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

Lecture – 53
Gene Delivery Vectors

Hello everyone, welcome to another lecture of Drug Delivery Engineering and Principles. We are at Gene Delivery module and we are discussing about various aspects of gene therapy either by cells or without. So, let us quickly do a recap of what we learned so far.

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So, as I said we are talking about gene therapy. So, the first thing we talked about is for gene therapy is let us deliver a cell that carries the gene of interest. So, this could be a native cell itself that is expressing that gene and contains that gene or this could be a cell that you have engineered first in laboratory to express this gene of interest and then deliver it.

So, that is one option and that is very similar to what we have discussed earlier in the previous classes where you can encapsulate this cell in a semi permeable membrane that will prevent any kind of immune response from acting in, but the cells can continue to express and deliver the protein of interest. The problem with that is it is not fairly successful because there are several small components also present in the immune

system that will still be able to go through this membrane such as cytokines and complement and they will not let these cells survive.

So, that is one challenge and so what is seen and we discussed an example in a mouse setting that if you have an immune deficient mouse these things survive at least for a few months and but if you have a healthy functioning immune system carrying mouse then that those things do not really survive they within a month or so we saw that that mouse rejected the implant. So, that is a problem. So, that is where the second strategy would come in is let us not talk about non-autologous cell and so any cell that is not from your own body use the cells that are in your own body. So, why cannot we just deliver the gene hopefully the cell will go the gene will go to the cell that you wanted to express and that way the immune system will not really attack the cell as much.

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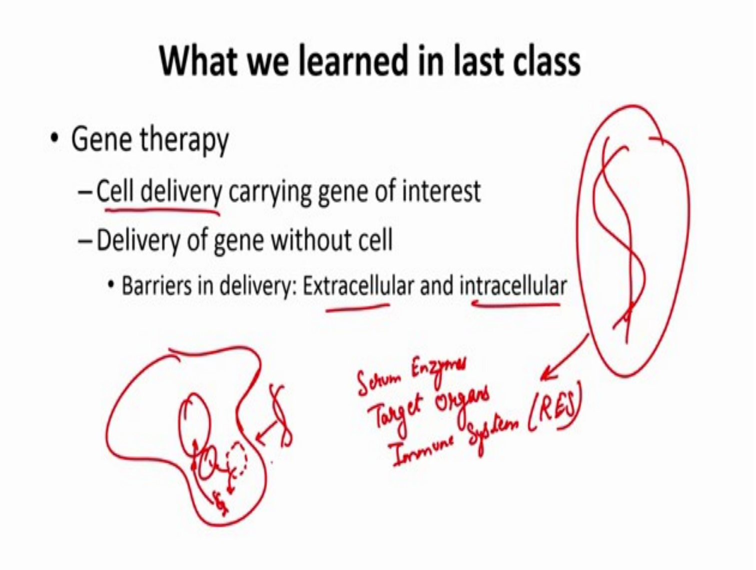

So, in that regards we discussed several barriers that are present of course, it is all well and good to say that you can deliver at DNA, but then the delivery of the DNA has several challenges because first of all when you deliver this there are several accessible barriers. So, if serum contains lots of enzymes that can degrade it. It is hard for this to target the organ that you want. So, let us say if you want the expression to be in lungs it is difficult that all the DNA will actually go to the lung and then the immune system again we will clear out quite a bit of it immune system or you can also call it the reticular and the reticular system.

So, all of this we will clear out quite a bit of it and then there are some of intercellular barriers again. So, even if it reaches your target cell how does it go inside the cell. So, let us see if this is a cell. So, first of all you have to figure out how does your DNA pass the cell membrane because DNA is a fairly charged in large molecule. So, it cannot go through. So, it has to go through endocytosis. So, if it goes to endocytosis one of the problem is that lot of it will go to lysosomes and get degraded.

So, that is one barrier. Another barrier is how do you if you want to prevent that how do you burst throughout this particular endosome to come out in the cytoplasm and once it is come is out in the cytoplasm how does it able to move from the cytoplasm from it’s location all the way to the nucleus because again it is a large molecule since the diffusion is fairly limited. And then once it reaches the nucleus membrane how does it go beyond that how does it go inside that because again the same barrier is present on the nuclear membrane also.

So, several barriers are present. Nonetheless it is not as bleak as it comes seems to be people use it all the time and at least from the literature reports and from the experiment it does seem that you can get gene delivery into a cell despite all these barriers and that is what we are going to discuss today.

