DR. ELAZER EDELMAN: I am going to depart as some of the recent speakers have from what I had initially intended on speaking about to talk more about what I've learned today - how the confluence of biology and imaging come together to propel technologies forward.

I will use a few illustrative cases to make these points, highlighting work on adhesive and erosive degradable materials, drug-eluting stents, and tissue-engineered vascular therapies to show how imaging technologies have been extraordinarily helpful but also where current interactions and technologies are lacking.

Adhesive materials are extraordinarily important because they serve an important and poorly served medico-surgical need. There are surgeries where the inability of the anastomosis, the connection between tubular structures, to stay intact has devastating effects. Gastrointestinal surgeries have a leakage rate which is as high as 30 percent and a mortality and a morbidity that is astronomical. The problem is that sutures and staples and even the clinically available adhesive materials really don't do the trick.

Just to give you some idea of what we're talking about, this is a $2.5 billion market which is only served to about 15 percent of its potential by existing technologies. So clearly there is a major need, and clearly an unmet need. Many of you are aware that fibrin glues and cyanoacrylates and even glutaraldehyde and formaldehyde can

be used to seal tissues. But the problem is that as you move in this direction, you sacrifice safety, you increase toxicity and you move in this other direction you don’t get much adhesion – you get coating.

Fibrin glues are wonderfully biocompatible, but they really don’t do anything to seal anastomosis. Cyanoacrylate is wonderful as an adhesive. But, like formaldehyde and glutaraldehyde, has devastating tissue effects.

We have developed a class of adhesive materials that circumvent these drawback. I am going to talk about the challenges in developing the materials and understanding how they work, how they could be clinically useful and how imaging added to that insight. We borrowed from the glutaraldehyde/formaldehyde chemistry and material aldehyde group-tissue amine chemistry to create these materials. We created star-like aldehyde moieties which allow for very flexible materials with limited penetration to restrict harsh or hard binding between tissue amines and aldehydes on the material.

Like most of the other speakers we found that imaging can drive program development.

What you see here is a four panel image of green fluorescently labeled materials adherent to different tissues. Cell nuclei in the tissues stain blue and tissue stroma in red. Imaging opened up an extraordinary vista for us and created for us an entire new research domain. The very same material adhering to four different tissue types

had completely different interfaces and internal structures. They are three different domains in the material-tissue interaction. There’s the domain within the adhesive, there’s the adhesive tissue domain, and then there’s the tissue itself. In a fascinating manner differences in the tissues drive differential adhesion between material and tissue and cohesion within the material. The same material adheres significantly to duodenum (hence the solid band at the interface), in a more network-like fashion at the liver, very lacy at the lung and as a more branched process at the heart.

Enhanced interaction begets enhanced adhesion AND cohesion. Each of those interfaces reflected a different kind of load bearing or adhesion strength at the material interface and cohesive strength of the material.

What all of this allowed us to do was to understand that what we had done was to create a smart material, by using material aldehydes that lock onto amines on the tissue to determine how much binding there will be and internal amines to bind free aldehydes. These free aldehydes are tissue toxic and by chelating them we remove them from adverse tissue potential AND we also use this binding to enhance material cohesion.

We confirmed that this was the case using or borrowing from AFM technology by creating aldehyde fluorescent microspheres and painting them onto tissues and showing that the ability of the aldehydes to seek out the amines

correlated directly with the ability of the material to form at different interface.

Imaging not only validated our hypothesis was valid, but it allowed us to move beyond. What we hadn’t initially anticipated until we saw the images was that the dynamic processes allowed the material to titrate its adhesion to every specific tissue at every specific state. We now have a material that can be used widely across a vast array of tissues, a vast array of applications and in each case modulate its adhesion to precisely what is needed.

Now what I want to jump to is to show you at least two other things that imaging allowed us to do. And since a lot of the theme of the day is about tissue engineering, what we can do with these very same materials is not simply put them on as adhesives but we can take those materials and other materials we have worked with for tissue engineering and drug delivery.

Most specifically I will present the case of a three-dimensional matrix that we’ve been working for many, many years to support endothelial cell tissue engineering, and we’ve used it to examine endothelial cell biology. These three-dimensional matrices allow for endothelial cells to grow to vast numbers. They coat the inside of the interstices of this three-dimensional sponge. The panel in this slide is an image of a double dye exclusion assay. Every green cell is alive. Every red cell is dead. There

are virtually no dead cells; all embedded endothelial cells are healthy. We used these cells to control vascular repair. If you create vascular injury by removing the native endothelium, then a reaction occurs signified by this donut of tissue hyperplasia - the extent of this growth is quantified as the intimal:medial area ratio depicted in this histogram. The I:M is significant for injury alone and made worse if the artery is wrapped with empty gel or gels with cells that are genetically modified to lose their inhibitory ability. In contrast gels that release the agent that is missing inhibit tissue hyperplasia but only embedded endothelial cells virtually obliterate the lesion.

