Drug Delivery Principles and Engineering

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Lecture – 54
Gene Delivery Polymers

Hello everyone, welcome to another lecture of drug delivery engineering and principles. We have been talking about gene therapy for the last couple of classes and we will continue that discussion, but before we go forward let us just quickly do a recap of what we have been talking about in the previous class.

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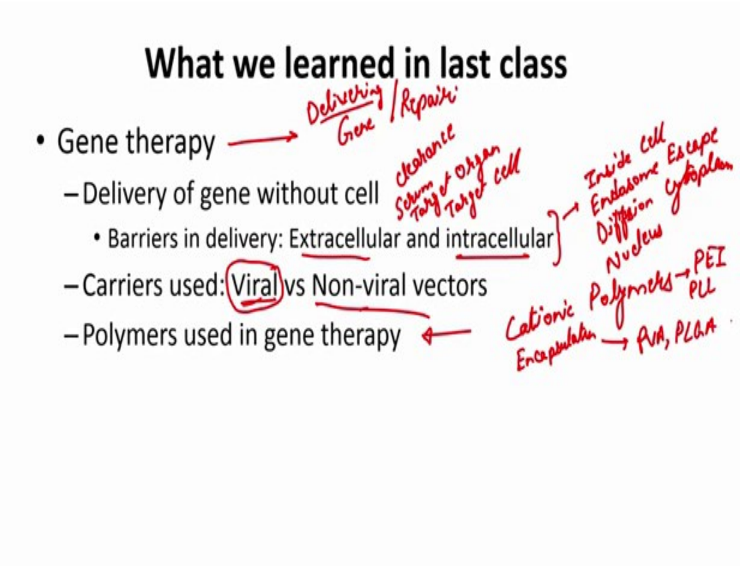

So, in the previous class we were discussing as I said gene therapy which involves delivering gene or repairing gene. So, this could be delivering gene, this could be repairing gene or this could be something else as well. So, the delivery of the gene could be in the form of just the whole cell itself that carries your gene of interest and then if the cell is not autologous; that means, if the cell is not from your own body then you have to device your drug delivery vehicle in such a way that it is protected from the immune system. And we have again discussed quite a lot about this in previous classes and then the other way that we were discussing in the last class was delivering directly the DNA and making sure that the DNA then goes to whatever site we want.

And we discussed there are several barriers to that. So, delivery of gene without the cell itself which is the gene itself and there is several barriers both extracellular and intracellular. So, extracellular barriers would include degradation by any kind of nucleases that are present in your serum. How it is going to reach the target organ. How it is going to reach target cell and then inter and of course, clearance by the immune system and then intra cellular barrier included how it is going to go past and inside the cell.

How it is going ,most of the time because this DNA is fairly large molecule as well as ionically charged it has to be through endocytosis. So, how it is going to escape those endosomes and then once it that escapes the endosome how it is going to move around. So, diffusion cytoplasm and then finally, how it is going to reach the nucleus.

So, all of these barriers were there and in the process of that we said that it will be easier we use some kind of a carrier. So, we discussed two viral and non-viral. Viral vectors as the name suggests is using the naturally occurring virus and modifying them to carry your own gene of interest and we discussed several pros and cons with that and then we discussed non-viral vectors which are typically polymers and lipids then we are in this case we are basically trying to mimic these viral strategies and remove the cons out of it while keeping the pros. But it is not that simple and we discussed again several pros and cons for both of these strategies and depending on what do you want to do, you may choose one over the other.

And then since this course is drug delivery and we have mainly talking about polymers and other biomaterial. So, then we dive deep into what are the different polymers that are used for gene therapy. So, some of them we discussed were cationic polymers and other class we discussed was just encapsulating within the polymer. So, they may or may not be cationic. So, examples of cationic polymers were PEI polyalysine encapsulation could be through PVA PLGA and other polymers. So, that is what we had discussed in the last class, we will take this discussion forward in this class.

