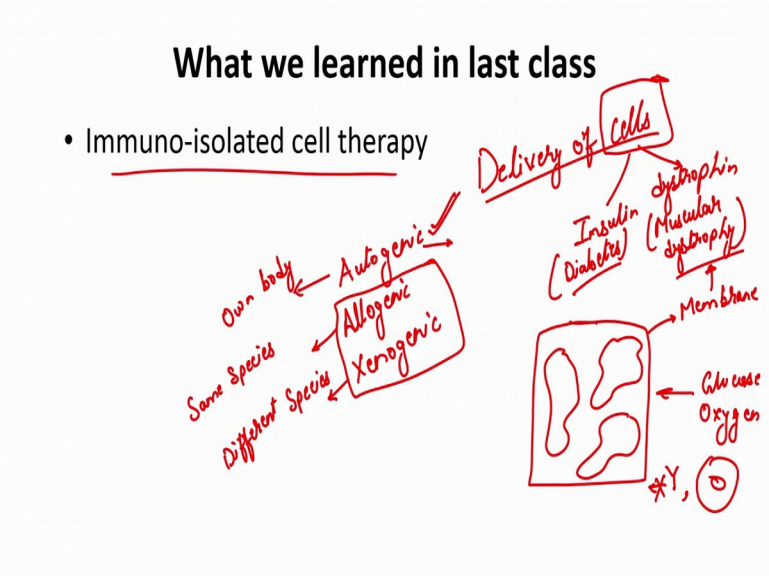
Drug Delivery Principles and Engineering
Asst. Prof. Rachit Agarwal
Department of BioSystems Science and Engineering
Indian Institute of Science, Bengaluru

Lecture – 52
Immuno Isolated Cell and Gene Therapy

Hello everyone, welcome to another lecture for Drug Delivery Engineering and Principles, let us do a quick recap of what we done in the last class.

(Refer Slide Time: 00:37)



So, in the last class we majorly talked about one major topic which is Immuno isolated Cell Therapy and this is in the immune module that we are currently at. And here we were looking for delivery of cells; and when I say delivery of cells, a drug in this case is cells where actual drug is actually something else that is being produced by the cells. So, this could be insulin, this could be dystrophin, insulin of course, if you are suffering from diabetes, dystrophin is for muscular dystrophy or this could be something else also.

I mean these are the two major applications here, but you can potentially look for any disease that require some protein as intervention and you can then deliver cells that are producing those proteins. So, that is what we have discussed. So, in terms of delivery to cells, we had talked about that certain parameters because most of the time the cells you will get, will be from some foreign source and there could be. So, essentially any source of cells could be divided into three categories; one is autogenic second is allogenic and

the third is xenogeneic, where autogenic represents something that is derived from your own body. So, if you are using autogenic cells, you do not really need to worry about immune system a whole lot because your immune system will recognize it as yourself cells yourself protein. So, it is not going to attack it.

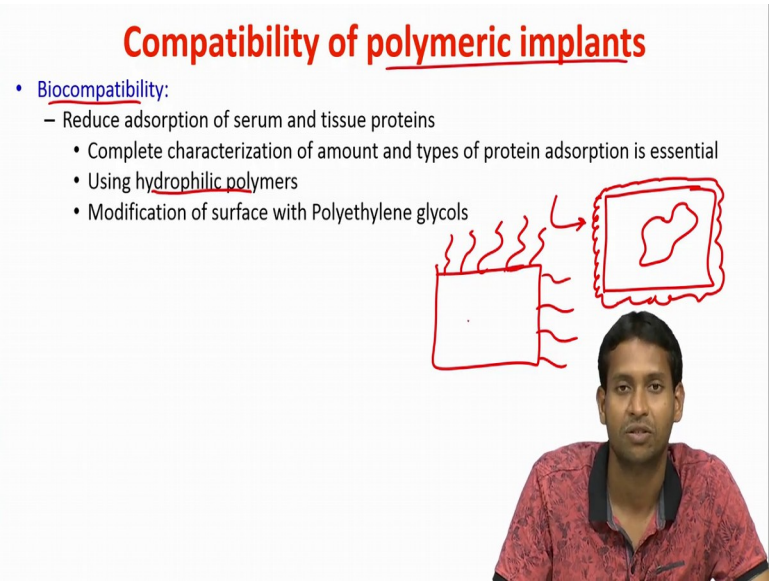
So, these things survive much better, but the problem is the source of these cells is fairly low and in most cases are not there. So, I mean if you are suffering diabetes your own pancreatic cells are not working. So, that is why you need more cells from some different source. So, autogenic will not work in that case, similar with the muscular dystrophy as well, where your proteins are not in the correct conformation or not in the correct orientation and so, your own cells will not work. So, you will need cell from somewhat different source which have a functional copy. And so, in that case allogenic is the most preferred which means that getting it from same species.

So, since we are humans, you are talking about getting it from another human donor. So, in this case the immune system will act against it, because the immune system will recognize this as a foreign cell and that needs to be protected. And of course, xenogeneic is even more stringent in terms of immune response because this is from a different species. So, this could be that let us say I get organs or cells from a pig and then try to implant that in my body, but; obviously, those are so different from my own body cells the immune system will immediately recognize them and try to kill those cells.

