SPEAKER 1: So we'll go ahead and get started with CTSA Grand Rounds. It's a real pleasure for me to introduce Dr. Dennis Wigle, who's going to give the presentation today. Dennis is a thoracic surgeon here at Mayo. He's got a joint appointment, both in the cancer center-- he participates in the CTSA-- and he's also a member of the Department of Physiology and Biomedical Engineering.

In addition to his skills as a thoracic surgeon, he also has a PhD in anatomy and cell biology from Queens University, so brings extensive basic science background to his work. He has been actively involved in lung cancer and lung regeneration. And it's a pleasure to have him talk today about prospects for lung and aerodigestive regenerative medicine.

DENNIS WIGLE: So first of all, welcome. I really appreciate the turnout here. I have to be honest. I'm not a regular attender of these rounds, and I didn't know they were so well-attended, so this is fantastic. But I guess lunch is always a good draw, right? So what I was asked to talk about today is some of our work in both lung and aerodigestive regenerative medicine. And how do I go forward here?

So what I was going to do today in terms of learning objectives-- so I want to just briefly review some of the recent technology developments in lung and aerodigestive regenerative medicine, and we'll review some of what the potential is for novel clinical applications stemming from these technology developments. And then hopefully we'll have a few minutes at the end-- I can just talk briefly about the Center for Regenerative Medicine Biotrust, since things are very much getting up and moving with that infrastructure.

Just two quick disclosures. So we do have a joint development agreement with a company called Nanofiber Solutions, which is the company that developed the artificial matrices to use in the first few tracheal transplants that have been done in the world. And this is largely around developing matrices for artificial esophogeal replacement. And then I am going to talk about-- this a bit of a stretch-- but the use of Alloderm, which is a product of decellularized human skin.

It's approved for hernia repair and breast reconstruction, and we'll talk a little bit about how we've been using it in esophageal reconstruction.

So first of all, I thought I'd put this right up front, just so that it's not given short shrift at the end. I want to thank, first of all, everybody on the team, and specifically Bill Carey from neonatology; Steve Cassivi, one of my partners from thoracic surgery; and then Matt Barron, who's a research fellow in our lab, sitting up front here, who's done a good chunk of the work that I'm going to present today.

There's some other key contributions from people. Specifically, Shari Suitor, who did most of the decell/recell work that I'm going to talk about. And then Tim Nelson and Frank Secreto in his lab, who have done a lot of the work with the iPS cells from the COPD trial that we've been running.

So just to start, you all are aware of the unmet need in regenerative medicine. And you know, it's very frustrating some days. Despite all the technology we can bring to what we do to treat patients, there are so many situations where you just have nothing left to offer. And this, unfortunately, spans a large number of both acute and chronic irreversible diseases, whether you're talking about diabetes, whether you're talking about heart failure, or kidney failure, liver failure, et cetera.

There are, unfortunately, a very large number of people that we can't really do a lot for. We're really treating palliatively, trying to make their quality of life as good as it can be, but without any real treatments or anything that looks like a cure for their disease. In many situations, there are some select people who are actually candidates for transplant for some of these organs.

But as we'll talk about, unfortunately, this is a very small subset of people who are really real candidates, largely driven by the fact that we just don't have a endless supply of donors for artificial organs. This problem is not getting smaller. It's getting worse, given the aging of the population. And unfortunately right now, there are many diseases that we don't even think about using for regenerative medicine applications.

Because we have such a big donor shortage, situations like the cancer example, where we might be able to actually do lung transplants, do liver transplants-- we already do that on a small scale for some diseases for cancer, but nowhere near to the breadth that you could actually do it if you had an endless supply of artificial tissues or organs to be able to transplant in people.

If you're awake and reading the literature, it's impossible not to be bombarded with information about the promise of stem cells and regenerative medicine technologies. There's just a couple of examples from *TIME* covers. I mean, this hype is everywhere. There's lots of promise for what could potentially be done with stem cells. But I think we're really getting past some of the really spectacular advances to the hard part of how, really, do you harness these technologies and these applications, and how really do you get them into the clinic for clinical use?

There's lots of promise here, but I think it's still very unclear in many different organ subsets what's really going to work. For example, are we going to be just replacing cells? Are we going to be blowing lung epithelial cells down a bronchoscope in order to partially reconstruct some of the function in a degenerative lung? Are we going to be making little subpieces of tissue and implanting those in order to restore function for people?