I’m not going to go through this in great detail other than to share with you some observations about how imaging propelled this project forward not simply through its pre-clinical stages but actually through now five clinical trials. Imaging helped us understand how to optimize the density, seeding and placement of the cells relative to the architecture of the device.

This is literally how long it takes to wrap one of these devices around a venous coronary artery bypass graft, and these data are an example of what happened in two Phase II clinical trials where these materials with cells are placed at the anastomosis of an artery and a vein for vascular access for dialysis.

These vascular grafts fail within about six months– only 15 to 25 percent of them are still patent at this time. This short term patency and the limitation in numbers of grafts available to a single patient is what creates the tension in dialysis patients. Most patients can only receive about five of these implants and they then run out of conduits and access sites. If the average duration of efficacy is 6 months then most dialysis patients can only sustain dialysis for only three years before they need a renal replacement.

The results of this trial highlight the potential of tissue engineering and a cautionary tale for imaging. Tissue engineered endothelial cell-based therapies doubled the lifetime of these graphs. At six months, 50 percent of grafts wrapped with endothelial cell implants were patent while only 25 percent of implants without cells remained functional. What we see here is a remarkable set of Kaplan-Meier curves - a wide displacement, and then the curves seem to come together and then a wide displacement. The convergence tells the tale of caution. There was a trial protocol mandated obligate angiogram at 3 months - a requirement in the clinical trial for imaging irrespective of whether there was a clinical event or not. And when the images arrived an ocular-manual reflex set in which bypassed every major part of the brain causing the interventionalists to intervene – patients with mild to moderate obstructions underwent angioplasty even though not

necessarily indicated by protocol. So that there is a danger in imaging, and I think that we can’t leave today without talking about that as well.

Now what I want to end the discussion with is two other aspects of imaging. Depicted in this slide is Cincinnatus who is celebrated for having stepped down as a great Roman leader to retire to his farm - serving as the paradigm of a material, which like the ideal leader, does what it or he is supposed to do and then fades away. Though the historical facts are far less clear this is a nice way of talking about erosive materials. This technology is important in the design and development of stents, stents that deliver drugs, stents that have a carriers on them and in particular stents made entirely of erosive materials. They, like Cincinnatus, do what they’re supposed to do and then they fade into the background.

But like the actual Cincinnatus, that issue is not so clear cut. One of the problems we have for those of us that use degradable or erosive materials is not only is the nomenclature entirely abused and unclear, but there aren’t very good assays for actually determining, detecting, tracking erosion and degradation in vitro, let alone in vivo. In general people weigh things either dry or wet and then actually sacrifice the samples or put them back into the system. And so what you invariably have are very different curves depending upon how you weigh the material

and what happens in vitro is virtually never predictive of what goes in vivo.

This is a third theme that I would like to highlight for our discussion - that one of the obligations of imaging as it relates to biology and translational biomedical sciences is to drive preclinical sciences especially to help determine that what goes on in a bench is predictive of what will happen in a human.

To address this issue in our specific case we took advantage of what must be second nature to very many people in this room, IVIS to track the fate of fluorescent-labeled material. We tracked the fate of the dendrimeric cross-linked dextran aldehyde materials. We characterized erosion in vivo, and then we determined what we would need to do to establish an in vitro condition to match what goes on in vivo. We made devices in the form of disks and blocks and, yes, even stents that are erodible and tracked their erosion kinetics in vivo and in vitro. Now what’s amazing is that if you look at the in vitro behavior and the in vivo behavior, you might say that these are not tremendously related. But in point of fact, when you plot the in vitro/in vivo ratio for any specific specimen over time, you get these remarkable isotherms.

The in vitro/in vivo correlations apply for large ranges of materials even one where the erosive kinetics are governed by very different properties. We used collagen-based scaffolds that are enzymatically as opposed to,

hydrolytically degraded, and defined the conditions under which the in vitro situation would mimic the in vivo - predicting precisely what the chemical composition of the specific space in which the materials resided would be. In this particular case, we implanted materials in three different body spaces and then asked what were the conditions that we would need to mimic the in vivo? And in all three of the cases, the chemical composition in vitro was precisely the chemical composition of the space of what you would find in the physiologic space.

Now what I'm going to close with is further insights, I hope, into where imaging is both wonderful and problematic. The development of OCT, IVUS, other technologies has helped us understand that the biological reaction to vascular injury is significantly more complicated than we've ever imagined. In this experiment simple imaging helped us understand the power and limitations of two similar drug-eluting stents, one that delivered rapamycin and the other paclitaxel. The students controlled animals' diet, making some atherosclerotic and others not and then placed stents in both animals arteries. They stained for tissue binding proteins for each drug and showed by immunohistochemistry that the dietary effects on the distribution of tubulin that binds paclitaxel and the FK-506 binding protein that interact with rapamycin. They showed that the two proteins are differentially affected by diet but who each pattern correlates with the distribution

of the drug. Counter to dogma we showed that the amount of a hydrophobic drug actually drops dramatically rather than increase as the amount of lipid increases. We took 256 of these serial sections and manually aligned them and overlapped them to get this linear correlation function. It took us years to get even to this stage defining the potential as well as the challenges.