So, these were some of the sources of course, now because we are saying that mainly the allogenic and xenogeneic is the sources that we have, we need to protect these cells before we put them in the body because the otherwise the immune system is going to kill it. So, what is done in this case is you take a compartment, which has a semi permeable membrane or some membrane, you put your cells within this compartment, the cells you are delivering and trying to protect from the immune system. And this membrane is such that it will allow small molecules such as glucose, oxygen to pass through; whereas, big larger molecules such as antibodies or other immune cells are not able to go through this.

So, that is the whole concept, behind it and we discussed several different conformations of these implants and all, but eventually this is what you want ok. So, let us take the discussion forward.

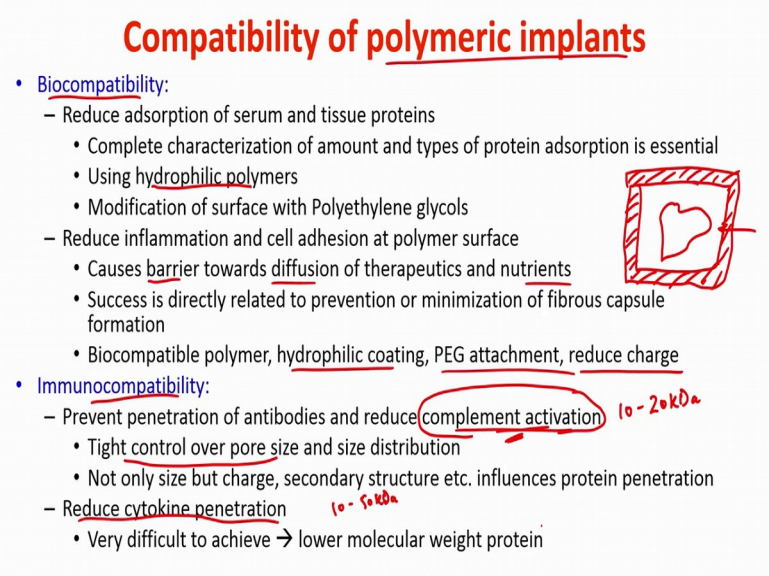
(Refer Slide Time: 05:07)



So, let us talk about what kind of polymeric implants compatibility we need in this scenario. So, first is of course, when we discussed a bit of it is biocompatibility which means that, it should not really adsorb a lot of serum and tissue proteins and that is a bit obvious requirement because let us say if this is your implant carrying your cell, if a lot of protein starts coating on it; it may first of all impede cell adhesion the cell transport of different nutrients as well as it can also lead to attraction of more immune cells coming in, as we discussed protein adsorption typically is a layer which helps in more cells to come in and attach to that site.

So, it is hence required that we completely characterize the type and amount of protein that are adsorbing on it, and we can use hydrophilic polymers as we discussed hydrophilic polymers are more similar to the environment that proteins are currently in which is hydrophilic water. So, they will have a less adsorption as well less confirmation change and sometimes even if you cannot really work with hydrophilic polymers, what you can do is on your implant you can decorate it with our favorite molecule here which is peg. And what peg will do is it will act as a windshield wiper, as we have discussed this several times in this course and make sure that none of the proteins are coming in absorbing, even if this is a hydrophobic surface. So, those are few of the conditions and requirements in terms of biocompatibility.

(Refer Slide Time: 06:46)



Then what about inflammation? So, it should reduce inflammation and cell adhesion at the polymer surface. So, it goes hand and hand with the protein data we already talked about, but even then it should reduce inflammation as well. So, that will allow the barrier to not being formed in terms of diffusion of therapeutics and nutrients in and out, and success is essentially for these implant directly related to how much you can minimize the fibrous capsule.

Because eventually if your capsule is good and the body cannot degrade it then and if a body does not like it then what we will try to do it will try to wall it off as we discussed. So, the body will first try to remove it if it cannot and then it will just wall it off, so that the rest of the body is protected from this particular capsule, but this is not what we want in our therapy, because we want it to continue to interact with various kinds of nutrients, with oxygen, as well as release of the product that we want from this particular device.

So, the whole success for this will depend whether a fibrous capsule is formed or not. So, again if a fibrous thick capsule is formed, then that is the end of the device, because at that point you cannot really have any kind of exchange happening at a substantial rate which you may need from this device. And again goes back to the requirement that you should have some hydrophilic polymer or at least hydrophilic coating, you can use peg to do that and typically uncharged surfaces will have less protein adsorption.

So, you can reduce the charge of your surface of these devices, and then finally, if we talk about immuno compatibility and that means, that the first of all our antibody should not be able to penetrate and we have already discussed this in the previous class that, the pore size should be such that antibodies are not able to go through. Because if the antibodies go through, then they will impair the function of these cells.