Or are we really going to be growing organs in a test tube that we might be able to use for transplantation? I think that's very unclear, in many different spaces, where we're headed. And it's also very unclear for specific disease types and for specific organ types what's going to work. What might end up working as a great regenerative medicine application in the lung might not be what we do in the liver, might not be what we do in the kidney. And there's many permutations and combinations here of what could potentially work and make real translation.

Unfortunately, I put this up just to remind me to say that, once you get past all these spectacular advances and all the hype, the real hard work of doing all this in phase 1 and phase 2 trials, and really sorting through what has the greatest potential for patient application, is going to take a lot of money, a lot of resources, and a lot of time and effort to really make this real for clinical use.

So as you know-- you've probably had lectures even in this forum on many different applications-- there are many different potential places that regenerative medicine applications are already happening. For example, in wound healing, in terms of what people are doing within the transplant center, and many different places where some of these technologies can be applied. And what I'm going to talk about today-- we'll talk about lung disease first, and then I'll briefly talk about some of our work in the aerodigestive space after that.

So first of all, let's just introduce what the scope of the problem is here in terms of people who actually have significant lung disease and actually die of it. If you go through and add up all the people with emphysema, with pulmonary fibrosis, with cystic fibrosis, it's a huge public health burden. I mean, there's almost 400,000 people that are going to die in year 2013 from lung disease, which is a huge number.

Unfortunately, if you contrast that with the number of lung transplants that are actually done, there's going to be less than 1,000 lung transplants done in 2013. So this is a massive differential between all the people that are suffering from these diseases, and the people where there actually might be a real transplant option in order to treat them. Obviously, this is just a fraction of those affected, and largely what we're doing in most situations is not really treating.

We call it treatment, but really what you're doing is just providing very good palliative care in order to provide as much quality and quantity of life as you can, with almost thumb in the dam type measures in order to be able to make someone's life with a really bad disease as good as it can be, but in no way being in a situation where you can cure it.

So let's touch briefly just on what some of the challenges might actually be in lung regeneration. And just to introduce that, I want to reflect a little bit on the almost dual nature of the lung as not only a very complex organ, but also one that might actually be very simple in terms of what you need to do in order to provide real therapies.

So first, from a complexity standpoint. You know, I look at it slides like this sometimes, and you think it's not even worthwhile to get out of bed. I mean, how are you ever going to be able to reconstruct parts of an organ or a whole organ where patterning is complex as this?

The number of steps that need to happen, and the number of things that need to happen perfectly, even just to get the outpouching off the embryonic foregut to form the right and left main stem bronchi, that is then going to form the pattern for where branching morphogenesis is really going to occur, takes a lot of very complex patterning to happen normally.

Beyond that, even once you've actually had branching morphogenesis occur, what it really takes to go through the sacculation mechanisms, and then really form real alveoli, is very complex. What you end up with when you start going through all the cell types in a fully-formed adult lung is over 40 differentiated cell types that are required in order to make the organ actually work.

The one statistic I always love is that if you take all of the alveoli in any one of us sitting in this room, and you lay it all out end-to-end, the surface area that you actually have for gas exchange is about the size of a tennis court, which is incredible.

So what about on the simple side, though? Well, you might look at that and say, my god, this is so complex. How would we ever make any kind of artificial lung, or any kind of therapy that could actually replace what the lung does? But the flip side of that is that to actually provide a mechanism for gas exchange to occur in a clinical setting, we've already been doing for over 50 years.

There's people right now this second up at St. Mary's Hospital on cardiopulmonary bypass for various heart and lung operations, and it's occurring right now in thousands of people across the world. And this is a very, very simple machine.

In order to be able to not only perform the function of the lung and the heart at the same time, you've got basically a pump, that is going to use a roller pump mechanism to take the place of the heart, and pump blood through a membrane oxygenator that looks something like this in cross section, where really all you've got is a wall of tubes, where you've got a permeable barrier there that allows gas exchange to occur such that you've got blood flowing on one side, you've got oxygen flowing on the other side, and the membrane set up so that you can diffuse CO2 one way, and you can get oxygen going the other way.

