Drug Delivery Principles and Engineering
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Lecture – 51
Immuno isolated Cell Therapy

Hello everyone. Welcome to another lecture for Drug Delivery Engineering and principles. We are discussing immunology and immuno engineering part of this course and looking at why the drug we have, is required for those aspects. So, let us just do a quick recap of what we learned in the last class before we go further.

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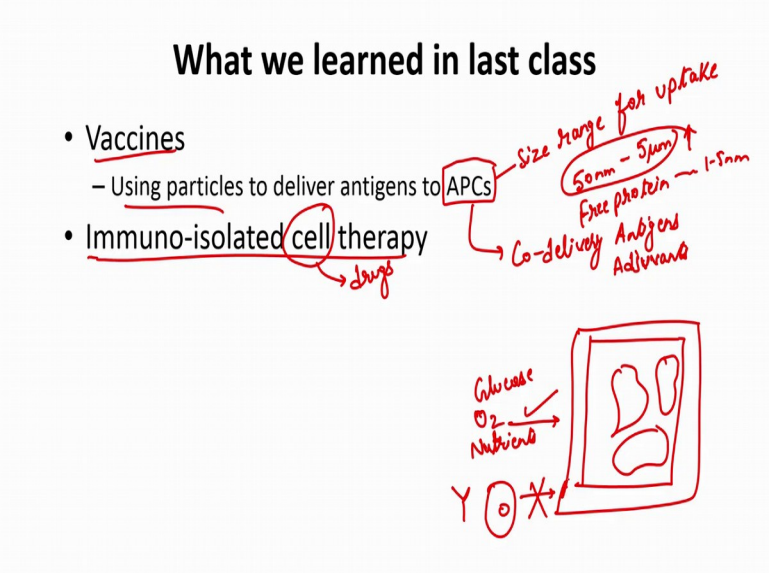

So, in the last class, we finished our topic on vaccines. So, we had discussed already different types of vaccines based on timing and content. And then in the last class, we looked at why we need to use particles as control delivery and sustained delivery to deliver antigens to APCs. And the major reason we said is first of all, it causes enhancement of the delivery of your antigens to the APC, the size ranges; size range for uptake is much better when they are in between let us say 50 nanometer to 5 micron. It is a big range and there is also ups and downs here, but it is much higher for anything compared to let us say free protein which could only be about 1 to 5 nanometer.

So, first of all you can then now deliver quite a lot of things. The second thing we talked about was you can do a co-delivery with antigens. So, you can deliver multiple types of

antigens as well as co-delivery of adjuvants. So, you can then ensure that since it is in the same particle, the adjuvant and antigens will be in the vicinity or in the same cell and that would make sure that the two-signal requirement that is present for activation of leukocyte is met in a more sustained manner. And then we of course, also discussed there are some challenges with these particles. It may denature your antigen sometimes that could be critical and so those are some of the formulation issues that need to be taken care of.

Then we started our discussion on immune-isolated cell therapy and as the name suggests, this is to deliver cells. So, in this case your cells are your drugs and as the name suggests here immuno isolated as we want to protect from the immune system. So, we do not want immune system to attack these cells which could be from a foreign source ,could be either from a different human or actually could be from a different species altogether. It could be from pigs for example, and so that is what we were discussing, and we discussed some of the concepts of first of all why that is required and the secondly, how potentially you can do it.

So, in that we were saying that we can encapsulate in some kind of a semi permeable membrane. So, your cells can just reside in this chamber in which the pores are small enough to allow glucose, oxygen, your other nutrients to go in, but it does not allow your antibodies and cells to go in. And that way you can have this isolated from the immune system to some extent and we will see how this goes on in today's class ok.

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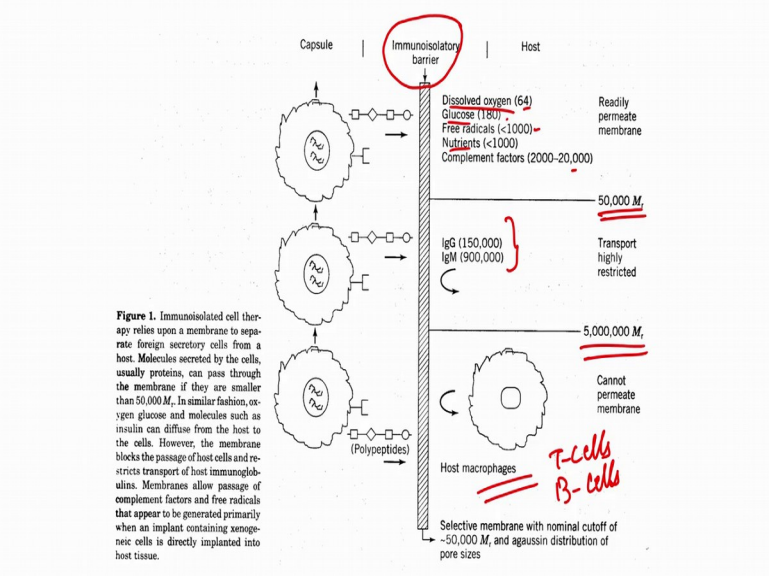

So, this is just a pictorial representation of what I mentioned. So, you have some kind of a barrier, it could be polymeric, it could be something else. This is your this is a barrier that will allow let us say dissolved oxygen, your glucose, some free radicals, nutrients and complement factors to go through, but it will not allow your big molecules like antibodies.

