Drug Delivery Principles and Engineering

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Lecture – 28
Tissue Engineering – I

Hello everyone, welcome to another lecture for Drug Delivery Engineering and Principles. I am Rachit and I am going to continue to talk about drug delivery and various aspects of it. So far what we have learned throughout this course were some initial concepts of how delivery happens. So, in traditional forms, its tablets or some capsules or IV injections, but then we pointed out that what are some of the definitions, some pharmacokinetics, how they distribute in the body and then we talked about some ways to sort of enhance that delivery.

So, we were talking about sustained release and controlling the system as to how and when to release the drug. And during the course of this subject, we have learned several strategies. We first discussed polymer drug conjugates, then we talked about some sort of macro-devices, either reservoirs, some pumps, or some erodible, non-erodible matrices. Then we talked about some particles in general - how they are synthesized, why they are useful, some injectable hydrogels, so all of that we discussed. Then towards the last 2-3 classes, we were discussing protein adsorption, which is basically a phenomena that happens on all foreign substances.

So, once you put in an implant, the proteins tend to adsorb on those implants. So we pretty much wrapped up the protein adsorption discussion in the last class. Now, we are going to move forward with Tissue Engineering. And this is again a very explosive field. These days, quite a lot of research is going on and its actually very exciting as well as very promising for patients.

So, we will briefly discuss as to what the buzz is all about. Tissue engineering, I think as you will see in this course, throughout the rest of the course we will discuss tissue engineering as well as drug delivery simultaneously. And tissue engineering itself involves lot of delivery of different bio-molecules or small drugs. And so it becomes important to talk about tissue engineering in a course of drug delivery.

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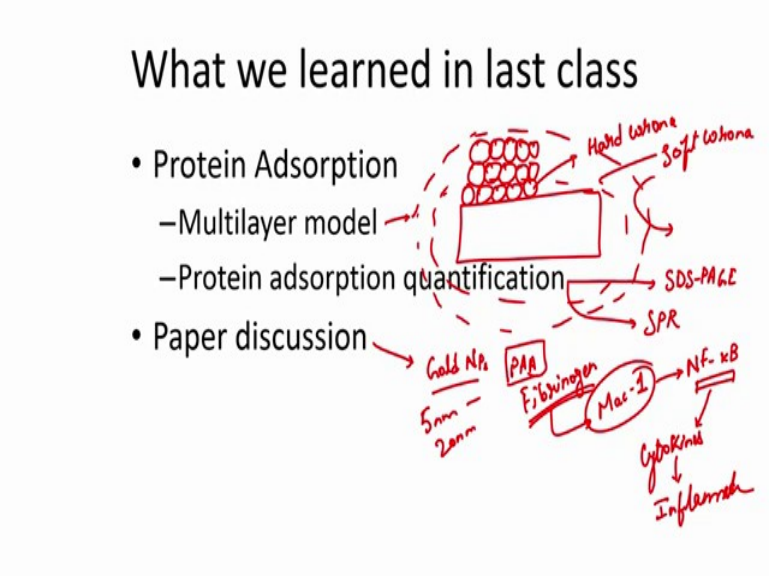

So, let us just quickly recap what we learned in the last class. We basically, as I said, finished up our discussion on protein adsorption, so why and how they adsorb? Towards the end, we were discussing about multilayer model which we were saying that if we have a surface it will not only form a single layer of protein, but it will have multilayers. And then we said that at some point there will be hard corona which is fairly stable and does not change a whole lot. And then the outer layer will be what was defined as soft corona and what is this? This is also protein getting adsorbed by some affinity but this has very low affinity. So, these proteins can actually go in and out, whereas the hard corona remains fairly stable.

Then we talked about protein adsorption quantification. So, there were few things here - one was the SDS page. So, you can take your material you can treat it with SDS, heat it up, SDS is going to denature all protein and will interact very strongly with the protein. So, that way all the protein that was on the surface will come out. And then you can run a PAGE gel to figure out what is the molecular weight, and then follow it up with some kind of a western blot for a specific protein to sort of give you semi-quantification of how much amount of proteins are there and then which are the different types of proteins that are also present.