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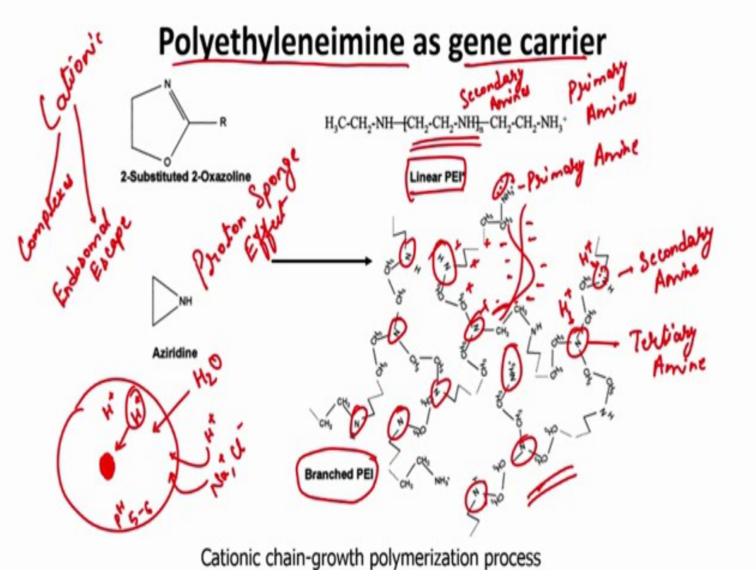

So, let us look at some of the major ones that I used. So, I mentioned PEI. So, it is polyethyleneimine; one of the most used polymer for gene delivery and the reason for that is it is highly cationic and so it solves two purpose one it forms complexes with DNA. So, at the right ratio you will get different size particles and you can choose what size you want and then the second is that since it is so cationic and contains lots of primary secondary and tertiary amines it helps in endosomal escape.

So, that is what we discussed, here is just an example. So, you can have a linear molecule of polyethyleneimine. So, in this case here is your monomer which gets polymerized and you can see that there are tertiary and in this case this is the secondary amine you have primary amines, but as you can see on the structure there actually no tertiary amine.

So, that is what the linear PEI is, but you can polymerize it further to get branched PEI and now what you have is you have all kinds of amines. So, you have you can see here you have primary amine. Here you have secondary amine and then in the structure you also have tertiary amine and all of these amines specifically the nitrogen has these lone pairs or this lone pair is already being used with this hydrogen, but all of this can absorb H plus ions through interaction through these lone pairs and what; that mean is let us say this is an endosome.

So, this is my endosome containing a particle with the these polyethyleneimine molecules, then what does that mean? That whatever is H plus that is available in this

endosome and which is in higher quantity than outside because the pH of the endosome typically is around 6, 5 to 6.

So, some of these polyethyleneimine can absorb these H plus ions causing the increase in pH, now the cell wants to maintain the pH to 5 or 6. So, it is going to pump more H plus ions and now as it is also pumping H plus ion it has to pump in to maintain osmotic balance. More sodium and chloride ions in there to make sure that the osmotic balance is there. So, now, what you are doing is you are creating in osmotic gradient in here. So, that is going to lead to water going in and now the size of this endosome is fixed by the amount of vesicles that are represent the or amount of lipids that are represent in the vesicle and as more and more water will go it will start to swell and after a certain limit it will just burst.

So, if you have these molecules in large quantities in the endosome which can absorb lots of H plus ion you can have this endosomal escape and we have already talked about this is also called proton sponge effect if you guys remember. So, if you want endosomal escape basically you want to make sure that your molecules of polymers or whatever your drug delivery vehicle is, is able to absorb lots and lots of H plus ions and again amines all kinds of amines, primary, secondary and tertiary are extremely good at this.

So, you can see even in this little small structure how many amines are being present here which I am just circling most of them here and hence this act as a very good carrier in taking your DNA and then making sure that the DNA comes out and now once it comes out the DNA of course, is interacting with all this negative charge on it is structure to the positive charge on the structure of PEI.