So, this is all you are seeing here is some molecular weight here so, but this will not allow anything which is higher molecular weights to go through which could either be big large protein such as antibody or could be macrophages and T cells, B cells; those things cannot go through. So, that is the whole concept here of course, this has lots of challenges and we will talk about that in today's class.

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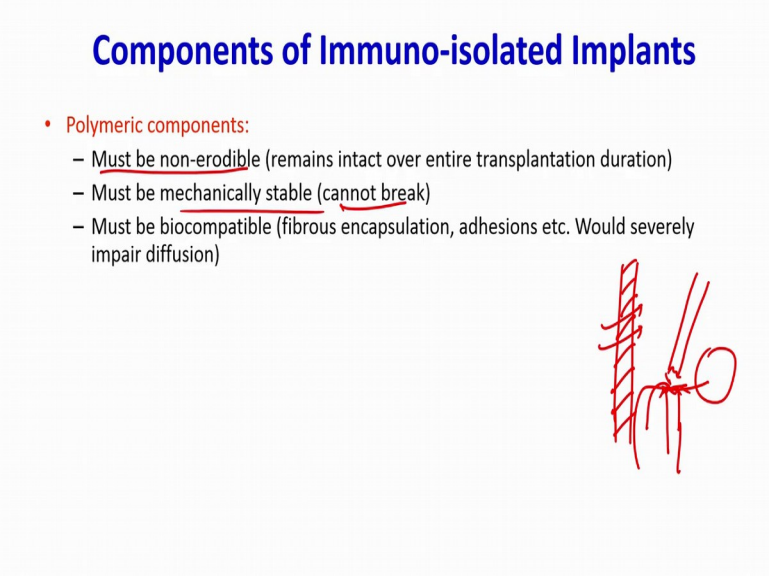

So, let us look at various components of immune-isolated implants. So, there could be a polymeric component, as I said this barrier could be a polymeric barrier. So, if this is the case and if you want this to be for life, this has to be non erodible right. So, if this is the barrier which is made out of polymer, it cannot erode. Because if it starts eroding, then these pore sizes will start to increase and then you can have antibodies and even cells to go in and out and that will not be good. This should be mechanically stable. So, of course, it is not like you are putting in bones, it should be fairly strong, but it should be strong enough so it does not crack.

Because what happens if let us say it cracks, then now you have created a big pore size through which, first of all these cells that you have put in can come out as well as these cells from the external and the antibody from the external host environment can go back in. So, it cannot break and of course, it must be biocompatible.

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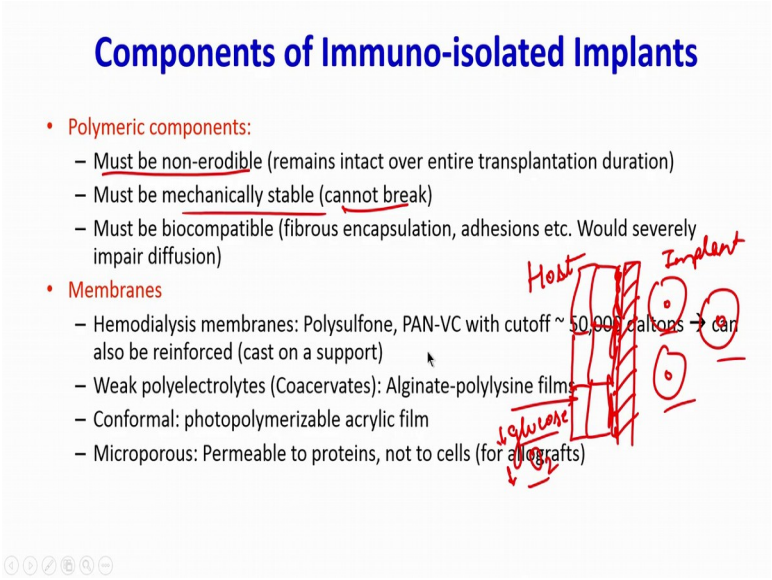

And this of course, is fairly intuitive because these cells that are residing here. So, this is the implant and this is your host. So, these cells that are residing here, they need to get a continuous supply of your glucose and oxygen for them to survive. And if this is not biocompatible, and if this polymer had that fibrous reaction that we had discussed previously so, what will happen is there will be protein adsorption first followed by a cell adsorption or cell attachment.

And it may even just fibrose out the whole implant and so in that case now your diffusion for this glucose and oxygen have gone down. And that is going to be a big problem because then the amount of glucose and oxygen these cells are getting is not enough for them to survive. So, it should be fairly biocompatible for it to work.

They will not be polymeric; this could be let us see some membranes. So, if we are using membranes and there are again some components and criteria that you need to take care of. So, one being that let us say if it is a hemodialysis membrane made out of polysulfone or something else, they need some support because these membranes are fairly thin and so, they need some support. These membranes could also be made out of weak polyelectrolytes.