And then another way to quantify this is to use something called SPR, which is the Surface Plasmon Resonance. It depends on the dielectric of a particular surface as

protein adsorbs to it, the dielectric changes and that is how you can quantify it, by running some standards. So, these are two of the methods we discussed. And then we had a paper discussion which had talked about gold nanoparticles which were coated with polyacrylic acid. And we showed, at least this paper showed, that what can happen is once these foreign substances go in the body, all kinds of protein adsorb to it. In this case, the protein that was involved was fibrinogen.

And what was happening with fibrinogen, it was getting denatured on the surface of these gold nano particles. Because of that there were some hidden sites which were getting exposed. One of those hidden sites was interacting with a receptor called Mac-1 and this Mac-1 then further caused upregulation of NF-kappa B which is again a master regulator for sort of a initiation of immune response that resulted in lots of cytokines being released and all downstream processes that are responsible and controlled by NF-kappa B.

So, what we learn here is that even though we feel that gold is a very suitable material, it does not have much toxicity, but depending on its configuration and depending on surface charge, surface coatings, we find that it can actually cause inflammation. So, I mean all of this is essentially leading to inflammation, which could be toxic to the body. So, if you inject too much of this and the inflammation really goes out of hand, the patient may actually have to go to the hospital and we have to get anti inflammatory drugs and things like that.

So, this is just one thing and what we found is if we replace PAA with some other polymer this, at least the fibrinogen based inflammation, wasn't happening. And then similarly the size of the gold - 5 nanometer was giving a lot more inflammation, whereas 20 nanometer - not so much. So, there are several factors that play here. So, just on the basis of the size or the surface coating or the charge, as we saw, you can have very different outcomes of whatever your therapy is. So, these are some of the important things to consider when you design these materials for use in human and patients.

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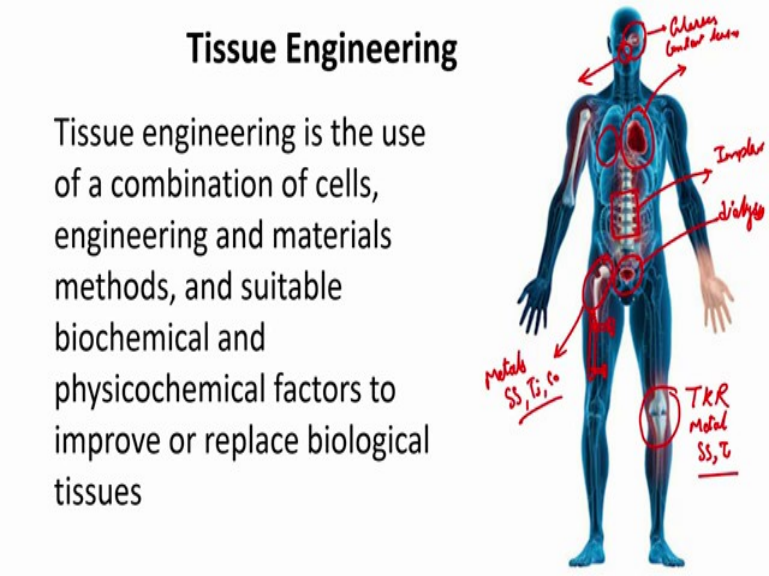

So, with that let us jump into tissue engineering. As I said it is a very exciting field and quite a lot of buzz is around it and we are also going to discuss it in the next few classes. And so what essentially is tissue engineering? Tissue engineering is nothing but is the use of combinations of cells, engineering and materials to improve or replace some of the biological tissues that we have in our body. Some of the examples here are and some of them you are very familiar with. So you might have seen hip joint replacement. So, a lot of time metals are used here, because of course the strength is required, the hip joint actually carries the whole body forward. So, metals such as stainless steel, titanium are very widely used for this, sometimes cobalt and chromium and all.

You have heard about heart pumps. So, let us say if your heart cells are not able to pump very well, then an external pump is put in which gives heart signal to pump at a certain rate. You have heard about total knee replacement, which is essentially if you are suffering from osteoarthritis and a lot of your cartilage has gotten damaged then you can go in and again this is also based on metal materials such as stainless steel, titanium. So these actually are very widely used in current clinics.