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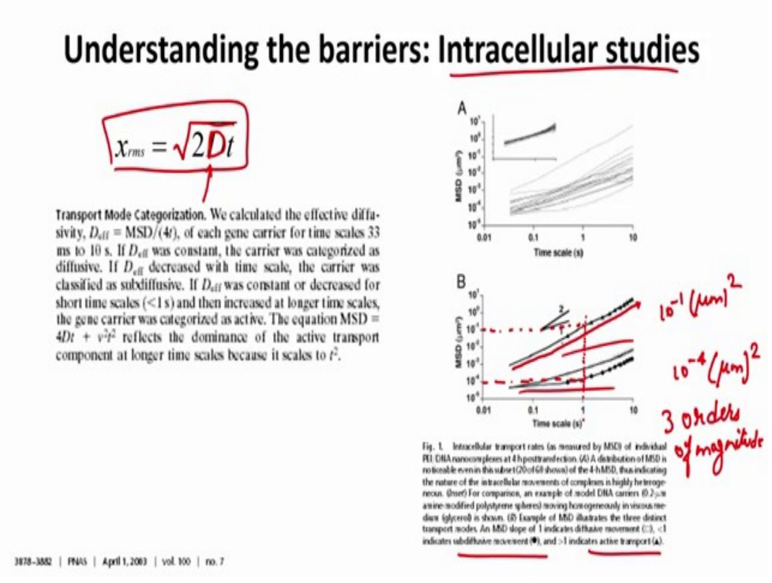

So, let us understand some of the barriers. So, let us talk about intracellular barrier the movement it itself and so again you can characterize the root mean square speed their

velocity here where you are trying to figure out how quickly the DNA will move in there. So, it will depend on the diffusion co-efficient of the DNA in that environment and again you see that there is quite a lot of variations you can see the MSDs can vary quite a lot depending on the different condition used. So, if it is slow it is like sub diffusive whereas, if the slope is higher then you are talking about quite a bit indicates an active transport.

So, if you have somewhat an active transport you see that the MSD Mean Square Displacement is almost. So, let us let us say let us take at the time 1 second, if somehow we can get an active transport. So, we are looking at 10 to the power minus 1 micron square here and 10 to the power minus 4 micron square here. So, the diffusion has actually increased the movement is actually increased by 3 orders of magnitude which is a huge improvement in translocation. So, some how you can get the active transport to work you have much better chance of getting your cargo into the nucleus.

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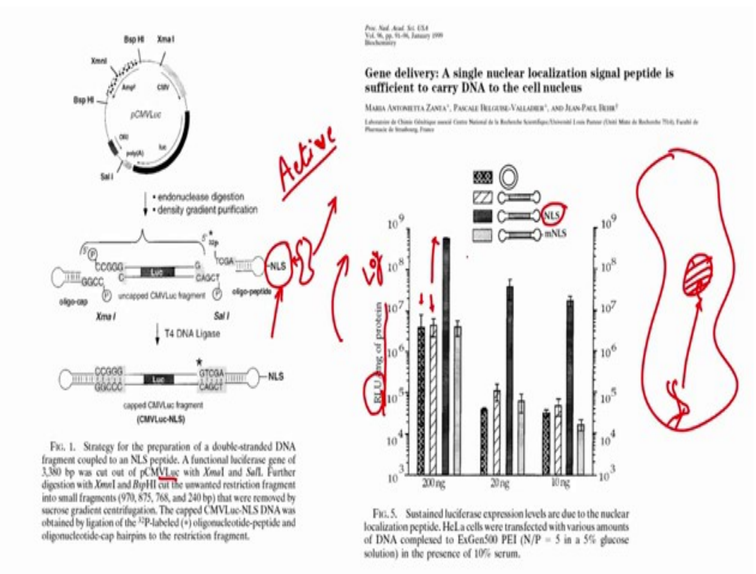

So, that is what these authors have tried. What they have done is they have designed a plasmid that has a binding site as a nuclear localization signal. As what this is, this NLS binds to a protein that can signal to the cell. So, let us say if a protein gets bound to this when bound to this DNA will signal the cell to move it to the nucleus. So, now, you are actually relying on active transport once you do that. So, you put this nuclear localization

signal and then the active transport will help it at least overcome one of the barriers that is movement first of all into the site through the cytoplasm.