And once it comes out the ionic concentration outside is high and because the ionic concentration is high in the cytoplasm this interaction between the positively charged polymer with the negatively charged DNA is not as strong and this DNA can break off from the particle and then can try to diffuse around and reach nucleus.

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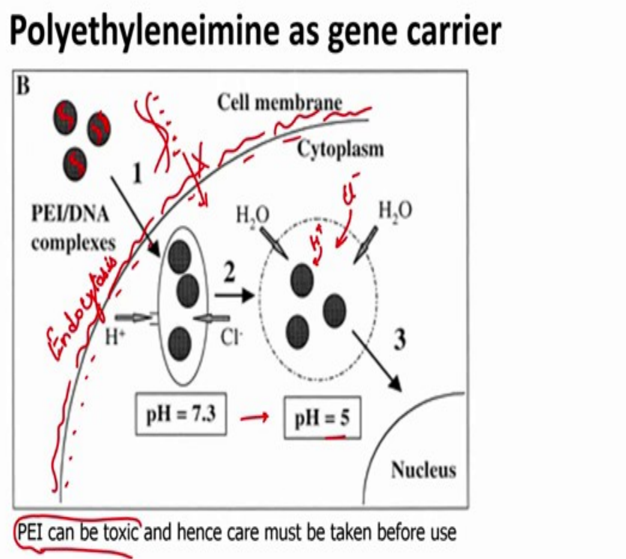

So, here is just pictorial representation of what is just said. So, instead of having a DNA molecule which is negatively charged, it is not going to go in the membrane, you have now formulated it in a particle formulation where all this DNA is making the particle and along with the PEI of course, and this can be then taken up through endocytosis and once it goes there the pH as it drops to 5 or as it starts to drop this is going to keep on absorbing more and more H plus causing more and more ionic imbalance and ions going in and increasing the osmotic pressure and eventually it is going to burst.

However, one note of caution is most of these cationic polymers, if they are highly cationic can also be toxic and one of the reason for that is these since they are ionically charged and they are positively charged we have already talked about that cell membranes are slightly negatively charged.

So, they can then ionically interact with the cell membrane in huge quantities. So, what can happen is your PEI polymer will start to interact with the cell membrane, thereby first of all and disrupting the function of all these receptors that are there on the membrane. Secondly, also causing instability in the lipid membrane layer, so may even lyse the cell. So, that is why it has to be taken into account that PEI can be fairly toxic and if you are going to use PEI you have to make sure that you are using it at a concentration and using in a formulation that is not actually killing off your target cell.

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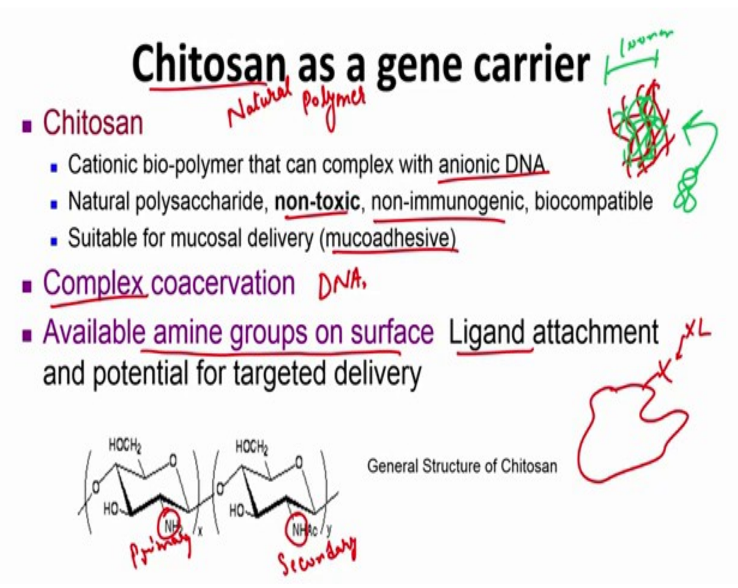

So, then chitosan which is a natural polymer PEI is synthetic is again also used for this application and as you can see it also contains in this case primary and secondary amines. So, very similar concept; however, this chitosan is not as heavy intensity of these amines and positively charged. So, they can still complex with the anionic DNA, but they are not as charged as your PEI is. So, this is not as toxic it is also a natural molecule. So, it is fairly compatible, and it is also non immunogenic it does not really generate immune response in a body.