And that's all you need in order to have an artificial lung. It's amazing, really, when you think about it. Now, there's obviously a lot of problems with that. This isn't a very comfortable thing to be walking around with hooked up to you, and that doesn't really work very well. One of the main problems with it is that you basically need to be under general anesthetic to have it. And if you're not fully anticoagulated, this all clots off.

As you can imagine, having a big cannula in your aorta or your femoral artery is a huge nidus for clot formation. And the only way these systems can actually work is to be fully anticoagulated. So you could be on this for a few hours, relatively safely. There was just a recent report you may have seen on the web page here at Mayo Clinic about a young girl who was on, for I think, almost 180 days on ECMO.

But when you're starting to measure things in terms of days, or weeks, or months on these kind of machines, you're really stretching the limits of what can be clinically accomplished.

It, in no way, also takes the place of the amount of area for gas exchange that you've got. So even on a standard cardiopulmonary bypass machine, you've got about a meter squared of surface area for gas exchange. That works real well when you're lying flat under general anesthetic, but it doesn't work very well if you're going to go for a run, or play tennis, or do something like that. So when you contrast that with the amount of surface area you have for gas exchange in a system that looks after preventing blood clotting through it, it's not the real thing.

People have made advances beyond this in order to try and go beyond the bulk and the complexity of a cardiopulmonary bypass machine. So this is a device called the Novalung device that's designed by a company in Germany. And it's been used as a bridge to transplant for people that just have pure lung failure.

So they have totally preserved a cardiac function such that you can use their own heart as a pump, and all it really is is the membrane oxygenator wrote of a cardiopulmonary bypass machine with things set up such that you've got an inflow, for example, here, and then an outflow coming into the device. And then this membrane gets oxygenated such that you're using the patient's own heart as a pump.

And you can actually be ambulatory on this. They've described patients in Germany and many other centers having these devices in place for weeks, months as a bridge to a transplantation. Again, it requires you to be fully anticoagulated. It requires you to have cannulation devices in, so you've got an inflow and outflow, but it actually works.

So how do we move beyond this, though? Even though, in the simplest terms, we already have technology and devices to be able to take the place of the lung, clearly walking around attached to a cardiopulmonary bypass machine is not great quality of life.

So let's talk a little bit about some of the recent advances in treating lung disease. Like many fields in regenerative medicine, there's been a convergence of a couple of key breakthroughs that have really got everybody very excited about what the real clinical potential for this might be, and I'll talk about a couple of them.

The first is the whole decell/recell concept that you may have heard about, given that the first descriptions of it happened just down the road from Doris Taylor's lab at the University of Minnesota. We'll talk a little bit about iPS technology, which I'm sure you've heard, also, lots about. And then, also, the issue of how do you go from what might be a progenitor cell or a pluripotent cell into some of the differentiated cell types that make up a fully-functioning adult lung.

The first breakthrough I'm going to talk about came from a couple different labs, but most prominently from Joe Vacanti's lab. Harold Ott was actually a surgical resident taking two years off to do time in the lab when he actually did this work. And he spent some time in Doris Taylor's lab to understand and learn about the decell/recell concept in the heart, and then basically came back to Harvard in Jo's lab in order to try and apply this to the lung.

And basically what they did is use the same concept of what they did in the heart-- of trying to take a fully-functioning, normal lung, and decellularize it. You can do this relatively simply with many different detergents or other agents in order to keep the matrix structure of the lung intact, but just kill all the cells within it. And what it looks like when you do that is something like this, where it's white because all of the blood's gone, so you don't have any of that pink color from a normal tissue. And what you're left with is just all of the matrix proteins that go up to form the scaffold of the lung.

And what they did-- the reason they had a Nature Medicine paper out of this-- was the striking result that when you take a lung, decellularize it, and then basically set up a bioreactor like this, and perfuse the vascular side with human umbilical vein endothelial cells-- even though it's a rat lung that's been decellularized here-- and then human A549 cells, which are basically a cancer cell line as the epithelial line-- if you pump those through their respective parts and you wait long enough, you can get something that looks like a lung.

Now the title of the paper was along the lines of creating a fully-functional bioengineered lung. Really what they did is they transplanted it back into a rat, and it lived for about four hours before the whole thing clotted off, is really what happened. So in one sense, you can look at that cynically and say, well, that's not that huge an advance. But really, I mean, that's spectacular. The fact that you can actually build something that was capable of gas exchange, that didn't cause the rat to exsanguinate when you implanted it and took the clamps off, is just amazing.