The second thing is we should reduce the complement activation. So, now, this is very tricky, because in terms of most of the other immune system-based molecules and cells, they are fairly large, I mean we are talking about antibodies which are more than 100 kDa, we are talking about cells that are in microns. So, it is easy to not have them penetrate and small molecules penetrate for glucose and oxygen, but when we talk about these complement proteins, as we discussed in couple of classes ago that these complement proteins are also fairly small. So, we are talking about 10 to 20 kDa complement proteins floating around.

So, it is becomes extremely hard to prevent complement activation from these surfaces, but there are different strategies that we talked about such as an activating c 5 a coating this with some molecules that may not let this complement activate. So, those strategies can be adopted in the scenario. So, that is why you need a very tight control over pore size to minimize these complement proteins as much as you can and still have a good flow of nutrients across this membrane.

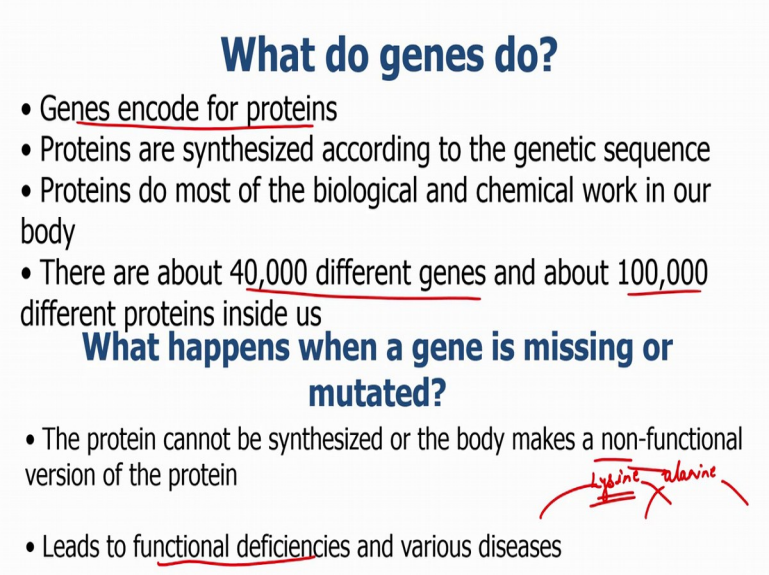
And not only size, but the charge, the secondary structure all of this will influence the protein penetration through this membrane. And you also want to reduce cytokine penetration because these are also small proteins just like complements that can go through. Again ten, some of them are at least 10 to 50 kDa which then you want to prevent and of course, you also want to have enough pore size for the glucose and insulin or whatever it is that these cells are producing to come out.

So, it becomes quite challenging and that is why there is a limit to how much these implant lasts, typically when patients get these implant these implants survive for maybe 1 or 2 years, but after that within a 5-10 year survival is fairly low and so you left to get another implant. And so, that is puts more pressure on more organ donation, which is anyways quite low at this point. So, these are some things to consider in mind when you are designing your vehicles for cell delivery.

(Refer Slide Time: 11:05)

So, now we were going to talk about gene therapy, which is again very similar to what we just discussed, but a little bit twist to the cell delivery.

(Refer Slide Time: 11:17)



So, let us talk about first of all what is genes and what do they do. So, what do you think genes are? So, to be very simple basic definition will be, genes encode for proteins. So, anything that is encoded, that information is in genes at this point, and proteins are synthesized based on what is the genetic sequences. So, you can change the protein sequence on the basis of the genetic sequence. Proteins do most of the biological and

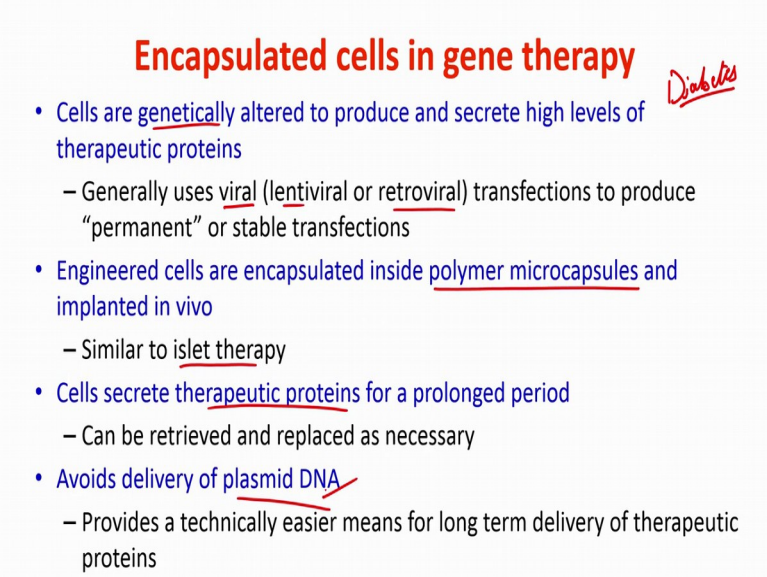
chemical work in our body, genes are basically just storage units which are storing the information so, that every cell can make more and more proteins.