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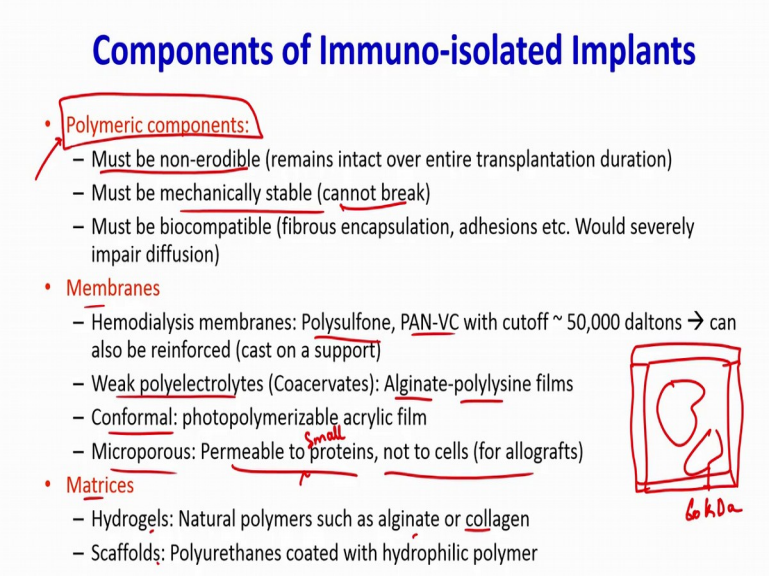

So, this could be some charge interactions, alginate with poly lysine films can be formed, but needs to be a careful with that because these charged interactions are fairly weak. And they may come off in certain conditions maybe the salt concentration increases and the dielectric changes and then these interactions may become weaker and may fall apart.

The other examples could be that they need to be conformal. So, what that means, is let us say if this is going go on the site which might be not straight, but they are round. So, these things can then surround that in a round shape as well. So, this should conform to the surrounding. As well as this should be microporous which again goes back to what we discussed earlier that it should allow permeation of some small proteins, so, permeable to small proteins, but not cells.

Obviously, this is all we are talking about immune system, but if your cells inside your implant or the membrane, they are producing a protein which is let us say 60 kDa, then you need these membranes to be permeable enough. So, that they can allow 60 kDa proteins to go through because, eventually the whole purpose of the implant is to get this protein.

So, those are some of the criteria's that you will have to consider while designing either the polymeric components or these membranes. And then there could be matrices; these could be hydrogel. So, again polymeric components is a wider term and these membranes and matrices are coming under it. So, these could be hydrogels or scaffolds.

So, these could be alginate and collagen hydrogels could be polyurethane scaffolds, coated with some hydrophilic polymer. So, again all of this is going back to what we discussed in this part of this criterion components that it should satisfy all of these before it can be used otherwise the therapy may fail ok.

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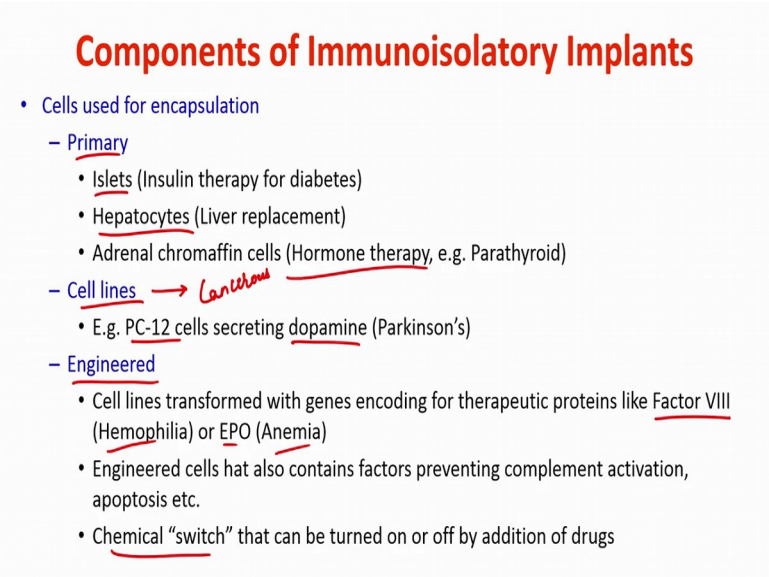

So, let us look into further the next component is your cells. So, one was the shield which in this case we discuss about polymers, but then what about the cells themselves. So, these cells can be primary cells which means that they are isolated from a being and their primary they are not being cultured in a lab or something. So, a classic example is islets. So, this could be from an organ donor, maybe you are getting islets from somebody who has just died for some other reason and their islets are still alive and functional.

So, you can get islets. Those are primary islets. This could be hepatocytes. So, this is for liver regeneration maybe liver had some fibrosis or maybe there some damage to the liver due to some accident. So, in those cases you can use these hepatocytes from some other source or there could be some other hormone producing cells that you are getting from some other source, that is not self. So, these are primary cells.

Sometimes you can use cell lines. So, again in this case you are planning to immune-isolate anyways. So, you can use cell lines, the problem is the cell lines is of course, that these most cell lines are cancerous that all cell lines have been transformed. So, that they

continue to divide and in those cases you want to make sure that it does not end up causing cancer. So, here some another example in this case, you are trying to get some dopamine so using PC-12 cells, or this could be engineered. So, this could be engineered with either cell lines or with the primary cells. So, that means, that maybe there is a cell that you think can be used for this application, but it does not produce the protein that you want. It might be suitable for other properties that may allow enhance survival in harsh conditions for those cells.