There's obviously still a ton of hurdles that need to be surmounted in order to really make lungs that might be useful for clinical applications. But you can see from this concept how you might get past all of the issues of how do you really come up with the right matrix scaffold to be able to repopulate for organ regenerative purposes.

One of the things we were very excited about when we first saw this paper-- I mean, it's one thing to do in a small rodent model, but there are many examples throughout science and medicine where just the scale-up from what can be done in a rat up to a larger animal system, like a pig, en route to what you can really use for human applications, creates a whole bunch of hurdles.

This is work that Shari Suitor did, when she was with us, where we actually tried to tackle the problem of what would it really take in terms of solutions, in terms of pressures, in terms of pump flows, et cetera, to really just try and decellularize a pig lung. And so what you're seeing here is what a pig lung actually looks like. We made a number of trips down to Hormel.

This was done very low-budget. Filled up the trunk of the car with lungs from the slaughterhouse, so it's not like we spent a whole bunch of money to do this under barrier-free conditions or things like that. But did that solely for the purpose of trying to obtain lungs to sort out the issues of how do you go about decellularizing.

What you're seeing here is a simple cannulation set up where the airway is cannulated, so this is the main stem bronchus on this lung, and then this is the pulmonary artery cannulated here, and then this is the atria cannulated here. And what you see-- I'll show you a movie of the whole process-- is if you go through this, you can decellularize relatively effectively. It's not that hard to do.

It probably took Shari about a year in order to sort out all the nuances of how hard should the flow rate be, where should you have the pressure pop-offs, how long did you need to do it for, what solutions did you need to use. But to be fair, it was relatively straightforward to set up.

Shari was fantastic, and I used to joke and call her MacGyver because she would go to Fleet Farm and pull out all these bins, and cannulas, and staff. This was really done on the cheap when she did it, but she was able to basically come up with this perfusion set up like this using just standard roller pumps in the lab that we did in the hood. And this is a view, here, of two different lung setups going at the same time, being perfused with a mix of decellularize solution, and then just PBS to wash everything out.

And so what that actually looks like-- I have a movie here-- we can talk around it. So this is a set of time-lapse pictures that Sherri actually took during the process of the lung actually being decellularized, which is kind of cool, which is why I wanted to show it to you. Most of the decellularization that you see to form this sort of whitish structure all happens within the first couple of hours. This whole movie was taken over a span of about 16 hours, but most of the action is right up front in the first couple of hours.

And like you see here, right at the start of the video, the whole lung is relatively deflated just because you've cut the airway at the start. So a lot of what you're seeing, initially here, is the perfusion solutions distending the whole lung, both from the vascular side and from the airway side, until it hits an equilibrium point here. And then what's happening after that is you're just getting slow, ongoing clearing of all the hemoglobin that's in there, which is what makes the red color, en route to something that's completely decellularized.

So as I said, most of the action is pretty quick. So by six hours, you get something that's basically completely white like this. And the whole process-- we did a number of sections anywhere from 12, 16, up to 18 hours, in order to look at how effective the recellularization process really was. I won't go through all the details, but bottom line is, it works.

This is still a work in progress. The obvious next steps in terms of doing recellularization, though, are obviously a lot more complicated. And I think that's where there's going to be a lot more time and money that are going to need to be spent in order to sort this out. In terms of the decell part, this is successful by histology and other criteria that people use. The recell part's a lot more difficult.

We've done a number of experiments so far using not only the cell types that Harold Ott tried to use in their original description, but also a mix of things like embryonic stem cells, iPS cells, various fetal airway types. I'll sum up all that data by basically saying, I don't think in the pig, so far, you can say that we've got anything close to a functioning lung that would really make sense to think about transplanting.

When you actually cut sections, you can find with fluorescently labeled cells-- you can find cells, but I have to be honest. I'm not really convinced yet that you've really got ones that are actually there growing and repopulating the organ, versus ones that are actually just stuck there and are still fluorescing. So this is still going to take a lot of work yet.

And we haven't done anything yet-- I mean, there's obviously a lot of cross-species issues here, or theoretic ones, anyways, even though you've gotten rid of the cells. And we haven't done anything yet with any pig cell types, or done anything yet with human tissue. Although theoretically, the pig lung's about the same size. That should be a relatively straightforward thing to use everything. More or less the same for the decell part in a human situation.