And we have about 40,000 different genes in our system and those then combine together to form about the 1,00,000 different proteins; so, all fine and good. So, what happens when a gene is missing and mutated; and we have briefly discussed about it in some of the disease circumstances, but of course, if a gene is missing, then that protein cannot be synthesized by the body or if its mutated then the protein could be non functional.

So, let us say there is a gene that codes for let us say lysine, and then there is another gene that codes for another sequence that codes for let us say alanine. So, if somehow the gene got mutated that the lysine gene sequence is now coding for alanine, then your eventual protein that will form will instead of having lysine will have alanine and maybe this lysine was fairly important in the function.

So, now the proteins will lose their function, they might even change the structure because of few proteins. So, then that leads to functional deficiencies, because maybe this protein is involved in important function let us say its insulin; so, maybe insulin is such important protein that if we do not have it, it is very difficult for person to survive and various other diseases.

(Refer Slide Time: 13:27)



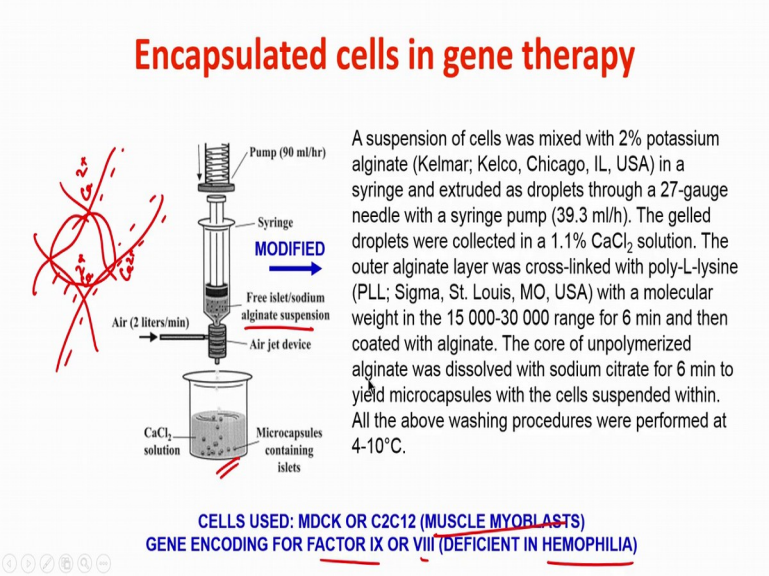
So, then what we can do is to overcome that, we can then like we talked about in the previous two classes we can encapsulate cells to do gene therapy. And so cells are we are genetically altering them to produce and secrete high levels of these proteins that are missing from that person. So, in the previous case we were more talking about diabetes and in that scenario saying the immune system destroyed the whole cell. So, the gene was still there, but that cell completely got destroyed. So, it is not getting produced. So, now, all you can do is, you can now genetically modify these cells and then have them produce insulin that is just one example.

So, this process generally uses some kind of a viral vector such as a lentiviral or retroviral and these viral vectors are extremely efficient in delivering a protein or a gene inside the cell and then have them integrate with the DNA and then have them produce several copies of that. So, if you have a gene mutated, you can take your own cells, you can then mutate them through this lentiviral or retroviral process and then have them get a stable transfection so, that is one way.

The other way is you engineered these cells, are encapsulated inside a polymer micro capsule and implanted in vivo similar to what we discussed previously for islet therapy. So, once you have these cells, you can then encapsulate them and use them for delivery in our body and then these cells will secrete whatever proteins you want for prolonged period. If you want them to be retrievable you can have them in some micro device which you can then just take out. And this way you can avoid delivery of plasmid DNA which we will cover in our next module.

So, it is much easier in that regards. So, it is much long-term solution rather than delivering a plasmid DNA and again we will come back to plasmid DNA in one or two classes.

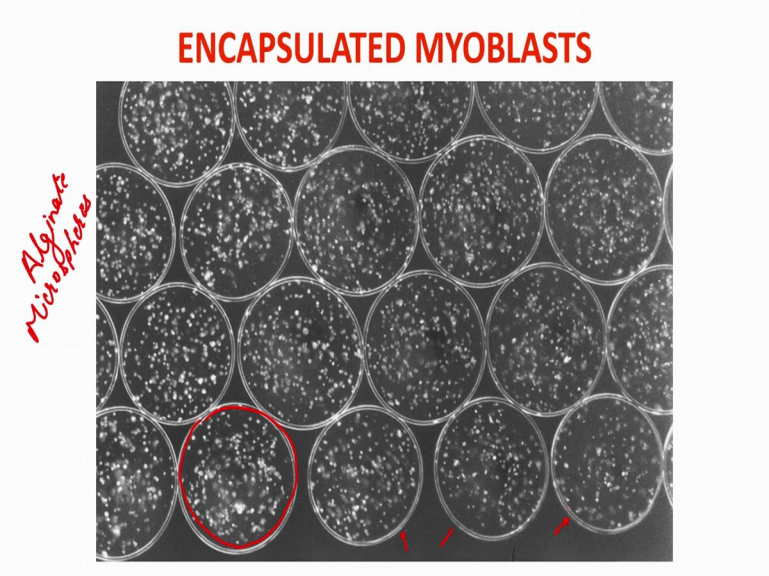
(Refer Slide Time: 15:27)



And again, just like the previous case it is a very similar system. So, again this is the same example it is probably the same figure. So, you have alginate polymer and you have calcium chloride solution. So, alginate is a negatively charged polymer and then calcium of course, is a divalent cation. So, what it will do? It will form bond between two different chains carrying negative charges and that is how these chains will form an hydrogel and whatever was present in the alginate gel initially before touching the calcium chloride solution, will get encapsulated within these chains.