So, in those cases, what you can do is you can take those cells, you can then genetically engineer them to produce whatever protein that you want, let us say factor VIII in case of hemophilia or EPO in case of anemia. And then once they have done so they can produce now these proteins which the originally were not present. So, they have been engineered, but then you can utilize some other property for these cells to integrate well with the tissue and survive in harsh conditions, something like that.

And so, these engineered cells can then help in preventing any kind of immune response and enhance survival. You can actually even have some chemical switch so, maybe not always they are producing it, but only when the requirement is. So, maybe they are under some feedback loop where when this ends high glucose only then the produces insulin or it could be something else for some other protein. So, this should be some trigger that can be put in as well. So, gives you a lot more control over these cells ok.

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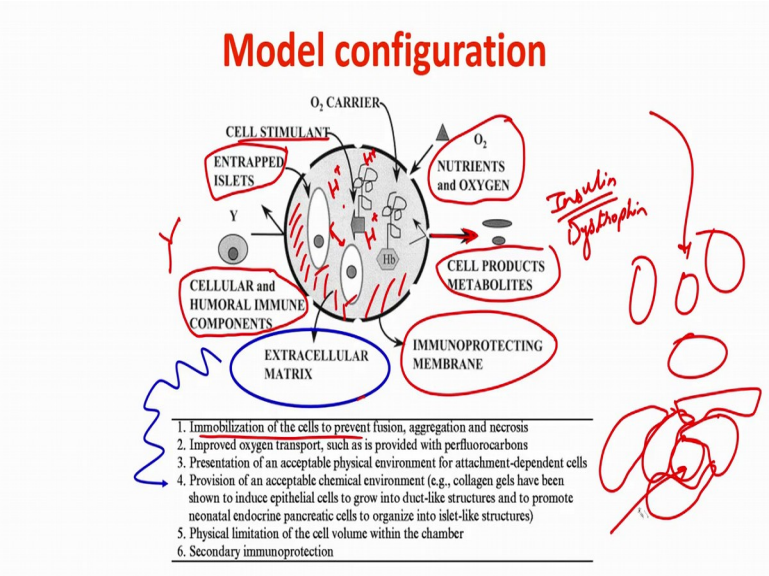

So, here are some model configuration that I will go over. These are a few of the configurations that have been used in the past. So, let us go over this. So, first of all we are looking at some sort of a capsule that has some pores, the cells are of course inside you may even encapsulate some growth factors inside. So, in this case the refer to a cell stimulant to make sure that these cells are happy initially. So, let us say that these were islets or the pancreatic beta cells.

So, now, what you are doing is with this capsule you are preventing any kind of cellular and humoral immune components. So, any cells cannot go through, any antibodies cannot go through, there is also some extracellular matrix that is present. So, ideally most cells would want to interact with some ECM proteins to be able to survive and have normal signaling.

So, now we are adding some extracellular matrix within this polymer or this implant to which the cells can attach to and be able to perform their normal signaling. Obviously, as I already said you do not have antibodies going through this, then whatever this is producing that is required for the body can actually also come out. So, that is also a big criteria that along with the nutrients and oxygen being able to go in, it should be big enough to allow let us say if is producing insulin which is fairly a small molecule, it should be able to come out or if its dystrophin which is not So, small that should be able to come out.

So, the self products should be able to come out and more than that the waste product should also be able to come out. So, what will happen if you do not let the waste product come out? Most of these waste products are acidic. So, if you do not let it come out, then you will have a buildup of the acidic environment. The pH of this area may drop and that may cause a death of these cells.

So, you want the waste product to also be able to have some sort of a circulation through this membrane and then just to highlight this external matrix this is actually very important. It is first needed to immobilize the cell and so, that they do not start clumping together. The cells do not find any support to attach to they will start to just attach to themselves and start to aggregate. Once they start aggregating, then instead of having separate cells to which oxygen can diffuse through.

What you will have is, you will have, cells forming these aggregates which will cause another diffusion barrier for the innermost cell to receive oxygen. So, to prevent that you want to make sure that the cells have attachment sites around them of course, you want improved oxygen transport.

Sometimes what is done is you can add some components such as perfluorocarbons initially, to allow oxygen to be released within this environment over some period of time. These are molecules that will release oxygen for an initial duration, but then later on you then you rely then, these cells will induce some more blood vessels to grow in and around this so, there is enough oxygen around. So, maybe initially you want this oxygen shock not to be there. So, you give the oxygen in the implant which through some chemistry and then you rely on the fact that these cells will induce blood vessels to come in to this environment very similar to the way cancer cells do it.

Then there are other factors as well you want; obviously, immune protection. So, you want the physical limitation of a cell volume within the chamber as well as immuno protection from any antibodies. And again you want to ensure that the chemical environment is acceptable. So, like for example, collagen gels have been induced shown to induce epithelial cells to grow duct like structures. If they if you are trying to produce some endocrine based islet cells so, those things should also be fairly comparable. So, it is not necessary that you can put any ECM depending on the cell that you are choosing you may want to choose a particular ECM protein over other proteins.