So what about the issue of the cell types that you need to actually put on a matrix, or any kind of scaffold, in order to repopulate a lung? There's obviously been a lot of recent hype and excitement about the idea of induced pluripotent stem cells, given that you could use those in an autologous manner for a patient to theoretically create any cell type that you want it.

The Yamanaka description of being able to take a small cocktail of transcription factors and basically transform any somatic cell-- but most commonly described, a skin fibroblast-- into something that is pluripotent is mind-boggling. But it does actually work. The idea that you then might be able to use these iPS cells, either for cell or organ-replacement technologies, or even do things like, for example, in the lung disease cystic fibrosis, cut out the cystic fibrosis diseased gene, and splice in a normal functioning copy of the gene, and then use that for cellular replacement tissue therapies is very, very striking.

To think about this in a lung context, a couple of years ago when we started doing this work, we had the very naive idea that maybe we could just take skin biopsies from patients with lung disease, do the skin fibroblast cultures, do the iPS reprogramming using the technology that was available at the time, and then do our best to try and differentiate that to various pulmonary epithelial cell types in order to start doing repopulation-type experiments in the lung.

And one of the things I was concerned about right up front was that, it's one thing to just describe a one-off in the lab. But how do you actually really translate that to something that's going to be robust and efficient for somebody who's 70 years old, a pulmonary cripple who's on oxygen chronically, is malnourished-- I mean, there's a lot of big issues in doing these kind of things in real patients that is going to be part of the next step of translating all of these technologies.

So we started, a little over three years ago, a phase 1 study to try and just look at the safety and the reproducibility of doing iPS cell generation in patients with severe COPD. We've enrolled 34 patients as of a few weeks ago. We were able to make skin fibroblast lines, which is a relatively straightforward thing. We had one line that got contaminated, but this is pretty robust, and is not that difficult to do. The iPS part is a much more difficult thing to do.

So we were one of the initial 10 lines through Tim Nelson's company, ReGen Therapeutics, in their production pipeline. We haven't made iPS lines for all of the patients that have been enrolled in the trial, but we've done 10 so far. One of these was unsuccessful, but we had nine lines from the patients enrolled to date.

And in two of these lines, Matt's done a great job in trying to show that you can really take that pluripotent stem cell from a patient with severe COPD, and really differentiate it down a number of different lung lineages, and get cells that express the marker complement that you would expect for differentiated lung cell types.

So just to look at some of the clinical data on the study, we had a total of 15 males and 19 females. Again, these are older patients. The mean age was 71 years. The mean FEV1 was 1.32 liters, which is roughly around-- well, our range here was the 0.36 liters up to 1.96 liters. And just to give you an idea of what that means in terms of percent predicted, so these are people-- you had to have less than 60% predicted FEV1 in order to get on to the trial.

And some of these people were pretty sick. I mean, 0.36-- there's people on the lung transplant list that are healthier than that, in terms of what their lung function is actually like. So this is pretty severe. FEV1, just for those of you who don't know it, it's one of the pulmonary function tests that we do in the pulmonary function lab. And it basically measures-- you take a big breath in, and it measures how much air can you blow out in one second.

And if you have a disease that has significant airflow obstruction, like emphysema, or other forms of COPD, your numbers aren't very good.

So what does it actually take in order to differentiate a pluripotent cell and make all of the differentiated cell types that you see in the lung? I'm not going to go through all of the detail on this figure. But this is from a review in Morrisey's lab about a year ago. And as you know, the definition of a pluripotent stem cell type is that you can make all three germ layers.

So you're going to be able to make tissue of the ectodermal lineage, the mesodermal lineage, and the endodermal lineage. From the lung, pretty much everything comes certainly from the epithelial component from the endodermal side. So in order to make some of these cell types that are more differentiated within the lung, you've basically got to go from a embryonic stem cell or an iPS cell. You've got to be able to make definitive endoderm from that.

And then you've got to be able to coax that definitive endoderm into cells that actually express Nkx2.1, which is indicative of the lung progenitor field. And then from there, using various cocktails, you can get down into some of the differentiated cell types, and that's actually doable. There are a number of even commercially-available cocktails now that will allow you to be able to push iPS or embryonic stem cells in specific directions.