Today we are rich in imaging modalities but poor in understanding how best to use them. At the end of the day we are still lacking in vital tools that we need to drive translational programs. The marriage of imaging and biology is the next critical step for translational biomedical science. There must be increased cross talk between the different domains. All too often one branch – that is the imaging or the biology or the technology – advances well ahead of the others, and then the others need to catch up. There’s need to be a link to computational sciences which we haven’t talked about all that much. This is a marvelous way of marrying pre-clinical observations with clinical observations and working backwards. It is precisely how you correlate in vitro and in vivo findings. And what we must be respectful of Dr. Heisenberg’s uncertainty principle that because you can look doesn’t mean you should, and there’s sometimes a problem with looking.

In closing I am delighted to be here, excited about the future but as always indebted scores  of post docs and graduate students who have dedicated them for these

projects. This is our lab and the tree on which I rest and I am so grateful to them. I'm always delighted to talk about the work they do. Thank you very much.

[APPLAUSE]

DR. EDELMAN: Questions? Comments?

[NO RESPONSE]

DR. EDELMAN: Chris, go ahead.

DR. CHEN: I'm curious from your perspective in terms of priorities, which parts of the black box would you like to build better now versus what you could wait five years.

DR. EDELMAN: Multi-scale, multi-axis, multi-dimensional imaging is my highest priority. Such technology would enable us to correlate multiple observations and events simultaneously.

We can measure many different factors but virtually none of them at the same time.

MS: OCT in a mouse, or was that taken from some other animal –

DR. EDELMAN: We have stented mice but not the OCT images are from pigs and humans. The mice work was taxing and remains a testimony to how extraordinary some of the people in the lab are.

Yes, Charles.

MS: So why doesn't the drug concentration correlate on the –

DR. EDELMAN: So the wonderful thing about the drug-eluting stent is that everything we thought was going on

with the stent was entirely wrong. So when we started working with drug alluding stents, my Ph.D thesis was on the technology of drug-eluting stents. So –

DR. CHEN: Please repeat the question.

DR. EDELMAN: The question is why don't the drug concentrations correlate directly with the amount of lipid? If it's a hydrophobic compound, why doesn't it do that. And the short answer is because we really had no clue of what we're talking about when we started working on this technology.

First – the coating on the stent is not really a controlled release device. Second, the reason that the drugs work is not because they're hydrophobic, not because their conduction velocity or convection velocity rather through the artery is slow, but because they bind so avidly to tissue-binding proteins. The drug-eluting stents that work, work because the drugs that are released bind specific tissue- proteins. And when you have a lipid laden artery, the lipid actually displaces the binding proteins.

Rather than serving as a pool to bind these hydrophobic compounds, they remove tubulin for paclitaxel, the FK-506 binding protein and all the complexes that rapamycin and its analogues bind to, and you actually get a precipitous drop. That's what happens, and it took a long time to figure that out. It took many, many years. Yes?

MS: So does the potential of the drug –

DR. EDELMAN: Well, it's a long conversation.

MS: [Inaudible]

DR. EDELMAN: Prasad is asking a two-part question. Is it a reservoir, and is it a diffusion barrier. So it's much less of a diffusion barrier, much more of a reservoir. These are very low molecular weight compounds. Paclitaxel and rapamycin are less than 900 Daltons. And so what actually happens is there is a little bit of a diffusion barrier, and you can be released over long periods of time. But it's very, very rapid first order release. What ends up happening is it is solubilized slowly. So it's more solubility limit, or they're trapped and they're erosion limited, and then they bind to their tissue elements and then you have release. Ralph?

MS: Was the drug that you showed you called a drug. Was it taxol – fluorescent taxol?

DR. EDELMAN: It's paclitaxel. Taxol is the drug with cremaphor

MS: Is that the C-11 or the C-7 position label?

DR. EDELMAN: I believe the fluorescent label on Oregon Green 488 paclitaxel is attached by derivatization of the 7-β-hydroxy group of native paclitaxel.

MS. So if it turns out that it is C11 then this compound doesn't bind to microtubules any more.

DR. EDELMAN: We did the binding studies but will need check this. As far as I recall the binding was intact. This is an important problem because when you deal with small molecular weight compounds where the label is on par with

or may even be larger than the compound, you interfere with a lot of the physical chemical elements.

MS. And so how do you – just for my own knowledge, so how do you hypothesize that the paclitaxel actually works? Do you think it is purely a microtubual inhibitor and it simply, you know, inhibits proliferation of cells or kills dividing cells? Or do you think there's something else happening?

DR. EDELMAN: As with many drugs it is difficult to understand fully how these drugs work. We know what rapamycin does to mTOR  and what paclitaxel does to microtubules are important. But the dose responses and the binding chemistry imply that there are a lot of other things going on, and we don't know what they are right now.

With that, in order to stay on time, I thank the speaker for an illuminating presentation, and I will ask Peter Zandstra to come up and talk about feedback control of endogenous signaling to guide stem cell fate.