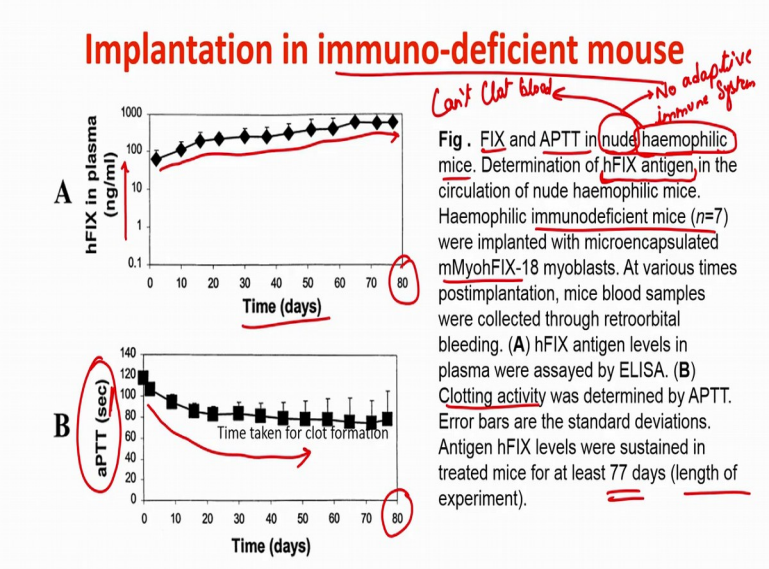
So, again very mild process you can use all kinds of cells. So, you can use muscle myoblast, you can use let us say if you deficient of factor 9 or factor 8 this is involved in blood clotting mechanism, it causes a disease called hemophilia in which if there is a small cut the patients will bleed out. So, you can use these kinds of cells, deliver them and they are encapsulated in a matrix. So, surrounded and protected by the immune system, if they are not self.

(Refer Slide Time: 16:43)



So, here is just some example. So, these are the alginate microspheres, and as you can see each of these microspheres is encapsulating several cells inside, this microsphere could be about a millimeter or in that size range and it carries several cells and you can get millions of these microspheres and depending on how much is the requirement for a patient, you can put these cells in there which this barrier then prevents it from attack by the immune system.

(Refer Slide Time: 17:26)



So, here is some literature example of when this was used. So, first they did an implantation in an immune deficient mouse, which means that the mouse does not have a good immune system and so let us see what do you see. So, in this case they were using the hemophilia factor, which was absent in this mouse and so what do you see is basically two things they are missing, when is the FIX and another is APTT in a nude hemophilic mouse, which the nude mouse here means that the immune system is knocked out.

No adaptive immune system and then hemophilic basically means that they cannot clot blood. So, these mouse get an injury or something this those even if some minor injury, those injuries could be potentially lethal just because a blood will not clot and so they will remove all their blood in due time and of course, the lack of blood they will die because they will not be enough oxygen going to brain and heart ok.

So, this was one then they measured this antigen which is the hFIX antigen, this is nothing, but is produced by the cells that they have implanted. So, this normally is not present in the nude mouse, but since now that you have implanted these cells, they are also apart from producing these factors that are needed for reversing hemophilia, they are also producing other proteins. So, they are measuring one such protein which is called hFIX and what they find is and this is of course, the unit is in time and this is showing the protein amount; and so, what they find is after implantation they see increase in this amount of antigen, this hFIX that is present in the mouse system.

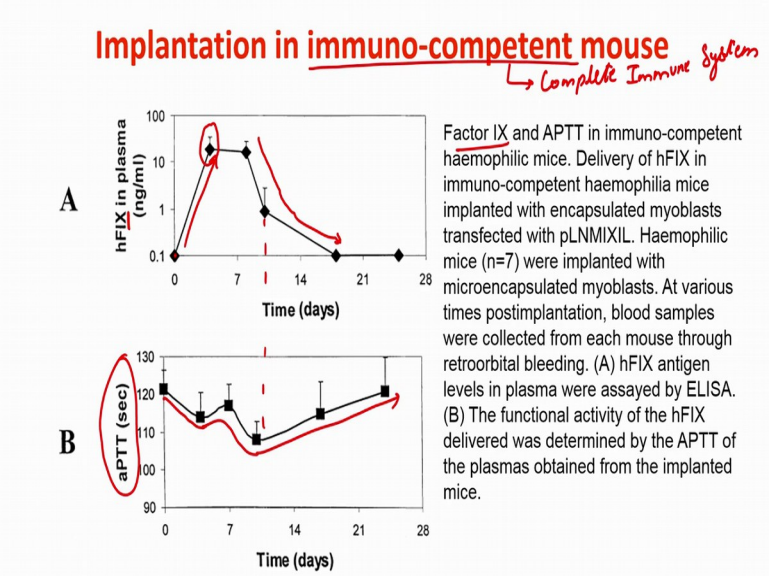
So that means, that the cells have actually grafted and actually survived all the way up to 80 days and this particular antigen is also being produced so cells are healthy. Now, the implanted cells are doing what they are supposed to do along with other functions and other survival proteins that they are producing. So, this is great and this was done in the n of 7. So, 7 mouse were used for this seven mice were used for this and these are the cells that were used, then the other thing that they looked at was the APTT level and what is this? This is you can consider this as a reference for clotting activity.