So, let us say this is a big cell, here is a nucleus, here is your DNA. So, all the way from here to here this analyst first of all will take care of this movement as well as movement across the nucleus membrane. So, that is what it is being shown here. So, if you have different conditions if you only have the plasmid, you do not really see quite a lot of production of the proteins. In this case the protein that is being used is in this case the protein that is being used is something that is a luciferase gene.

So, it can be easily measured using light production and this is on log scale. So, you see you can change the type of plasmid here you using the conformation on the plasmid you do not really get quite a lot of enhancement, but the moment you put an NLS you actually see quite a big jump in the localization or actually the production as this is more a function assay in the production of the protein through this.

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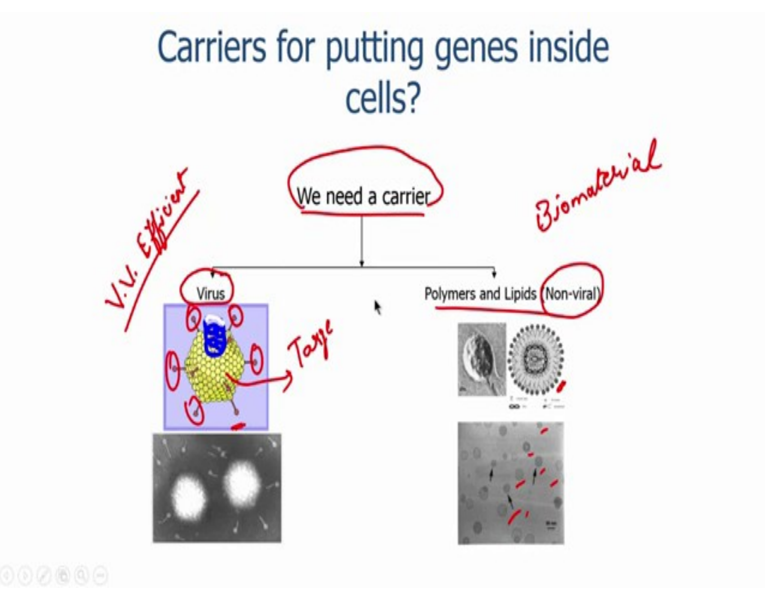

So, that is one ok. So, that is good we can overcome one of the barrier what about the rest of the barrier what about going into the cell what about coming out from the endosome and what about the extracellular barriers the proteases the nucleases present in the environment how do you target a particular organ how you target a particular cell. So, for that we need a carrier. So, it is fairly clear that because of so many barriers if you

just inject the DNA by itself it is probably not going to be able to reach in sufficient amount to your target site.

Now that we establish that yes we need a carrier we have 2 choices; one as we go to the nature itself and use a virus based carrier and this is nothing, but a virus that is already adapted it itself to infect human beings. So, it has overcome all those challenges if we talked about. So, it has a shell of protein shell which protects it from any extra cellular barrier it is a different size now. So, it can translocate it has receptors to target a particular type of cell in organ and that can be then used to deliver your DNA.

So, you can take this virus remove the viral genome put your gene of interest into the virus and then let the virus go and deliver your gene of interest to your target cell and this is actually very efficient in the whole reason is that they are well adapted to do this. So, this technology that is being made by evolution is very efficient, but the problem is of course, that and we will discuss this in more detail, but obviously, let us say if you are not able to remove all the viral genome you are talking about causing now a new disease, the other option is to use polymers and lipids.

So, what we are discussing throughout the course are non-viral vectors. So, some kind of material and mimic some of these viral strategies either their size put some targeting ligands on to this change their charge on the basis of that get different size particles and then package these particles with the DNA and then deliver it. So, these are two major sort of carriers that we can use for this application.