So, you will actually find people already with some of these sorts of implants. Another thing that you will often see is in case of complex or sort of large fractures what you will find is the doctors will put a plate and some screws to sort of stabilize these plates and the fractures. And again that is required because this is a structural organ it needs to bear

the weight and so you need to have quite a bit of stability around it. And these are some things that are very widely used. In fact if you really look around you might find some people you know that have these implants.

Then we have also heard about, obviously we've heard about lenses. So, lenses quite a lot of people use. So, these could be either the glasses themselves which is not really under the class of tissue engineering, because this is something external that you are using. But then you have contact lenses and sometimes these contact lenses may also be made out of polymers and all. These are very widely used in clinics. Then you have artificial kidneys and external dialysis that happens. So, those are again part of that. The ribs and the spine also have implants which are used to stabilize them.

So, this is basically just an overview. I mean there is a lot more that is being done now these days with human tissue engineering, and you will find quite a lot of that. You may find people having some sort of deformities in their nose and then their nose, just for aesthetic purposes has been added with some polymers and you find silicone breast implants. So, all of those things are there. So, we will discuss briefly about few of these things. It is again as I said tissue engineering could be a whole course by itself. But we will touch upon some of the concepts that are required especially in drug delivery scenario through this tissue engineering module.

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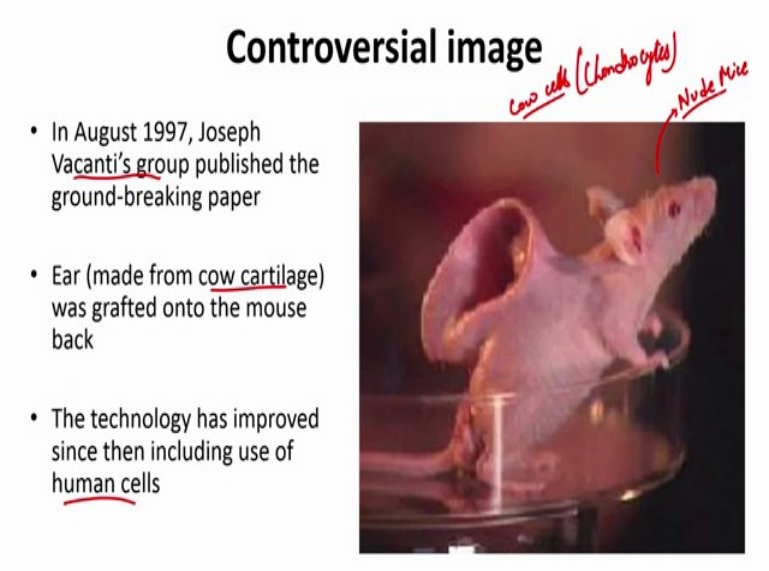

So, this was one of the very controversial image that was published quite a while back now. This is essentially what you are seeing here is a mouse, a laboratory mouse, in '97, Joseph Vacanti's group actually published his ground-breaking paper. And what they had done is - they have made an ear out of a cow cartilage. So, they took cow cells, in this case chondrocytes, which are the cells of the cartilage (major cells of the cartilage). And then they did some tissue engineering and grafted it into a scaffold and put it on a mouse back and let it grow. And after a certain amount of time, they get a structure like this where you can see a sort of very similar to a human ear looking like structure on a mouse's back.

So, this was proposed as one of the ways that we can tissue engineer some of these tissues and get them ready for humans. So, whenever a human needed, at that point he will euthanize this mouse, take this implant and use it for humans. I mean of course, that was the vision at that time. We know that there are lots of hurdles that were needed to be crossed. Some of them are crossed now and then some of them still need work before it can become a reality for humans.

So, as I said and then further the technology has improved. So, in this case, they were using cow cells now actually several groups have reported similar results using actually human cells. So, you can actually take cells from a human body, culture it for some time, implant in some animal either mouse or some other animal and then have these tissues ready if you need to put it in humans. Again, none of this is actually done so far, but then there is quite a lot of excitement as well it is quite a lot of debate about how and whether to do it or not and that is currently going on.