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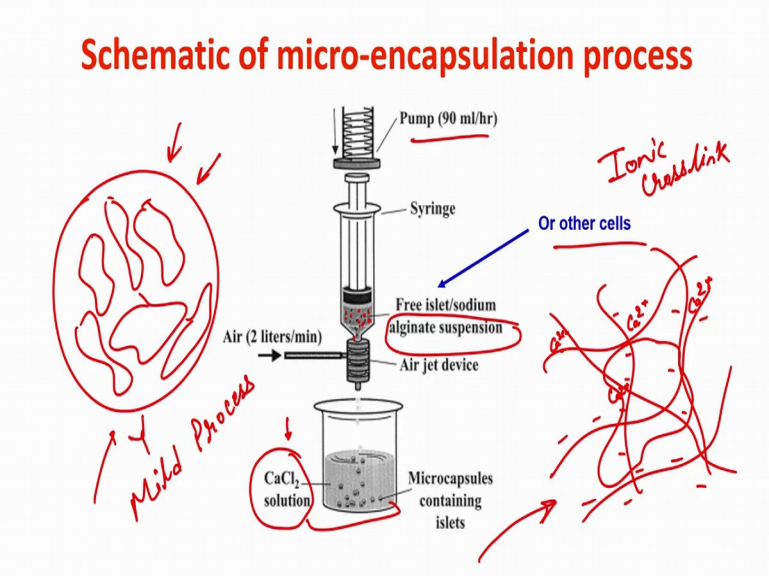

So, here is just a very crude example of micro encapsulation process. So, in this case what you are looking at is and we have discussed this before and this process. You are looking at an alginate-based solution. So, this is the hydrogel. So, you want something like this that there is an alginate which is encapsulating several cells and the pore size of the alginate is such that it will allow the oxygen and the glucose and the waste product to move in and out, but it will not allow your antibodies and the immune cells.

So, what you have done is you have suspended these alginates at a certain concentration in which the pore size will be such that it follows what I just described. You can put islet sort of some other cells for that matter and then there is some pump which is then pumping this alginate solution containing cells through a syringe with a certain diameter and it is being pumped into a calcium chloride solution. So, what will happen is, if these are alginate chains and again we have discussed this in a hydrogel class, these are alginate chains which at this point are not cross linked. These calcium ions, which are divalent will start to interact with the negative charges that are present throughout these chains.

And so, it will go and start cross linking these chains using ionic cross link and whatever cells are there gets entrapped in here. It has enough pore size to allow the movement of a small proteins, but more cells cannot come in. So, that is one way you can make them. Of course, in this case you have to be careful, alginate is not an ECM protein. So, if you

want the cells to be fairly happy in terms of cell signaling, it depends on the type of the cell. You want to ensure that you put some ECM proteins also along with the solution and they get incorporated and stay at the site. And then this process is very widely used and the reason for that is it is a very mild process. So, if you look through the whole process, there is no organic solvent, there is no high stress condition what the cell is facing them. All its seen is some high concentration of calcium which is anyways a physiological ion that is present. So, in most cases this process works very well with the cells and the cells are fairly happy in the end product.

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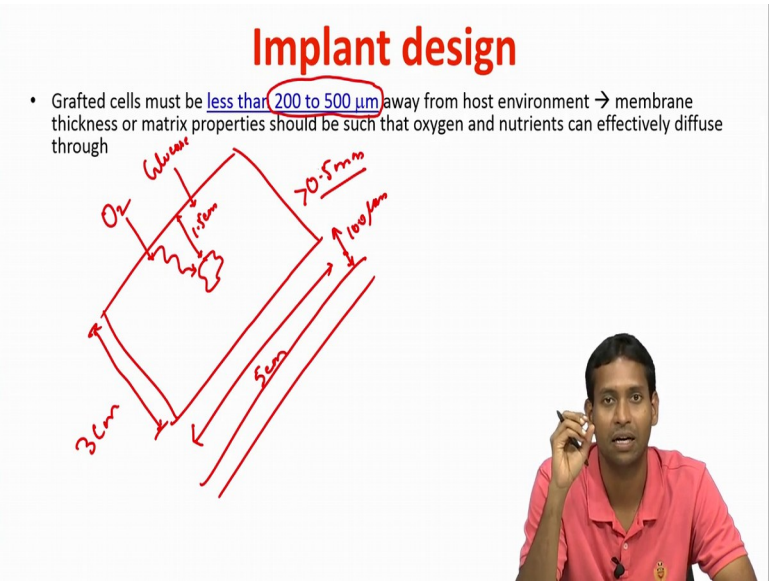

So, let us look at some of the implant designs now. So, the grafted cells must be less than 200 to 500 microns away from the host environment. And where does this 200 to 500 micron number comes? That is because you want the oxygen and nutrients to effectively diffuse in the system. So, now, let us say if you put an implant which is let us say 5 centimeter by 3 centimeter just for example. Then what will happen? The cell that is residing here is almost 1.5 centimeter at the very minimum is about 1.5 centimeter away from this edge.