So, one of the other property of the chitosan is also mucoadhesive so; that means, that if it is given through a route which is a mucosal delivery and if you remember from our route of administration topic you will remember that mucosal delivery can be of several types, this could be oral, this could be nasal. So, any route that will encounter mucus in the surrounding will be classified as mucosal delivery. So, you will see that all of these routes are feasible and of course, inhalation will also have mucus, you will have vaginal rectal all of these are mucosal delivery routes. So, if we deliver this chitosan particle what will happen is they will tend to bind to the mucus very well. So, it is mucoadhesive; that means that it binds to the mucus and that could increase it is residence time at the location.

So, instead of just getting up taken by random cells these particles will stay there and depending on the application this could be good or this could not be good, but you get

the control with chitosan and again like PEI or any other cationic polymer it is going to form these complexes with that DNA. So, these will form complex with DNA and like most cationic polymers this is going to result in a formation of particle you can change their ratios to get different sized particles and it will be good to encapsulate your DNA. So, as I said there are amine groups attached to the surface because of this chitosan primary and secondary amines. So, you can then also attach potentially ligands to the cell that you are targeting maybe you want to target to a cell that is expressing an X receptor which is not present in let us say other cells. So, you can put a ligand for the X. So, let us say XL is the ligand.

So, then it has a more chance of going into these cells and other cells. So, you can get it to be more targeting you can also do that with PEI. So, all of that is there one thing to note is why are the complexes are important. So, not only the endocytosis, but once you have this let us say red is your polymer and let me just pick another color and let us say green is your DNA. So, it is forming a ionic complex like this maybe the size of this is about 100 nanometer. So, if you have a complex like this what will happen. So, now, you injected it into the blood and we were initially talking about one of the barrier is that the serum components such as nucleases will degrade it.

So, now if you have a nuclease, it cannot really access the DNA very well. So, the DNA is also protected violates traversing in the serum. So, you have basically killed 2 birds at a time, you have made sure that the serum stability is high and you have also then ensured that this is going to be taken up through endosomal pathway and can also escape those endosomes.

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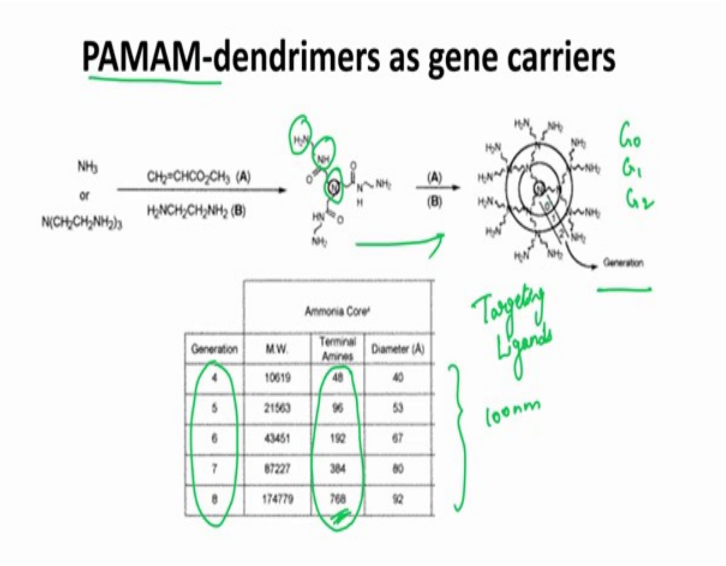