So, this is showing these seconds. So, how much time does it take for the clot to form and so, you see in hemophilic mouse earlier it was rarely high, but once you put this you see that the time for clotting has decreased. So, that means, that there are more clotting factors now in this mice blood and they are able to form a clot which so that means, that

the hemophilia is somewhat cured they will not bleed out completely or immediately; and then again the same thing you see is up to 80 days I think that is what the authors or 77 days, that is what the authors were looking at for the length of the experiment and they continued to see these cells produce what was required.

Now, of course, this is immuno deficient mouse. So, you would expect these cells to be fairly easily surviving just because immuno deficient mouse is no adaptive immune system to attack these particular cells they do not have any immune system.

(Refer Slide Time: 21:21)



So, what happens in immuno competent mouse? So, immuno competent mouse is fairly a healthy mouse, that is complete immune system is present, but there is still hemophilia in this mouse and so, now, they do the same thing again and what they see is the factor IX the hFIX I was talking about earlier. This initially of course, before implantation there is no antigen for this factor IX which is missing in this mouse and as the time increases you see that this implantation has worked and this some amount of this particular antigen that is now floating in the mouse circulation, but then as time increases beyond seven days you start to see a big drop. And eventually by the time 15-20 days is passed you see complete removal of this particular antigen.

What does that mean? That the now immune system is attacking these cells and it took a bit of time for the immune system to start developing response against it, but once it did you see even with the micro encapsulation strategies, we are seeing that these cells are

dying. And that is because there are several cytokines and complement factors that are not letting this cell to survive. So, big problem and the same thing you can see with the time for clotting. So, initially it went down a bit, a little bit of variability, but then eventually it starts to go back up, as soon as this level starts to go down.

So; that means, that this mouse is returning back to hemophilia, is going back to that 120 second that it was before the implantation and that is obviously, not good and so, this is the major challenge here. So, if it is not working in a mouse system, it is unlikely that this is going to work in a human system, and this is what lots and lots of research has been going on to even in an immuno competent mouse how to get this to work better.

(Refer Slide Time: 23:32)

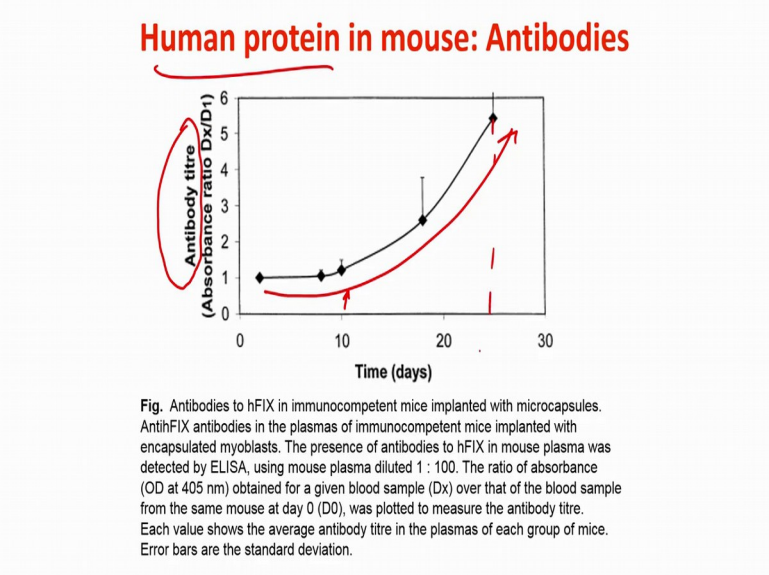
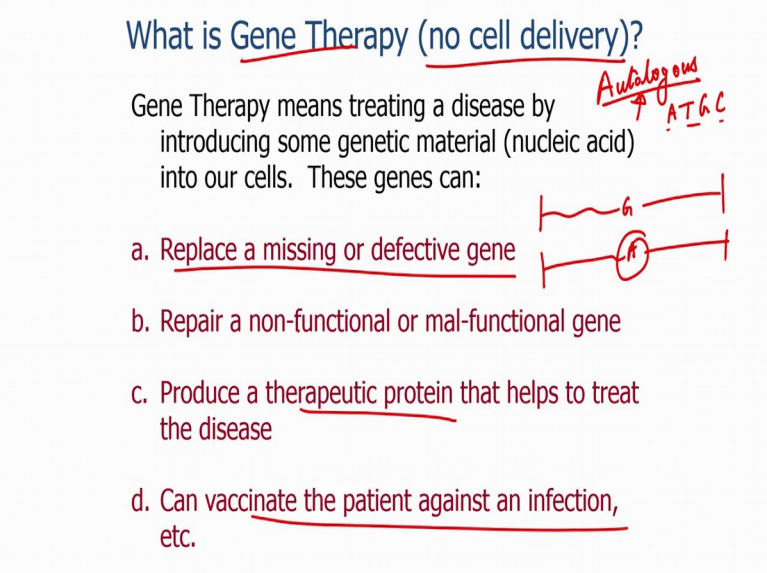