So, now the oxygen that is here or the glucose that is here not only has to diffuse through this semi permeable membrane, but also has to go and find its way all the way to the cell. So, what is seen is if you increase this size range to beyond 0.5 millimeter, it is greater than 0.5 millimeter then these cells do not survive very well; they do not get enough

oxygen. So, and that is of course, relying if the blood vessel is passing right through here. The blood vessel it could be further away; the blood vessel could be further away from this implant edge by let us say 100 micron.

So, now that this distance is further increased so, that is a major limitation that comes in the implant design. So, ideally you want the implant to be such where the cells are not away from the host environment by more than 500 micron because, if we do that you would find that the cells do not survive for very long. They need this oxygen and glucose to come in fairly regularly in high amount.

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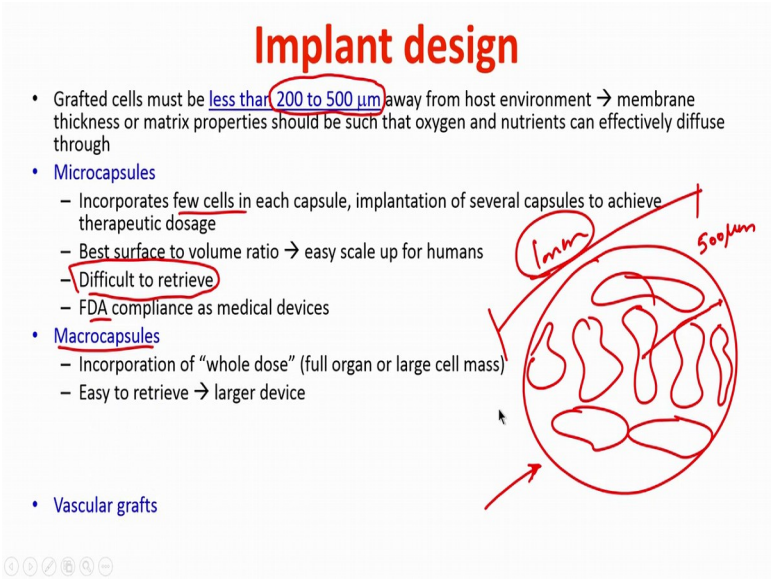

So, that is why you go to microcapsules and the reason for the micro capsule is you can make these capsules of the sizes that about 500 micron or lower instead, but at least ensure that from the host environment, you are not more than 500 micron away.

So, in that gives your because its only 500 micron and let us say your cell is about 10 micron, then you can encapsulate quite a few cells not millions of cells, but at least each capsule can have quite a bit of cells and then each of these cells is not more than 500 micron away. So, if you make it 1 millimeter, all the cells are within 500 micron of the edge. This is also good because now what you are doing is instead of making a big device like head earlier drawn in centimeter, size, skills, you have now used the same amount of material, but instead you made several of these devices. So, the now surface area to volume ratio is very high and that ensures that quite a lot of diffusion is

happening, and you can still in capsulate the same amount of cells or more; however, this has a problem it is a difficult implant to retrieve.

So, let us say if something goes wrong with this particular implant, maybe the cells are turning tumorigenic, they are forming tumors or if something is; something is problematic with this implant. These are fairly small spheres and they can move around a bit in the body and you it may be hard to then retrieve this. So, this is a sort of a regulatory requirement that our regulatory agencies such as FDA put that if you are putting something in the body; if its retrievable, the clearance is easier because of course, if something goes wrong you can take it out.

What if these cells now started producing something else that is causing toxicity to the body or what if they are producing too much of your insulin or too much of something some other function that you wanted these cells to perform and that could also cause toxicity. So, you want them to be fairly retrievable which is a problem with such design nonetheless this is still very widely used.

And then you can have vascular grafts and microcapsules or macro capsules in this case. So, macro capsules is incorporation of the whole dose. So, basically talking about full organ or large cell mass to be implanted, it is easy to retrieve because it is a big device, it is not going to go anywhere. So, it will remain in the body.

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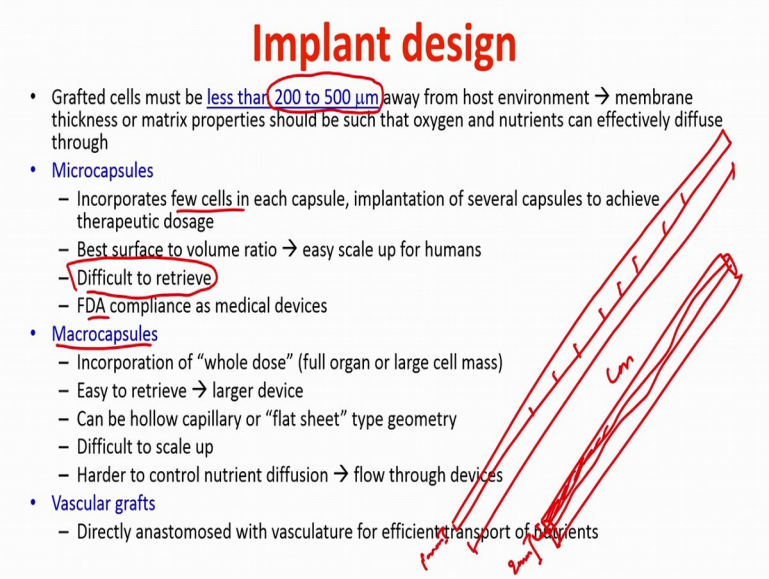

To allow more surface area, you may not want to use sphere, maybe you take a long sheet which is let us say 1 millimeter wide and the these dimension could be in centimeters. But that would ensure that all of your cells are not more than 500 micron away and then this device can be also retrievable or you can have a hollow thing in the middle going as well.