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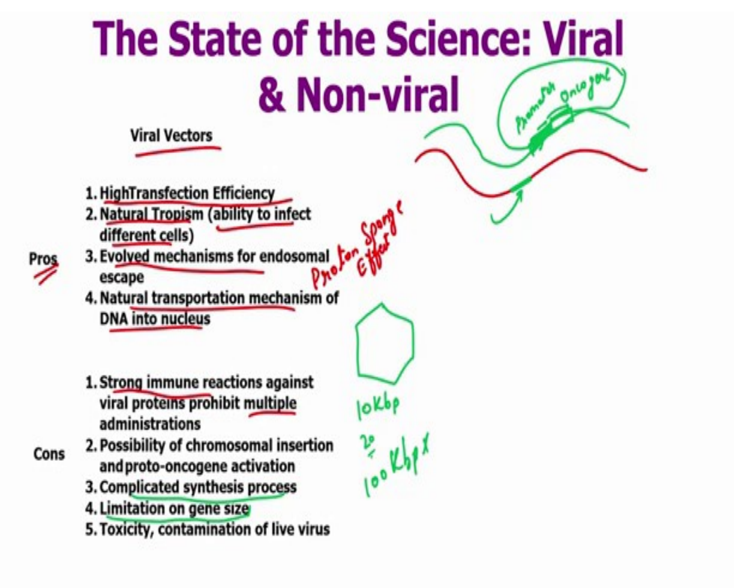

So, let us look at the state of the current science with both the viral and non-viral vectors. So obviously, viral vectors as I said has lots of pros it is a very high transfection efficiency. So, you are talking about pretty much more than 50 percent efficiencies almost more than half of your cargo will get transfected and result in functional protein being produced. So, it has natural tropism which means that there are viruses existing that infect only a particular type of cells and then there are viruses existing that infects many cells and then also being selective in the type of cells that they are infecting. So, that way you can choose what virus infects your target cell and use that.

So, you do not really have to do much work you know the nature has already produced all this to help you do it then these viruses have actually evolved mechanisms for endosomal escape. So, they are very well versed in how to rush out from the endosome and go back into the cytoplasm because eventually these viruses also want to inject their DNA and want the DNA or the genetic material to go in the nucleus, so, they can do that. In fact, one of the strategies that we talked about the proton sponge effect was something that we learnt from these viruses. So, in this case they are coat protein was such that was acting as a proton sponge and then they also have mechanisms for transportation of the DNA into the nucleus.

So, again they are well adapted in overcoming all these challenges and that is why there is one of the most ideal systems when you consider their efficiency in gene transfection;

however, there are some cons and one con again being that now that these viruses are not something that is a part of your own body. So, now, you have introduced a foreign material.

So, your immune system will start acting against it. So, you get a very strong immune response against these viral proteins and that means, that let us say if you have done it once and you wanted to do it again and by the time you are ready to do it again the body is already aware of how to handle that particular virus because you essentially vaccinated the body by giving the first dose and the second time we do it the body's going to clear it even before it can do any of it is function.

So, prohibits multiple administration. Now the problem is that these viruses even though they. So, one of the thing that these virus will do is remember this chromosome that we have discussed and let us say this is the gene that we want to put, but these viruses again are not very specific in where this gene is going to go ahead and insert itself. So, it could happen that this gene will insert on this chromosome it could happen that this gene may go and insert in a neighboring chromosome let us say here. So, on the basis of where it is in where it is getting integrated this may lead to several consequences first of all the expression may change, the second problem is what if now gene you have a promoter in a gene as well. So, that it can express what it is carrying.

What if downstream you have some oncogene. So, now, along with your own protein you are also making the cell cancerous. So, that is a big problem actually there was a lot of enthusiasm for these viral vectors and they went even in human clinical trials, but in one of those trials what they found out is more than 50 percent of the patient actually developing cancers and the patient actually died because of that.

So, that decreased the enthusiasm when using this evaluator of course, this was quite a long time ago and technology has since improved quite a lot, but there is still a concern as to something like this could happen and lead to more serious disease then what are you trying to cure. It is a fairly complicated synthesis process it is not trivial to take a virus and then load it with your own DNA and let it go and do whatever you want it to do. It also is a limitation on the gene size that you can put.

So, this virus coat protein that you may have it may only be of a certain size and there is a limit. So, let us say the virus genome is about 10 kilo base pair then you can't really

load more than 20 or so I mean it may have some stretch ability. So, you can add maybe few kilo base pairs, but eventually you cannot tell you put 100 kilo base pair in there. It is just too big for this to be loaded into that space that is given in the viral shell.

So, if you if your gene is big you have to look for some other way. And again there is toxicity associated with this because the immune system there is also chance that you may contaminate your thing with the live virus and if you do that you again cause a different disease and then what you are trying to cure and that is never good. So, there are few cons that exist with these viral based vectors and so that is why the field has now also tried to look into other alternatives to what these viral vectors offer.