Fig. Antibodies to hFIX in immunocompetent mice implanted with microcapsules. AntihFIX antibodies in the plasmas of immunocompetent mice implanted with encapsulated myoblasts. The presence of antibodies to hFIX in mouse plasma was detected by ELISA, using mouse plasma diluted 1 : 100. The ratio of absorbance (OD at 405 nm) obtained for a given blood sample (Dx) over that of the blood sample from the same mouse at day 0 (D0), was plotted to measure the antibody titre. Each value shows the average antibody titre in the plasmas of each group of mice. Error bars are the standard deviation.

And then here is another example. So, now, in that particular assay, they try to figure out if there is antibody against this human protein and what they do fine is yes as the time increases, the antibody titer against this human protein that they put in a mouse has also increased.

So, even though that human protein was functional for a few days once the antibody starts to come in, it is causing the immune system to get further and further active against these particular cells and eventually causing death of these cells. So, you can see how these antibody titers are increasing over time. So, before that we were saying up to 80 days there were 77 days there is no problem, now we are seeing that even at day 25 or 24 these cells are gone.

(Refer Slide Time: 24:32)



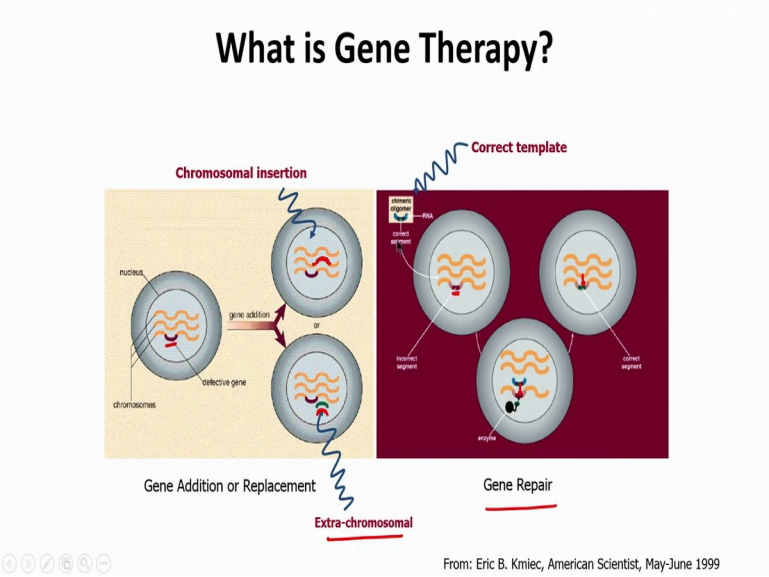
So, what about if it do not deliver a cell. So, now, we are saying that we are delivering these cells, this is what is causing all this immunogenicity, the cells produce several other proteins maybe some of them are causing the immune response to get triggered at a higher level.

So, what if we still do the gene therapy, but remove cell from the picture or cell delivery from the picture? So, that means, that we need some other cell so in this case what about we use our own cell. So, going back to your autologous concept; what if we use their own cells somehow genetically modify them and in that way and when we put it back at least the whole cell itself is not immuno genic maybe a protein is maybe its not, but it is much higher chance that these autologous cells survive a lot longer in our system and would not be as immunogenic as the other ones.

So, again these genes can be to replace a missing or a defective gene. So, if a gene is completely missing, we can try to add one or two copies of those genes or if it is defective, we can try to do the same thing. We can repair a non functional non malfunction gene. So, maybe its genes are big. So, genes can be anywhere in kilo bases long, what if there is only a point mutation maybe there is one sequence maybe let us say A has changed to G, ATGC is the genetic code right nucleotides. So, it is essentially composed of one or the other nucleotides throughout its genome.

So, let us say a G has changed to A and everything else is the same in that gene. So, why do we change everything? Why cannot we just repair this A back to G and that way we will have a normal functioning for that particular protein. And then other case is when we produce the therapeutic protein that helps to treat the disease. So, do one of these two things, produce this protein which will help in treatment of the disease and they can also be then used to vaccinate the patient against an infection, and I will come to that in a moment, but let us process this information that I just gave you one by one ok.