So, let us say you do not want this, you want it to be let us say about 2 millimeter, but what you can do is you can have some hollow pores. So, all of this area can be exercised by the host. This is what is protected from the host and that will still mean that you are still less than 500 micron away from any of the edge of the host, but these are typically difficult to scale up, it is not easy you are talking about manufacturing each of them separately.

So, they are difficult to scale up and of course, they are harder to control the nutrient diffusion through the flow device because they are big. So, it they may be non uniformities throughout the whole implant because if its huge thing that you put in maybe here the diffusion is slightly different than what is here. so you will have non uniformities throughout the whole device and then there are vascular grafts.

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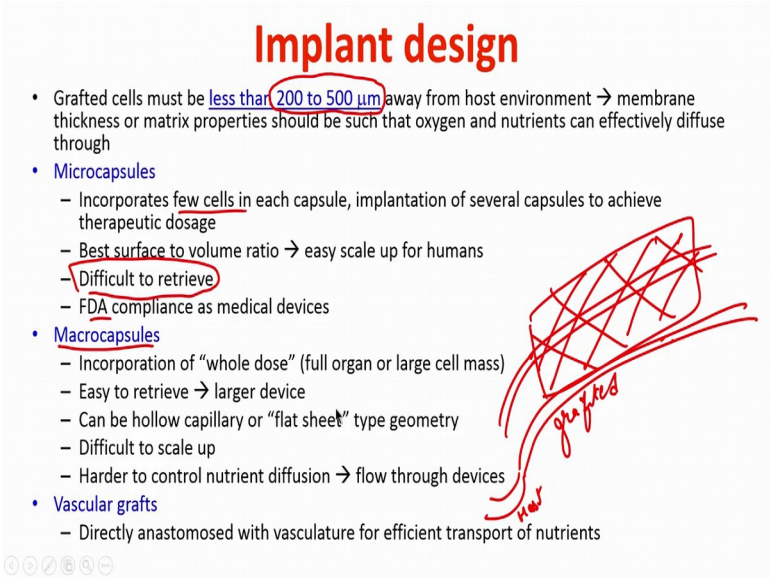

So, that means, that let us say, I have an implant. What I can do is, I can graft a vessel next to the implant or something like this and then connect this vessel to the host vessel. So, this is host this is grafted and so, at that point you are directly grafting it. In this case,

these may not contain any cells because this is exposed to the environment. This is where your cells are and then they can then act on whatever is required and still get lots of nutrients. So, maybe this vessel is going right through a vascular graft.

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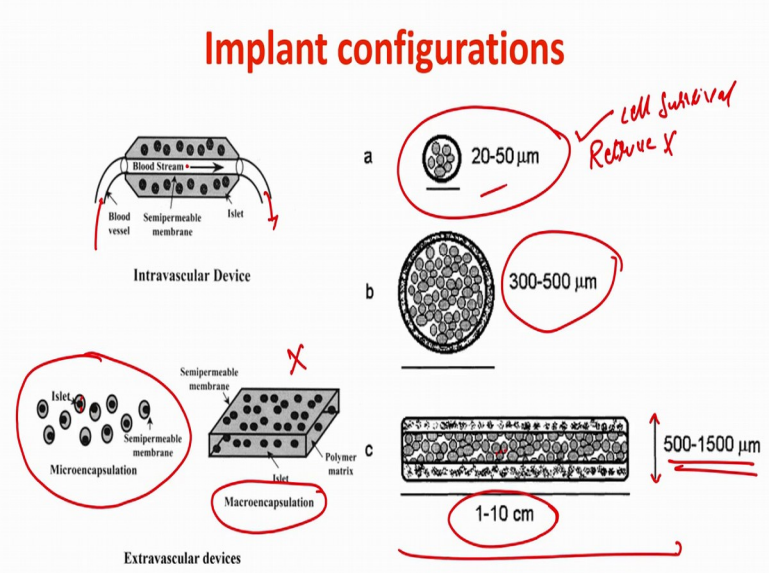

So, here are some implant configurations. So, this is one of those things I talked about. So, you have bigger device, but if you have the whole device filled with these cells and immune-isolated and then what you will find is that the cells at the center are not getting enough nutrients. So, one way you can get out of that is to have a blood stream going through right through. So, now, you can then make it thicker. Other example is what I discussed before.

So, you can make very small spheres such as 20 50 micron and put few cells in here alternatively, you can have even more micro coatings in which you put yourselves and very very thin layer. So, this is very nice in terms of the diffusion that can easily happen. Then you can have a semi-permeable membrane and put in a polymeric matrix. This is micron capsulation not preferred in this case and this is not going to last very long. You can have big alginate spheres that we discussed similar to here; obviously, this is better in terms of the cell survival.

However, in terms of retrievability, this is not good. Similarly this is better in terms of retrieving, but still not as good as let us say this in this device and the cell survival is lower than this particular device. And then you can as I said you can flatten it out. So,

that the dimensions on the width is in microns. So, that these cells can get any of nutrients whereas, this device is long; so, they can still survive. So, these are just some of the models that are out there that you can use to go forward; each has its own advantages and disadvantages and it just depends on what application you are trying to target.