And just a side note I mean this is of course, a nude mice. So, if you are wondering why these mice are not rejecting these cow cells or human cells, because this is a nude mouse. And what I mean by nude mice is these mice do not have any immune system. So, these mice actually have a heavily susceptibility to infections, they cannot really survive out in the wild they are only grown in laboratory conditions extremely clean conditions to make sure that none of these mice get infected with any sort of pathogen and only then you can put these cells which can then survive because the mouse immune system is not going to attack them since the mouse does not have an immune system. So, those are called nude mouse ok.

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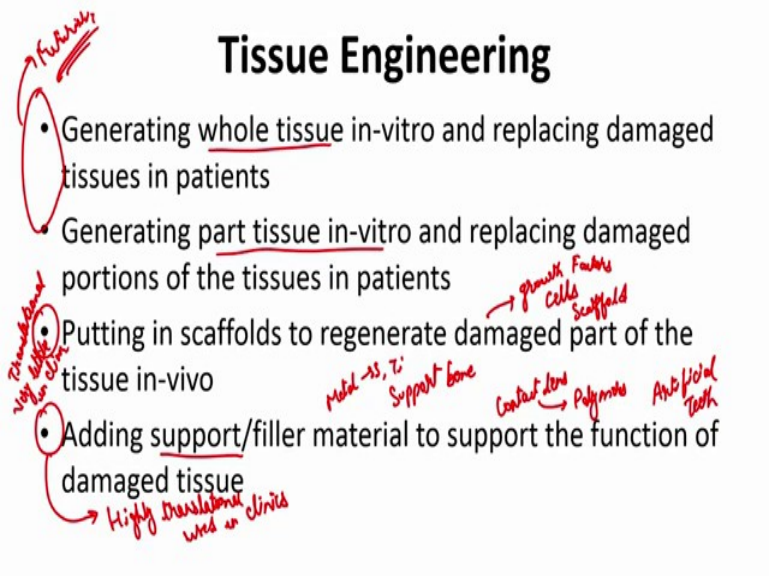

So, further on tissue engineering, so I mean tissue engineering can technically be divided into sort of four-five domains. So, I am just going to briefly describe these domains. One is you generate the whole tissue in vitro and then like in the previous image, and then you replace the damaged tissues in patient. So, in the previous image, it was done in mouse, but then that has lots of ethical as well as translational issues. What happens if you implant mouse, there are some of the blood vessels might be from the mouse. So, those are some of the issues there.

So, an ideal way would be to let us forget about the mouse just use all human cells and grow it in some kind of a culture dish, get to the whole tissue a level and then if the patient comes in with some let us say damaged lung, then you replace the lung from the culture tissue for the human. I mean again this is all, at this point, a hope that this will happen in future. Some of the results that have come up has shown that this can actually happen. But in the actual scenario it has never happened so far. And that the research and the technology still needs more work before this can actually happen. At least for all tissues.

Then the other sort of branch of tissue engineering is to generate part of the tissue. So, this is actually you will find is closer to human translation. So, essentially let us say if I come with a damaged tissue part of my liver is damaged and I can still live without it,

but my function is sort of hindered or it may continue to get hindered. So, I want to get some sort of a healing process to start maybe have that whatever the tissue is damaged.

So, my level is now operating it lets say 50 percent capacity, so I want to get that 100 percent capacity back. So, you can grow a part of the tissue in-vitro and then replace it with the damaged portions in the patient. So, that way maybe I won't get to 100 percent from 50 percent, but maybe 80 percent of the liver is now functional. That is the hope in that case. So, this is again still needs a lot more work before it starts to get used heavily in humans, but as you will see some of this is actually starting to translate and a lot more hope for this section of tissue engineering to actually go into the humans is currently among the scientists and the clinicians.

Then the next thing is let us not grow the tissue let that tissue handle how to grow itself, but what you can do is you can provide a scaffold. So, again the same example let us say half of my liver is damaged. It is only operating in 50 percent, I have tried to grow a liver tissue in my lab as a scientist and it is very difficult.

