement and encouraging repairs and regeneration, but also drug delivery and stabilization at the same time.

Okay. So, that's a little bit about the optics platform. The other side is electronic, so this 'll be a little briefer. And the idea is very simple. Can we take electronic devices? Can we implant them

into a repair mode, and when we're done, make sure that device goes away 100 percent as well? And, again, many of you, I'm sure, are familiar with the challenges here, biofa-[unintelligible] being number one. The interface is lost and, therefore, sensitivity is lost. Mechanical mismatches causes major problems with implantable electronics, et cetera. And then almost in every case, you had to surgically retrieve whatever you're using, and the whole point is to get away from that secondary surgery.

So, can we build devices, again, that have – maintain their electronic functionality – all the same things we talked about before. They have to be safe, tunable lifetimes again, and multifunctional, as we've alluded to for the optical platform, at the same time.

So, this started a number of years ago. This started first as a collaboration with John Rogers at UIUC. John's one of the world's experts on flexible electronics. And so we got together with John, and we took advantage of his ability to use PDMS stamps to build electronic components, and we asked a very

simple question in the early days: could we take those stamps and transfer those electronic components onto our silk films that I just showed you, keeping in mind again they're mechanically robust and thermally stable, again, above 200 degrees C.

So, we found in the early days we could do that. And if we did nothing to the film to lock in crystallinity, you put the film in water, and the electronic components float away – as you'd expect. And that's what you see here. I spared you all the videos, so be thankful for time, but here's the film with the stamped electrodes on the surface. Over time, you can see on the right-hand side the film has gone away completely, and the little bits of gold are left floating around. And we argue that the gold that we use is fairly safe, but we can debate that, if you would like.

And you can control this rate – okay? Obviously, you can make this fast, as I show you here. Or, you can make it last literally a year or more, depending on how much you lock in the structure.

And the other question is, if you do this, if you pattern metals or other electronic components on these surfaces, do they stay stuck? Because that's critical if you're going to build devices for the goals we're talking about. So, here's a free-standing, optically clear silk film. We've patterned little, gold electrodes in the middle, and you can see we're bending and torturing the film, and they stay perfectly stuck. So, the interfaces are really pretty remarkable in terms of the robustness that we need for these sort of inorganic-organic interfaces, and that's turned out to be a really important part of what we're doing.

The second part is to match compliance; and, really, that gets into conformal, related electronics. And so here you can build complex arrays, and if you have a very large-diameter tube all the way down to a very small-diameter wire, depending on how we've processed this film, you notice it drapes very, very nicely, regardless of the diameter. And that's all, again, in the processing. The point is when we went into – on the surface of the brain to look at how these electrodes worked as they were transferred onto the

silk matrix, you get very good, thin fits, or conformal fits, to those convoluted surfaces.

And so that was the goal – to get away from the typical, nondegradable, non-implantable polyamide-related electrodes used to monitor brain function and use our conformal silk electrodes. And this was all done in collaboration with UIUC and U Penn. And on the surface of the cat brain, we got outstanding sort of recordings driven by the ability to get better contact between these electrodes and the surface of the cat brain.

And that opened up a lot of different windows now that we're pursuing. For example, you can pattern silk films with all kinds of electronic components. Here you can see different kinds of resonators. All of these give you different features that can be deposited on the silk film by deposition processes, writing processes and so on. The list is quite large. At the same time, aside from the silk substrates, we always have to be conscious of the choice of the inorganics that we're using. Gold, magnesium, iron[?]. There're some others we are using as well.

All are very friendly in the human body. Some of these will dissolve away. Some of these will be small enough and inert enough to, presumably, not cause any long-term complications.

And here's just one example. We can make these split-ring resonators, pattern them on the silk surfaces very easily; and depending on the sort of morphology and features that we build into these, we can change the resonance frequency; and this gives us a window into tracking what's going on for these devices as we implant them and – and determine what's happening over time.

So, here's just a clever example of how you can use this. Not going to be in vivo at this point, but the idea is we can take a banana. You know how horrible it is. You buy a banana. It looks green, and before you get to eat it, it's turned ugly brown – right? And so here's one of our resonates[?] placed on a silk film, stuck on the surface of the skin of the banana. And the point is very simple. As you allow that banana to age or ripen, you can watch the peak resonance frequency shift according to the ripening

process. So, you're reading out what's happening physiologically around that banana.

The real value here is this is a completely implantable, edible device at this point. There's nothing that limits that. And so you can see how you could use this not only for foods and food contact, but also for implantable devices.

And so two other examples. I'm not going to belabor it. I always show cheese 'cause I love cheese, but we'll skip that.

[CHUCKLING.]

DR. KAPLAN: But the important one – what can I say, you know? The important one is over here. Here's where we just took one of these resonators and placed them on a lawn of – a Petri plate to track bacterial growth – okay? And over time, you can watch the shift as the lawn of bacteria grows – the point being you could start to track infections based on frequency shifts – resonant frequency shifts – with these devices.

And the last one just ‘cause it looks good – and you can do these on bananas. You know, not simple here is the fact that when you make these devices, depending on how we make them, they actually stick very, very well. So, these stick quite nicely to even very, very slippery surfaces. And that’s a big plus. The other: you can make large arrays, as you can see here. You can make pattern arrays, so you can get fingerprinting and take this to another level, all of which we’ve been doing.