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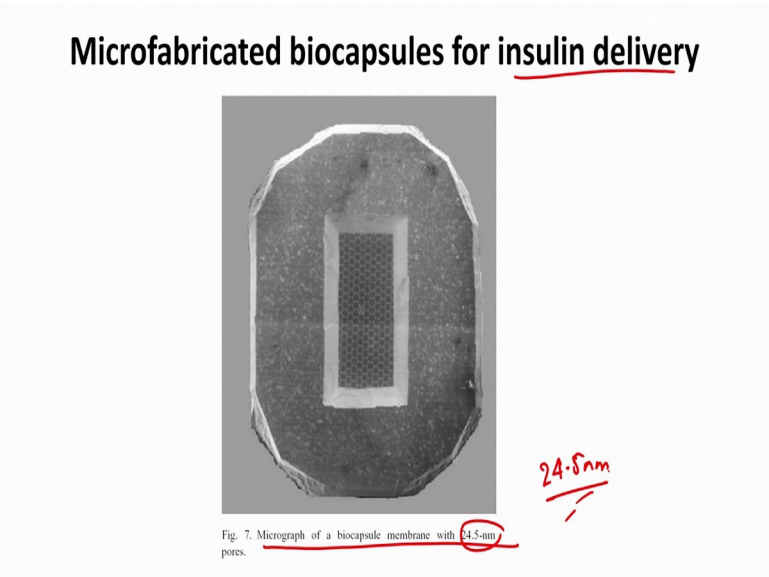

So, here is one of the devices that was used. So, this is used for insulin delivery and what you are looking here is a very porous membrane and it is actually very defined membrane. So, in this case you have pore sizes of about 24.5 nanometer and inside this device, you can have cells. So, this pore will actually allow your glucose and oxygen to go in and out whereas, it will prevent cells from going in and out; however, at that big of a pore size, you may still have some antibodies going through.

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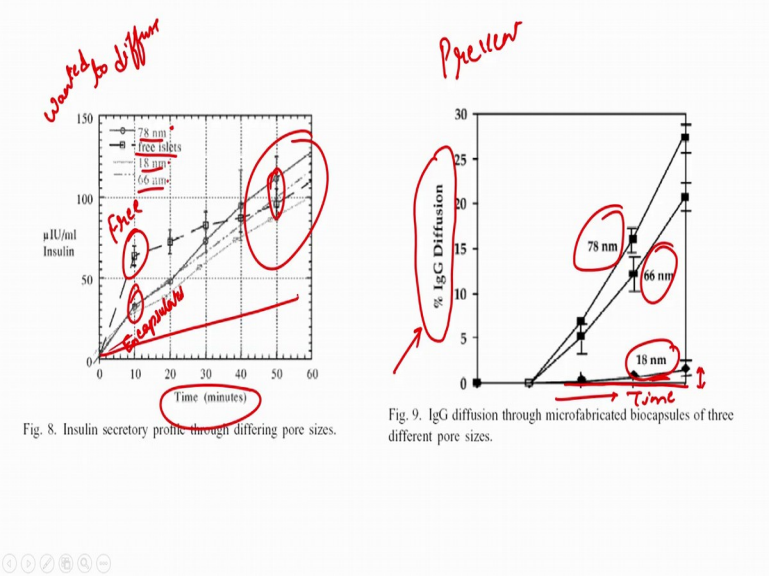

Fig. 8. Insulin secretory profile through differing pore sizes.

Fig. 9. IgG diffusion through microfabricated biocapsules of three different pore sizes.

So, this is what is done here. So, further analysis of what happens to the antibody at that size. So, you have free islets of course, and you are looking at the diffusion of insulin out from this device. So, you have free islets, you have different pore size devices and what you find is, the diffusion of the islet is fairly equal for a longer duration.

So obviously, free islets do very well with the rest of them. So, this is free islets, this is encapsulated, but eventually what we are seeing is what for all these size 18, 66 and 78nm; you get a fairly good diffusion of the insulin very comparable to free islets at a period of 1 hour. But what happens to an antibody which is something that you want to prevent. So, this is for something that you wanted to diffuse. This is something you want to prevent.

So, now, let us see what happens. So, what you are seeing here is that as you are going up. So, this is time again. So, for 78 nanometer you have quite a high diffusion of IgG. So, this is not good at 66 nanometer you still get some IgG diffusion slightly lower than 78, but still fairly high, but at 18 nanometer you kind of eradicate it quite a bit although some of it is still going in.

So, if you want to make a device, you would rather make a device with 18 nanometer because in among these 3, this is better in terms of preventing IgG from going in, and in terms of the functional itself, it does not really affect a whole lot. So, you do not really see a significant difference here. So, but of course, it may be beneficial to even try

something smaller. So that this IgG diffusion gets completely eradicated, but of course, we will have to make sure that the it is not something like the insulin goes down like this because, then it is not good. We will stop here, and we will continue further in the next class and discuss more about immunoisolated cell therapy and some of the various factors that need to be taken into account and some will also give some examples to see what people have done in the literature ok.

Thank you.