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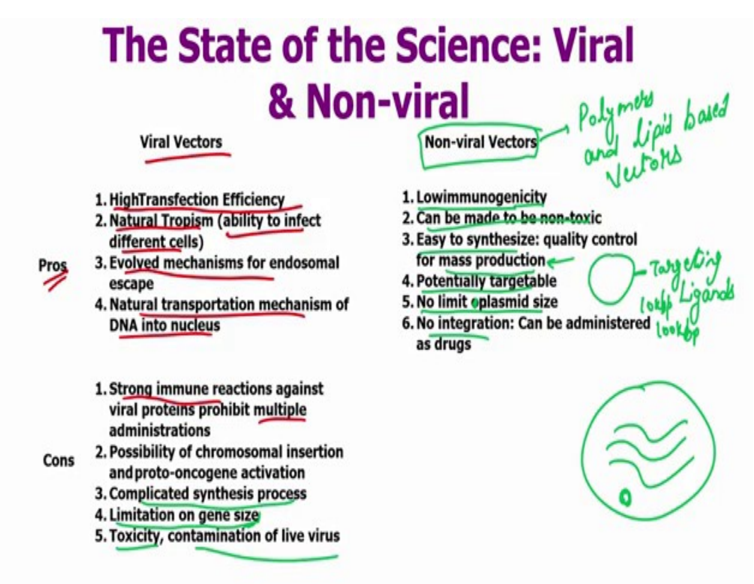

And so, once again as we discussed are the non-viral vectors. These are these polymers and lipid-based vectors. And one of the major advantages is unlike viruses they have fairly low immunogenicity we have seen this throughout this course that these polymers and lipids, there are so large libraries of it that you can choose fairly low immunogenic polymers and lipid. They would not typically develop an immune response against these proteins or these polymers and lipids, there is a big library. So, you can use non-toxic biomaterial, they are fairly easy to synthesize.

So, we know how to synthesize this very well and there is all kind of chemistry and you can do a very easy mass production. So, and that is a big plus because if you want to let us say use it in clinics and you expect to get 1,000 patients walking in every day in a

clinical setting, you want quite a lot of this polymer and you can get that you can potentially target that. So, you can once you form the particle you can conjugate it with targeting ligands and that would be enough to help with some targeting. There is no limit to the plasmid size, you can get as much plasmid size as you want. So, if you want 10 kilo base pair that is feasible you want 100 kilo base pair that is feasible. So, large genes are easy to take it through unlike the viral vectors that you are saying there is a limitation to the gene size and they do not really integrate

So, in this case if let us say this is the nucleus and these are your chromosome. So, the viral vectors would not integrate they will just reside separately and will produce gene from the separate plasmid. So, you do not have to worry about onco genes being activated and other things like that. So, they can be administered fairly without concern of causing cancer and other diseases.

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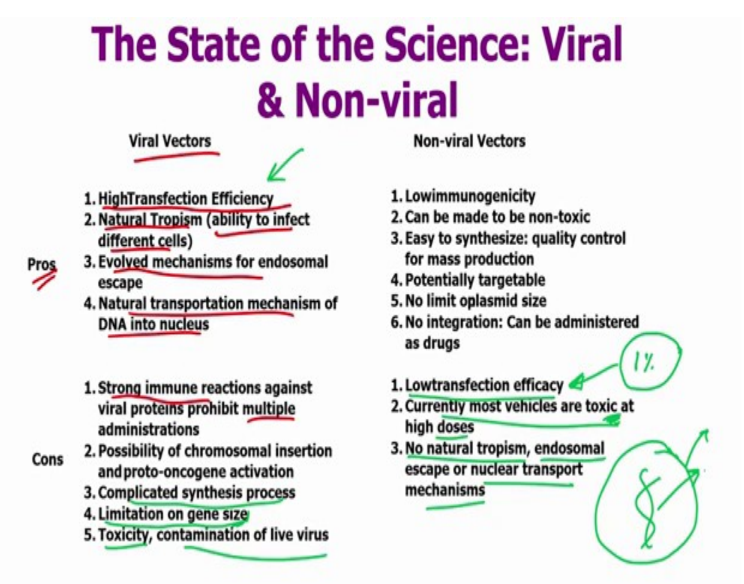

However, they like the viral vector there are some cons associated with these systems as well and let us look at what some of these cons are. So, the major the biggest con is it is a fairly low transfection efficacy. So, now, here you are talking about more than 50 percent of the cargo transfecting here the efficiency is actually very low. We have been trying to improve that, but we have picked as good as the nature is in increasing this efficiency.