And the last part, more diagrammatic, is you can even put these on very, very brittle hard surfaces all the way to very, very soft surfaces. So, thinking about tissues, implants and regeneration, this gives you a huge window of tools that you can start to use.

So, being that we have lots of wonderful grad students – [chuckles] – so, you can make these little – we call them these little “tattoos,” but you can make, you know, clear silk films with some of these, resonators on the surface. And you can actually place them on your skin. I’ve actually worn these home ‘cause my

13-year-old daughter loves tattoos that are removable – thank God. But –

[CHUCKLING.]

DR. KAPLAN: -- no problem. I mean they sit there fine, and you can use them in different ways. And the real value here for what we're talking about is starting to think of wireless ways to activate and probe what's going on as we implant these into repair modes. And so we obviously would have a transmitter. You could see the coil here – the transmitter – that would sit outside the tissue repair, outside the body. Here's the implant that would go in the receiver, and you would then carry out different functions to probe what's happening here.

And you can see what we've done so far. We've made transmitters, LEDs, heaters. All these have been made successfully. All of these have been implanted in vivo, and you can see the operating distance to drive this with the transmitter.

And so here’re just a few, quick examples. Here’s under the skin. I think this was a mouse. Don’t hold me to that. We’ve placed one of those devices, you could see here, under the skin; and over time, if we activate with a transmitter, you could see we can locally heat a spot here, if we put the appropriate heating element in the design, and we implant it. So, the idea here would be from outside the body we could decide we want to locally do something at that repair mode. We can activate it externally, have it happen, and over some programmed timeframe, this entire device will disappear – again, be it in a day, a week, a month, or a year.

And, again, this is just a close-up. You can see the transmitter here, the external coil here; and you can see the local heating going on here, in the animal as it’s activated. And this could be a passive device until you need to activate it. It really depends on the readout and what you’re trying to do.

So, quickly – I need to finish. So, you can also put LEDs on here. Now, the LEDs today are not going to be degradable, so please understand that; but the point

is you can place these under the skin. You could see a four-LED array with the same technology I'm talking about, that's under the skin; and you can actually see all four lights flashing. Here's the histo. It looks good. Everything goes away now except for the LEDs, and it would be great to work on how to get that part fixed as well, and that's part of where we're going.

So, quickly, to conclude, what we've really been working on is a toolkit – two kinds of toolkits, I'd say. One is optical devices that can be used in mixed and matched modes to build systems that would allow us to track internal repairs optically in new ways. And I showed you some of these. I didn't have time to get into lens arrays and all that, but you can see the list, and it's a growing list all the time. All of this, you know, gives you sort of ways to start to meld materials in new ways with programmed degradation.

And the same with the electronics world. I showed you some of these as well today that, you know, we're starting to really build this kit nicely. And there's all sorts of variables we can measure. I didn't have

time to get through all those today, but you can look at mechanical issues. You can look at mechanical vibration and tension, flow and so on with the same concepts I presented to you today.

I tried to show you at least one really good example where you could take a device, like the prism arrays, and track it for enhanced optical sort of reflectivity and, at the same time, functionalize for heating or localized drug delivery. So, you can start to envision how to build multifunctional optical devices to carry out tissue repairs and diagnostics and feedback as well. And this is where we're trying to go.

All of this is really informed because we have a really remarkable, robust material, like this protein, that we can generate into all sorts of material features that is, otherwise, quite difficult to do when we look at sort of other options today. So, I think that gives us a really good opportunity to build these kinds of devices.

So, back to where we started. Our goal is to get, you know, through some of the challenges that exist today in electronics and optics. We're making progress here. You see some of the progress, hopefully, this morning; but there are lots of challenges ahead, and this is where we're working today, you know, expanding the materials base, expanding device designs, expanding therapeutic opportunities; and then we can debate whether you eventually want to implant the power sources as well. And, you know, at some point, I think that should be feasible. We're not there today.

Let me close by thanking my awesome group of graduate students and postdocs, who do the work. I failed to mention at the beginning this is all joint efforts between my lab and Prof. Almonetto's [phonetic] lab at Tufts, who's a physicist. I have wonderful collaborators around the world, locally at Tufts, wonderful brand support from many agencies, which I'm thankful for. And I'll stop there and answer any questions.

So, thank you.

[APPLAUSE.]

DR. FRANK: Any quick questions that I can answer?

Q: Really fascinating. If you wanted to do something at, say, an MR frequency, like 300 MHz, what sort of cue[?] do you think you could get for a resonance structure?

DR. Kaplan: I guess I don’t know right now. I think you could certainly – we’ve built systems like that, but I don’t remember the values. That’s something Phil[?] could certainly answer, but I don’t have the numbers here.

Q: I’ll buy you lunch.

DR. Kaplan: Oh, fantastic. Okay.

[CHUCKLING.]

DR. Kaplan: I’ll hold you to that. [Chuckles.]

Q: David, thanks for a wonderful talk. One of your colleagues at Tufts is expert in phase conjugation, Mark Co-[unintelligible] – so I'm wondering if you have built [a] phase conjugation device out of silk, because that would really [the] improve tissue penetration scattering problem.

DR. Kaplan: Yeah, it's a great thought. Mark has not done any of that. The only thing we've so far pursued together with him is to build an azo-modified silk, so we can look at a *little* bit of holographic systems, but not a lot yet. So, lots more to do. Great idea.