There's actually commercially-available kits that allow you to make FOXA2 positive definitive endoderm. And Matt's done a fair amount of work over the past couple of months in getting some experience with these. The bottom line is they work. So from the two iPS lines that we have so far from the trial-- this is merged [INAUDIBLE] and Foxa2 staining over here on the right side for two different lines.

And you can get reasonable purity of lines, either just alone, or with flow cytometric cell sorting from the differentiation of iPS cells. To take that further, you can then use a number of different cocktails from there. So for example, the active in induced definitive endoderm can then be exposed to FGF2 and sonic hedgehog in order to push things in a direction where the cells actually express Nkx2.1, which is one of the markers of the lung progenitor field.

And this is an example from one of the lines with the combined [INAUDIBLE] and Nkx2.1 staining. Again, you can create cultures that are relatively pure that are indicative of lung epithelial differentiation. I won't go through all these details. There's a number of cocktails available in the literature that people have described to create cell types expressing various different marker profiles. I think they all work, to varying degrees, but obviously working with the commercially-available ones makes things much simpler.

The bottom line is, you can do this. You can go from an iPS cell. You can go from there to definitive endoderm. You can go from definitive endoderm to something that express markers that are indicative of the lung field. And then you can go all the way down to cells that express markers of epithelial lineages in the lung. And Matt's taken cells all the way to CC10 positivity. It's just hot off the press, so I don't have a good picture of it yet.

The other interesting things is that some of these intermediate-- so for example, going from iPS to definitive endoderm, you can actually suspend that for a certain amount of time, too, which I think is also going to be important issue for regenerative applications. So Matt's actually got one line of Foxa2-positive endoderm that he's actually taken through multiple passages, and still is able to maintain that in a relatively pure state.

It sometimes takes some intermittent flow sorting in order to keep it that way. But the idea that you don't have to go all the way back to the initial stem cell to be able to make the cells that you want, and you might be able to make suspended intermediates for clinical applications, I think is a very powerful observation.

So clearly next steps here. I mean, a lot of the hard work here about what cell types are really going to work best-- like can you just put an iPS cell on a lung matrix? And does that work just as well? Do you get differentiated cell types? Do you have to do some form of partial endoderm differentiation? Do you need more differentiated epithelial cell types? Bottom line is, right now, we have no idea.

The hard work, then, of taking all of these various cell types and using combinations of both decellularized matrices and doing even things in-vivo with cryoablation, lung injury models, and things like that, are really the important steps here to make progress towards real clinical applications.

I'll just speak briefly about the whole aerodigestive space. In theory, you think this would be a lot easier. I mean, you're talking about hollow organs in the chest. We already have vascular grafts. You can take a tube of Gore-Tex and replace somebody's aorta, and it works really well. It's really well perfused. It's a nice clean space. As long as you sew it in right and it doesn't leak, you can live like that.

If you think about it, if that works, why can't you just do the same thing for the trachea? Why can't you do the same thing for the esophagus? In theory, that should work. Unfortunately, a lot of the work that's been done so far to try and just translate what's been done in vascular grafts, for example, doesn't really work when you're talking about the trachea. It doesn't really work when you're talking about the esophagus. Either the organs fall apart, they stenos. Lots of horrible problems without really a lot of success so far.

But one of the startling successes that's happened in the last couple of years is the idea that you might be able to use some kind of tissue-engineering approach, where you have a scaffold or a decellularized donor trachea that's then been reseeded in order to use for clinical transplantation. And this is the paper from Paolo Macchiarini's group, from him and Martin Birchall, of a young woman.

This is a 30-year-old female who had already had a tracheal resection and reconstruction, but had a stent in for a long time period and developed a stenosis at the distal end of the stent. And she basically had almost a complete obstruction of her left main stem bronchus. This is a 3D reconstruction view here just depicting what the stenosis actually looked like.

And what they did-- the reason this was so startling is that they took a donor trachea, they decellularized it, and then seeded it with a complicated cocktail of mononuclear cells, biopsied tracheal epithelial cells-- I have to be honest. You can read the paper a million times, and it's not entirely clear what they did, but they basically took a bunch of cells, seeded it on the matrix, and then operated on her and re-implanted this thing. And it worked, startlingly.