So, what you can do is, you can instead of doing all this process in-vitro in your lab what you can do is you can put the scaffold at the damaged portion, supplement it lets say with whatever growth factors that you might require, so let us say you put some growth factors, maybe some cells, and the scaffold of course. And give it the right conditions, the right properties of these scaffolds for the stem cells in that niche to sort of populate this and start to make the damaged tissue and that way you can essentially help the patient restore some of the function and that was lost during the damage.

And then the final one which is actually now very widely used in tissue engineering and we gave some examples in the last slide is to adding support or filler material to support the function of the damaged tissue. So, this could be let us say if I need a structural support, so I am putting in a metal such as stainless steel or titanium to replace bone or to support bone.

This could be that my lenses are not working in my eyes, so I put in a contact lens that again is made out of polymers. Or my teeth are having issues and what I can do is whatever tooth is having problems I can replace them with some artificial teeth. And then there is several, several applications, the more you read about it, the more you will get to know of how different support and filler material are being used actually in clinics.

So, among all this, this particular point is highly translational and it is actually being used in clinics quite a lot, and more and more solutions are also coming up. The regeneration is again is somewhat translation not as translational as the last point. So, it is translational and very little is being actually used in clinics. And then these two point are at this point more futuristic and then the hope is at some point of time the technology will advance enough that we can reach at this goal. At this point I mean the idea is translational, but at this point it does not, in near future - at least in the next 2-3 years, it does not seem like this is going to be something that is going to be used in humans quite a lot.

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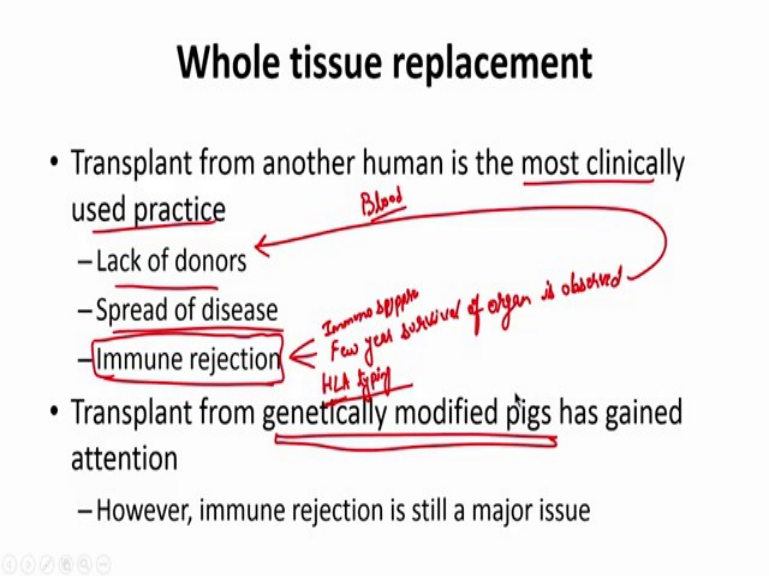

So, let us discuss again each of these points in a little more detail. So, at this point for a whole tissue replacement let us talk about what are the technologies that are out there. So, one is and you might have heard of this organ donation and all one is to sort of have it transplanted from another human.

And so let us say if a human is registered as an organ donor, if I am registered as an organ donor and I meet an accident and I am dying, at that point some of my tissues which are not damaged in the part of the accident, let us say it is eyes or lets says something else, those tissues can be then retrieved from my body. And then put into a recipient who is actually suffering from let us say some disease maybe the eyes or he is

complete he or she is completely blind and needs an eye implant. So, those can be then transferred.

And this is again very heavily used in clinics. What you actually find is there is quite a lot of demand for organs to be donated. In fact there are not enough organs to be donated. So that is something that we can consider to be signing up as an organ donor and that actually helps save life when we are not there anymore.