(Refer Slide Time: 27:00)

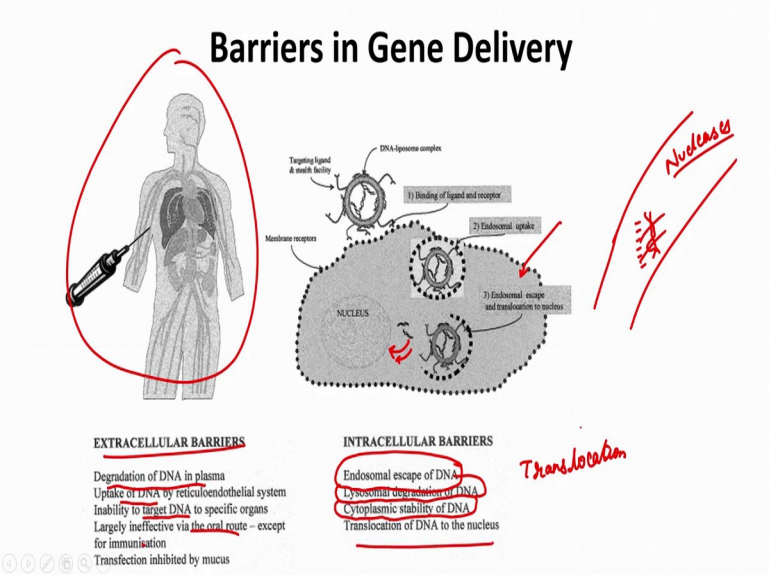


So, here is what we are talking about. So, let us say here is your cell, you have your genome. So, it says several chromosomes are there, in one of the chromosome we have a defective gene here.

So, one thing you can do is you can add a gene. So, this gene can integrate into the genome at some other site as it is shown here or can just lie outside the genome, but in the nucleus separately. So, those are two options in gene addition or replacement. So, if you are actually you are talking about. So, this is an extra chromosomal which means that its outside the chromosome, but it will still lead to protein production. This is chromosomal insertion of course, because now it has integrated with your rest of the genome. And then this is gene repair where what you are doing is let us say if a portion of your gene is not functional So, you have an incorrect segment, the rest everything is correct. You come in and you then insert the current segment by replacing the defective

segment. So, that is gene repair and it sounds very futuristic, but it is actually fairly feasible and we will talk about it in a moment as well.

(Refer Slide Time: 28:27)



So, here is your correct template and you can then put it. But then there are several barriers to this, I mean it is good on the cartoon that yeah we can do this, and there is a quite a lot of enthusiasm about it, but then there are several barriers of how to do this. So, first let us talk about some extracellular barriers. So, one is the DNA that you are delivering, if it goes in the plasma which is of course, one of the major fluid in the body, the blood vessels.

If the gene is let us say in here, the plasma contains several nucleases and these are nothing, but something that degrades any long nucleotide chains. So, this will get degraded and once you get degrade it is no longer functional. So, that is a big problem when you are delivering any gene, then the there is problem that these external DNA can get up taken and cleared out by that at reticuloendothelial system which is nothing, but your immune system, so RES. So, your spleen and your other immune cells may able to clear this DNA out. So, not everything goes to the site.

Then the problem is let us say if I only want the gene delivery to happen in pancreas, let us say if it is for islets or its for something else, then it is hard for me to go to that location and target only that location because these will go everywhere. They are largely ineffective by the oral route that is of course, because most of it is not be going be able to

make it to your circulation, although it does work relatively ok for immunization. Then let us say if we are using some other route that is mucosal route. So, something that comes in touch with mucosa, and then these gene do not really move around diffuse very well in mucous layers. So, you do not really get a good movement of these genes in those circumstances.

So, those are all extracellular, while traveling through body and then there are several intracellular barrier. So, let us say after all these barriers some of it has been able to reach the target cell. So, once it reaches the target cell, the first is how it is going to first of all go inside the cell because the cell membrane is not permeable to such large molecules as well as DNA is also charged. DNAs quite negatively charged it has several phosphate groups.

So, you are not going to be able to diffuse through the cell membrane. So, then it has to be up taken by some endocytosis method that we discussed previously, but then the problem is that these endocytosis methods will end up going to endosomes. So, if it does go to endosome then you have to worry about how do you escape out from those endosomes that is a problem. Quite a lot of it gets degraded in the lysosome. Eventually the endosomes will go into the lysosomes and that itself contain a very harsh environment and lots and lots of nucleases as well. So, that is not going to work.

Let us say if somehow this thing comes out in the cytoplasm, then how stable it is in the cytoplasm, that is another challenge because that is not an environment where the DNA was supposed to be and then let us say if it's in cytoplasm and it needs to go to the nucleus, and then how does it travel to the nucleus because it is a fairly large molecule, it has fairly low diffusivity through the cytoplasm. And then the same question arises again that when it reaches the nucleus that also surrounded by a membrane, how does the DNA go from outside the nucleus to inside the nucleus.

So, another barrier is translocation to the nucleus, I guess it's already written here. So, how does it go to the nucleus? And then finally, it is whether it goes to the right region in the nucleus, where it can get expressed. So, there is so, many barriers that this DNA will have to go through and this is a good point where we will stop, and we will see how we can overcome these challenges in the next class.

Thank you.