A lot of people have been very critical of this because without a lot of large-animal data to show what's really happening to the cells, what's really happening here in terms of ingrowth, many people have even suggested that even the graft itself that was implanted-- that all the cells don't actually hang around there. They just act as homing devices for the important cells to come in and migrate to the graft. The bottom line is, we don't know.

There's now been 14 tracheal transplants that have been done in the world, and 1 in the United States so far, though. And I think for people that are really in a no-option situation, this is ready for prime time.

What about the esophagus? The esophagus itself is one of the other hollow organs in the chest. It has some unique issues in the sense that it's the dirtiest of all of them. You've got gastric and esophageal contents freely flowing through this.

And you've got the problem that, like the aorta, you can't really tolerate any kind of leaks. If you're leaking from anywhere on a reconnection point for somebody that's born a hole in the esophagus, or got a perforation due to cancer, or a problem with an anastamosis around the time of surgery, it can be a lethal event. Your chest does not tolerate gastric contents very well within it.

This is an example of a patient that we had about a year and a half ago with a not-uncommon problem, although we don't like to admit it. In thoracic surgery every now and then, for people that actually have esophageal replacement surgery, like an esophagectomy for espophageal cancer, where you've made a new tube out of the stomach, and you've brought it up, and hooked it up to the proximal esophagus to replace the esophagus, sometimes that reconnection point just doesn't heal well.

And when it doesn't, this is what it looks like. You can sometimes end up with these big gashes where there's not enough healthy tissue there to be able to put a suture on one side of the hole and actually bring everything together.

This is a very difficult problem because the textbook answer is that the only way you can really deal with is do what we call a defunctioning operation, where you basically bring the cervical esophagus out in someone's neck and put a stoma bag on it, remove the whole esophagus here, and then put a feeding tube in the stomach below, and just completely disconnect everything so that you can have a chance to heal, and come back, and fight another day with using either colon or small bowel as an interposition graft.

But it's a huge deal. It has a very high morbidity and mortality rate, and it's a big problem. This is an example where we've started toying with the idea of, can you actually use decellularized matrices in order to patch large holes like this, that you otherwise couldn't fix? And the commercially-available matrix scaffold that you can get-- although it's not FDA approved for this use-- is a product called Alloderm, which is basically decellularized cadaveric human skin that you can get off the shelf in the operating room.

It's approved for use in hernia repair and breast reconstruction, but theoretically could be used in any situation where you need some kind of matrix scaffold for cells to grow in on. This is a rendition of one of the initial patients that we fixed a hole like this. Basically, all you do is use the Alloderm product, get it hydrated again, and in this situation, you can either just sow down a patch-- something like this-- or do a wrap all the way around a hole like that.

This is basically what it looks like in the operating room. So this is the real rendition of what the artist is depicting here. And this can actually work. We've done a total of four patients so far. We have three of them where we have at least many month follow up like this initial patient. It's very interesting what happens here. This is one of the initial patients that we did, and what I'm showing you here is the endoscopic view. This is with the gastroscope down and through the esophagus, and then looking at the reconnection point.

And what you see here is where the Alloderm patch actually was. It's a bit hard to appreciate here, but what you actually see endoscopically is you see these little patches of where you've actually had pink reepithelialization occur. It doesn't completely reepithelialize within a four-month time period, but that process has already started at four months.

And having something to completely block this hole, and not have any leakage outside of it, was what made the difference for this patient to actually get out of the ICU, get out of hospital, and work towards being able to eat again on their own, and salvage the conduit that we had initially placed at surgery without having to defunction everything.

So can you go beyond that? I mean, it's one thing to plug a hole. What about replacing the whole esophagus? The development agreement that we currently have right now with Nanofiber solutions is to actually do just that-- to take some of the synthetic scaffolds that they've made with various chemistries, and Matt from the lab has already done a great job in deriving some pig epithelial cells, and is doing some in-vitro experiments.

I'm not going to show you any results today, but to try and sort out some of the issues of, what's the porosity that you need from the graft? What's the chemistries that actually work best in order to allow cells to be seated on it? And we're on track to sort that out so that we can do some pig experiments. This summer, we're actually going to do a thoracotomy, cut out a portion of the esophagus, and put these grafts in in order to really be able to see what works.