So, some of the issues with this particular thing as I just mentioned there is lack of donors. There is a chance that disease may spread. Let us say if a donor was suffering from some chronic disease, could be HIV, infectious disease or could be something else and then there is a chance that some of those HIV viruses will end up going to your recipient as well. So, the disease transmission is high. So, it needs to make sure that whatever organ is being used has been vetted very well to ensure that it would not spread the disease.

And then the final and the major hurdle with this sort of thing is immune rejection. So, again all humans are different. So, if I get an implant from another human, my immune system is not going to like it at all. It is going to continue to attack it and continue to damage it, so not only I will suffer from all kinds of problems, because my body is in a continual state of inflammation, but then the organ will eventually get damaged.

So, what you will find is most of the time when these organs are donated, some kind of immune-suppressants are given. So, immune-suppressants are given and then over quite a long time and then that then increases the risk of infection because now what I have done is to accept that donor implant we have suppressed my immunity and now because my immunity is suppressed I am more susceptible to get infections such as bacterial diseases, viruses, worms and all kinds of things. So, all of these become an issue.

And then most of the time you will find that only few years survival of the organ is observed. So, what this means is even though I am under immunosuppressant, my immune system is still rejecting it, the cells in this new donor organ that I received are not also very happy, because my immune system continues to attack it even with the immunosuppressants. And eventually because of all this the function of the new organ that I have got continues to go down and down and eventually it will get rejected.

So, for a few years maybe 5, maybe 10, depending on the organ and the site of the location you may end up getting quite a bit of help from this, but then it goes back to the issue of lack of donors, because what we are saying is one donor is not going to be enough for one recipient for a longer period of time. So, one recipient may need donors from very large amount of donors rather than just from a single donor. So, some of the strategy is to sort of reduce this immune rejection is done is what is called the HLA typing. And we will talk about more of this HLA and MHC when we get to the Immune part of the course, but what is essentially being seen is we all humans are divided into several types of HLA.

So, what I mean if I have a particular type of HLA, my immune system is closer to another person who has a similar type of HLA. If I have a similar HLA to another human, that means, that my immune system is very similar to that particular human. So, if I get an organ from those types of donors, then those implants will survive longer, because my body will not be as quick to reject that implant. It will still be rejected or still I need immunosuppressant, but the chances of the graft to survive are much higher. So, those are again some of the things that are done. So, you will hear this HLA typing term quite a lot when you go around and look for donors.

One of the tissue that has actually very well is the blood. So, blood donation you will see all the time I am sure some of you might even have donated blood or received blood. But then the blood donation works very well just because these blood cells have a lifecycle of only about 3 to 4 months and so and then their immunogenicity is also very low. So, immune system does not really reject them a whole lot and then anyways these cells get recycled every 3-4 months. So, this blood donation works very well in terms of the organs, but then when you talk about big organs and solid organs, those have always risk of immunosuppression.

The other way that is being now done is to get transplant from genetically modified pigs. So, pigs have been studied quite a lot for this and they have been found to be very similar to human. Well, of course, not as similar as another human, but still among all the different species, pigs are supposed to be the closest as well as in very similar and sort of the sizes of the organs as well. The problem again is going to come back to this issue of the immune rejection. However, what people are now starting to do is they are continuing to modify pigs more and more such that whatever different aspects of the

immune system that is recognizing that this is foreign and it is a pig thing not a human thing.

Those slowly and slowly with more and more genetic modification, all those things are getting removed from the pigs by genetic modification. So, what is resulting in the end is the pigs are becoming closer to human, more so than what they are in the natural state. These genetically modified pigs, their organs are getting very similar to human organs. And especially the regions that get rejected are being removed more and more. So, what you find is, it is becoming a viable alternative to the human donation just because these pigs are very ideal, because if you need an organ you can get it at a very short amount of time right because you can have a pig farm where you can get these pigs.

And let us say if I come in with a requirement for an eye, those pigs can be used to immediately get eyes for my body. But again, having said all that, this is still very far away from reality to be used in clinics. So, it will still need a lot of work before these things can become a possible alternative to humans. As I said our immune rejection is a still a major issue.