So, this could be as low as 1 percent actually it just depends on what you are using and that means, that almost 99 percent or more of your drug is getting inactivated or is

wasted. Most of the vehicles that we use are fairly toxic at high doses and the reason we need high doses is goes back to the transfection efficiencies now that because we have such a low transmission efficiency we want to deliver more of it.

So, that at least even if 1 person goes we have enough dose, but then at those high doses you can have toxicity associated with these vesicles and these non-viral vectors and then there is no natural tropism. So, you have to worry about how do we target a particular cell, how do you target different cells endosomal escapes become a challenge. So, we said we adopted some of the proton sponge effect, but obviously, we have not come to the technology which is as good as the viruses in proton sponge and then also the nuclear transport mechanism became an issue because earlier we were talking about DNA molecule having problems diffusing in the cytoplasm.

Now, we are saying that this DNA molecule is in a large particle. So, it is even larger now how this is going to diffuse in the cytoplasm is another bigger challenge. So, all of these combined result in quite a lot of pros and cons for each of these strategies and depending on what is it that is your application and concern you choose one or the other; obviously, this is a drug (Refer time: 21:40) course and we have been talking about polymers. So, we are going to focus on non-viral vectors. The viral vectors for gene delivery are still being used in research and they have gone to clinics in the past as well. So, this is another field, but we are not going to talk about the viral vectors much we will focus on the non-viral vectors.

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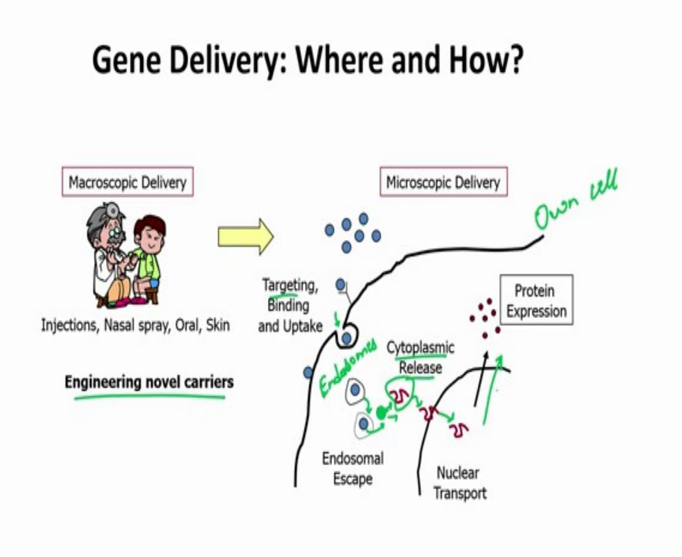

Okay so, gene delivery where and how? So, let us look at microscopically what is happening. So, you go to a clinic the doctor injects you something. So, these could be injections these could be in nasal spray orally you take something they put it under the skin muscle whatever then if you zoom into it as to what is happening there you have some particles. You use either some targeting or these particles inherently go into the cell.

They go into the endosomes and eventually if you have used proton sponge effect or something else, they burst this endosome and come out into the cytoplasm. Once they come out in the cytoplasm typically the best strategy is to let your DNA come out because this particle as I said in the previous slide is too big. Now we are talking about 100 nanometer earlier the DNA was maybe 1 to 10 nanometer, now you are talking about 100 nanometer big particle moving in the cytoplasm that is going to be a big challenge.

So, most vectors you can design in such a way that once they come out from the endosome the DNA can release and we will talk about how this release can happen, but we talk we expect the carriers to release in cytoplasm. And then either using NLS or some other strategy we hope that this DNA will translocate to the nuclear membrane and go into the nuclear membrane. And once it goes there we hope that this is still functional and start secreting the protein. So, this is the whole overview of gene delivery in a

nutshell; obviously, it has some advantages you are not actually now delivering cell. So, this is your own cell and so immune system is not going to act on that which is great. So, this is autologous order it is not really an implant. So, you are in this case now your drug is gene instead of being cell. So, we want to engineer some novel carriers that will facilitate this process and make it better.