	Ammonia Core		
Generation	M.W.	Terminal Amines	Diameter (Å)
4	10619	48	40
5	21563	96	53
6	43451	192	67
7	87227	384	80
8	174779	768	92

So, another class that we are looking at is PAMAM dendrimers. So, PAMAM is a polymer and again like the previous 2 example these also contains amines both tertiary, primary and secondary so as you can see here. So, you have primary amines you have secondary amines and you have tertiary amines. So, by the same logic this can result in quite a bit of proton sponge effect and what is done as you make dendrimers out of these. So, if you remember dendrimer is defined by the generation. So, you have G 0 G 1 G 2 and what you are seeing is as you are increasing the generation. Obviously, the molecular weight of the dendrimer is increasing and then the amount of terminal amines is also increasing.

So, you have got almost close to 750 primary amines that are available the diameter is still within the size range of about 100 nano meter that you want and because you have these many prime tertiary amines you can get it to complex with the DNA you can conjugate it to the DNA. Obviously, all of these amines can be used for any kind of chemical conjugation such as EDC-NHS or other pathways and you can also put targeting ligands as we discussed in the previous slide through these amines. So, that is another class of polymer that is being used.

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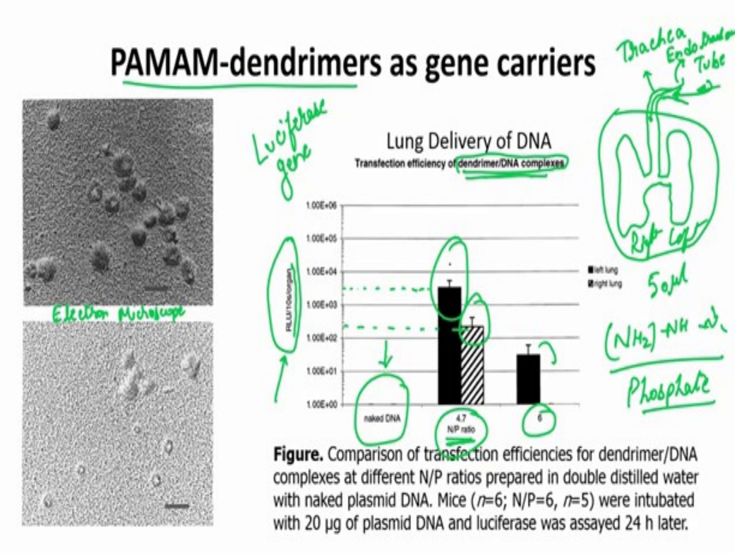

This is just some example on this is from a paper. So, what you are seeing is these PAMAM dendrimers were formed as gene carriers and they were mixed with some DNA and to form a complex the dendrimer DNA complex and this is just electron microscope image.

So, this is an electron microscope image and what you see here is when they were given to different mouse in through lung. So, what was done is so you have lungs right. So, you have your mouse lungs, you take the mouse, you anesthetize it you put a tube into the trachea. So, this is trachea and you put a tube. So, it is called endotracheal tube and once you put that tube you can then use this tube and to thread a syringe or needle through it and deliver whatever you want to deliver in a liquid formulation.

So, you can then just push the liquid through this tube; obviously, there is a limitation of how much you can push because you can potentially drown the animal if you put too much. So, usually for a adult mouse you can put about 50 micro liter of a liquid. So, what these authors have done is they have injected 50 micro liter of the liquid containing these complexes of dendrimer in DNA into the mouse and one of the things that they are delivering is a luciferase gene.

So, this is a proof of concept that is a proof of concept. So, they delivered this luciferase gene and then they monitored the animal to see if they are getting any uptake of these gene complexes into the animal cells and whether then it results in production of the

luciferase protein, because luciferase produces light in a presence of substrate. So, it is an enzyme. So, it is going to and then they are basically measuring the amount of light that is getting produced in 10 seconds per organ. So, they see if they inject naked DNA they do not really get anything it is almost 0, if they injected if they mix the dendrimers and DNA at a certain ratio in both the right lobe and the left lobe. So, depending on where you seeing it from the animal perspective.