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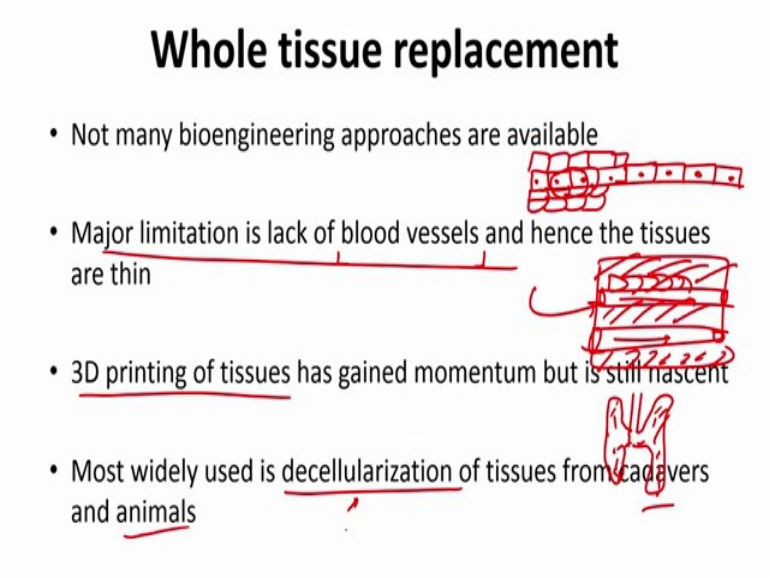

So, on this whole tissue replacement, there are not many bioengineering approaches that are available. So, you cannot really grow a whole tissue in the body or outside in a dish. The problem there is, it becomes the problem of size. So major limitation is a lack of blood vessels. So, if I have a single monolayer of cells that survives very well in a

laboratory setting. But as I start to stack up more and more cells together, the oxygen diffusion to the inner core of this tissue will become an issue and that is why in our body you will find there are lots or lots of blood vessels.

And their major job is to make sure that the blood, the glucose, the oxygen is going to every part of the tissue. And when I grow them in vitro, in a culture dish, it is very hard for me to get to that level of intricacy and that level of distribution as we see in our own organs or in animal organs and that is why you can only get very thin tissues, a millimeter at max. And once you start going bigger than that, you start seeing quite a lot of cell death in the center which limits the growth of the tissue.

So, this has been a major issue, lots and lots of people are working to figure out how we can overcome this, how we can also grow blood vessels at the same time to ensure that enough oxygen and glucose and waste material is getting removed from the system. So, one thing that has come up is 3D printing of the tissue. So, what people are doing is using 3D printers and essentially making these large tissue structures with let us say vessels going through them or at least the space for the vessels to go through them.

So, the media can actually go through and then the rest of the places they will grow their tissues and there will be another blood vessel, so that way they can get to little bigger areas. So, this is the flow and the rest is a tissue. So, that way they can get to bigger tissues, but still the problem is that it is still a new field, is still very nascent and still to be seen whether something grown from this is going to be very functional in a animal setting. Although quite a lot of research is being done on this.

And one of the most widely used strategy for a whole tissue replacement is called decellularization of tissues and so, what is essentially done is the tissue is taken, its decellularized. So, let us say if I take a heart or let us say a lung, let us say I have this lung tissue that contains all kinds of things that contains vessels which are everywhere in the lungs that contains lots and lots of cells, that contains lots of extracellular matrix that makes up the region on which the cells are growing. And so what is done is, this tissue is taken either from cadavers or from animals. This tissue is then treated with several reagents to make it decellularized.

And what that means is all the cells are removed, all the free proteins, free genetic material is removed. And once that is done, most of the things that are recognized by the

immune system are these cells and these small proteins that are different from the humans and once those are removed, those are fairly non-immunogenic material. And then you can come in and seed cells from a patient body or from a human source which will then result in a filling of these ghost of your initial lungs and they also have their blood vessels as well.

So, you can see it all kinds of cells, cells that are present in blood vessels and endothelial cells, the cells are present in the lung, epithelial cells and culture this and then you can use it as an implant. Again, very widely used in research not a whole lot of it is being used in clinics. So we will stop here and we will continue the rest in the next class.

Thank you.