Just a couple of minutes and then I'll wrap up on the Regenerative Medicine Biotrust. You've been hearing a lot about the Center for Regenerative Medicine. The Regenerative Medicine Biotrust you may or may not have heard in some of the talks given from leadership within the center. But I wanted to introduce the concept today since it's kind of cool.

The way I would envision the Biotrust-- and I give Andre and Mike Fenning a lot of credit for the foresight in supplying resources to build this infrastructure-- but the way I see it is that there's a very big gap between what we're actually going to do at the clinical application side-- so what's really going to go into a real patient-- and then at the other end of the spectrum, although it's not depicted here, what people are actually doing in the lab.

Like the Harold Ott example of decellularizing a rat lung, and then recellularizing it. There's a whole bunch of stuff that has to happen in between to really get to the point of real clinical application. And what the Biotrust is designed to do is to be a cellular repository where we have the opportunity to be able to collect, to be able to process, to be able to store, various cells and tissues for both research and translational purposes in regenerative medicine.

So just a couple of brief slides. The overview of this is that what it's going to be is one of the key infrastructures that's really going to enable regenerative medicine technology transfer within the institution. Within the first couple of phases-- and this is within the three-year plan-- the goal is to try and engage over 6,000 patients and have over 120,000 samples accrued from those 6,000 patients.

To split that up in terms of what that looks like, we really just got started in 2013 with a number of people who are sitting here in the audience being recent hires to the Biotrust infrastructure. And our goal in 2013 is to have 250 or greater patients enrolled on a number of the pilot projects that we're currently working on. There's a lot of work to get this kind of infrastructure up and running, and do things like standardized protocols for collection and processing of cells and tissues, for people to be able to use them.

But I think we're definitely on track to be able to meet all the milestones for 2013, and be able to significantly expand this, and launch it bigger and better in 2014.

So what's going to be in the Biotrust? Well, really many things. Both live cells, biomaterials, tissues-- I mean, any derivative that might actually have a research or a translational angle to it within regenerative medicine, we're interested in hearing about, and want to be involved in. The main idea here is to have the infrastructure set up so that we have all the systems and processes in place to be able to identify patients, to be able to enroll them, to be able to track process cells, and also to be able to create live cell products for use.

None of these things are trivial to do on a large scale. We hope this is going to be an infrastructure for both knowledge and technology transfer, both within and outside Mayo Clinic, and really position us as one of the leaders in regenerative medicine. So what's happening right now? We're, I think, beyond the rollout phase. I think we can safely say that, in terms of what's already been accomplished.

We've got equipment in place, even though we don't actually have dedicated space yet. It's coming. And there are a number of collaborative pilot projects that we've engaged people on in order to set the infrastructure and get things up and running. And I'll just run through a couple of those quickly here, and then we'll wrap up so we have time for questions.

So a couple of different pilot projects, one in the cancer space looking at a number of different SNPs within brain tumors, where there's an interest from Dr. Jenkins and his group of looking at specific SNPs and what that actually means for neuronal differentiation. Which doing that through iPS technology will be a great way to do for their needs.

We've been working with the Hepatocyte Tissue Registry in order to sort out the issue of harvesting human hepatocytes in the operating room and being able to process those for experimental uses. We've been involved in umbilical cord blood isolation, or mononuclear cell isolation, from babies over at Methodist. And to try and have these available for subsequent uses, I think, will be an important resource for investigators here within the institution.

There's projects ongoing in diabetes using iPS cell technology for treatment of diabetes. Same kind of idea in ALS and other various neuropathies. And the other important one I just want to mention is the development of the regenerative medicine consult service, which Tim Nelson is really heading, although my name's here.

And the idea of that is anyone who might potentially be able to access a clinical trial in regenerative medicine, get questions answered about regenerative medicine, has a means to be able to do that through the transplant center and the regenerative medicine consult service. And what we're trying to do is use that as a centralized place where people can have the clinical interaction, but also be able to access some of the activities of the Biotrust for things like iPS cell generation and another tissue harvesting and processing.

In terms of contacts-- so I guess, technically, I'm the medical director of the Biotrust. Andre and Mike, of course, are leading the center, but the people who really do the work are Mindy Rice and Zach Rasch, who are here, and then Cass and Kimberly who just started with us as technicians, and Lindsey, who's a recent hire as an RN study coordinator. So I think I'll stop there. I'm happy to take any questions anybody has. But thank you.

[APPLAUSE]