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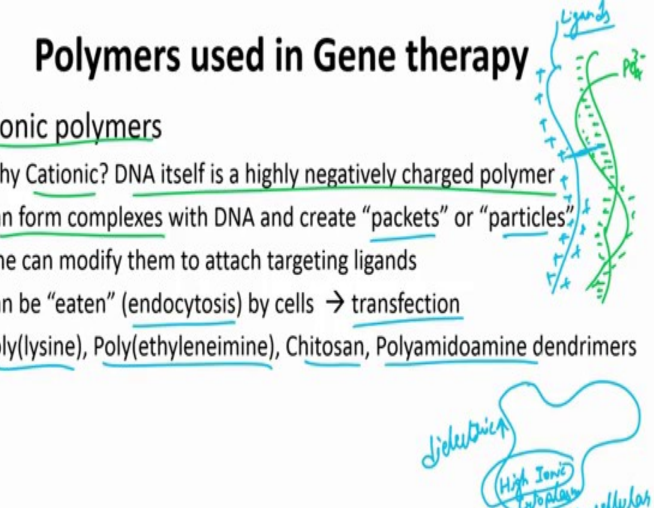

So, we then go to polymers as we have been doing throughout this course. So, mostly cationic polymers are used and why cationic is because DNA itself is highly negatively charged. So, as I said the DNA is composed of nucleotides which are composed of phosphate and this is several negative charge is present on each of those nucleotides and so you can imagine 100 kilo base pair DNA will have quite a lot of negative charge on it's surface or on in the structure and so you can use cationic polymers and that way these DNA molecule interact quite strongly ionically with your cationic polymer.

So, these end result in formation of complexes. So, here is one DNA then you let us say you take up a polymer which is positively charged and you mix them in the right ratio or you vary the ratio and at some ratio what will happen is they will start precipitating out and form these complexes or create packets or particles. And depending on the ratio you can change the size and all and then further you can modify these polymers with various ligands to target a cell receptor or to target some other function. Then these particles are big enough that they can be taken up through endocytosis and once they are taken up

through endocytosis you rely on their escape from the endosome to and then eventually release of this DNA. So, how does the release work? So, outside the cell, so, let us say this is the cell you have cytoplasm and you have extra cellular.

So, the extracellular environment is typically low ionic concentration is present, but in the cytoplasm you have high ionic concentration. So, what that does mean what does that mean? That means, that since there is high ionic concentration the dielectric of this particular cytoplasm is high and because of that this ion-ion based interaction. According to Coulomb's law, dielectric is in the denominator since it is high it is going to decrease and so; that means, that the bond is not going to be as strong and this can separate out. So, that is how when this particles will come out in the cytoplasm from the after the endosomal escape they will cause a release of the DNA and then the DNA can go in to the nucleus and transfect and start producing what you wanted.

So, this is the whole concept of using polymers. There are a few polymers that are particularly good at this and some of these are poly lysine, poly ethyleneimine, chitosan some polyamidoamine, dendrimers. So, here are some examples that I am giving you.

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Polymers used in Gene therapy

- Cationic polymers
  - Why Cationic? DNA itself is a highly negatively charged polymer
  - Can form complexes with DNA and create "packets" or "particles"
  - One can modify them to attach targeting ligands
  - Can be "eaten" (endocytosis) by cells → transfection
  - Poly(lysine), Poly(ethyleneimine), Chitosan, Polyamidoamine dendrimers
- Other polymers
  - Can slowly release genes (DNA) from polymer devices inside the body
  - Can be in the form of small particles or matrices or gels
  - E.g: Poly(lactide-co-glycolide) or PLGA, Polyvinylpyrrolidone (PVP) etc.

You can use other polymers also it does not have to be cationic polymer, but it is just seen that cationic polymers are much better at this than any other type of the polymers. And so, you can have a big polymer such as let us say PLGA, put your gene inside and when the PLGA degrades this thing can come out, but you then run into the problem that

they do not really have good encapsulation efficiency, not a whole lot of DNA is going inside your particles and then the release is also just relying on degradation rather than triggering when it's out in the cytoplasm.

So, that is one issue but then you will still use it. These can slowly release this gene once they going to the body, you can make various sizes as we discussed already with the PLGA particle. You can make matrices, you can make gels and some of the polymers that are used is PLGA, PVP etc. for all these applications. We will stop here and we will continue rest in the next class.

Thank you.