So, let us say this is right and left and so what you see here is both lobes have quite a high amount of light signal that is being produced; that means, that not only your particles have been able to go to the lung, they have been taken by the cell they were able to endosomaly escape the DNA, the DNA was able to then translocate into the nucleus and then was able to produce the signal.

So, quite all those barriers were taken care of; obviously, through the route of delivery they took care of one of the barrier which was how it is going to reach the target organ because this was directly injected into the lung. So, it was reaching directly the target organ and even that amount of racial intermixing then it also makes a big difference. So, this is basically is NP ratio and what does NP ratio means it means the amount of nitrogen present in the structure. So, amines could be NH2 it could be NH or it could be N only.

So, amount of amines divided by the phosphate and where does the phosphate come from? The phosphate is from the DNA because again the phosphate every nucleotide has a phosphate. So, the amount of phosphate that is present exactly corresponding to the amount of DNA that is present. So, that ratio makes a difference as well because you will get different stability different sizes of these complexes and that is going to change the pharmacokinetics of them the optic pathways and all those will change, but what you are seeing here is using these polymers they get much much better efficiency then let us say just a naked DNA.

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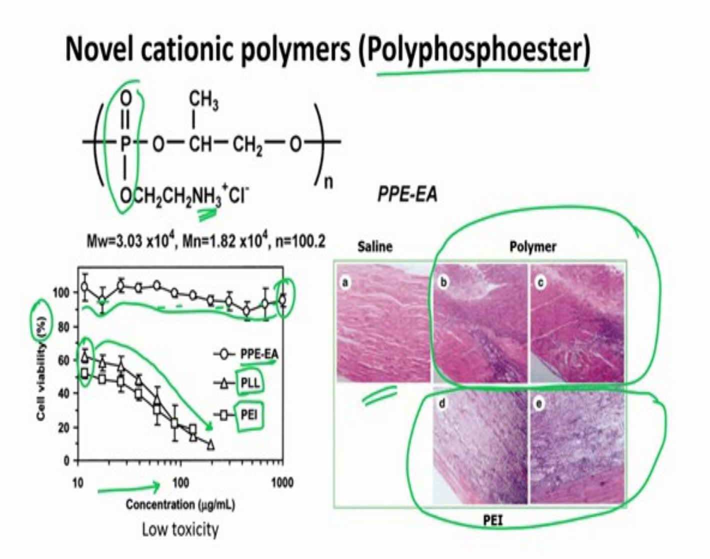

So, here is some more novel materials. So, these were the ones that are widely used, then there are some other reports in the literature. So, in this case they have taken of polyphosphoester. So, in this case they have taken phosphosphoester backbone. As you can see here is a phosphate group and they have then modified it with an amine to get that positive charge and the one major reason they are doing that as I said most cationic polymers are toxic. So, now, you can see. So, their first profound experiment to see whether compared to different polymers how is their toxicity.

So, you can see they have chosen 3 polymers, one is their polymer the polyphosphoester another is PLL which is poly-l-lysine, another is PEI which is polyethyleneimine, we talked about in few slides ago. You can see that as you increase the concentration of your polymer along with the cells, a million cells, you see that even at low concentration you almost have half the viability than what you started with and this is percentage and as you increase it more and more cells die up to a amount that even at 100 microgram almost all the cells are dead.

So, this is something definitely you do not want, but in case of their polymer, they do see even at high concentration, the amount of viability is almost 100 percent. So, that is what then they started with, this is for the confirmation in an animal setting. So, this is they are looking at what happens when they inject this polymer into an animal. So, you can see the tissue looks fairly healthy, it is well organized in a saline injected as you would

expect, but if you are injecting PEI, you see quite a bit toxicity and then with their polymer, it is much closer to saline then it is with the PEI. So, that is what they started with.

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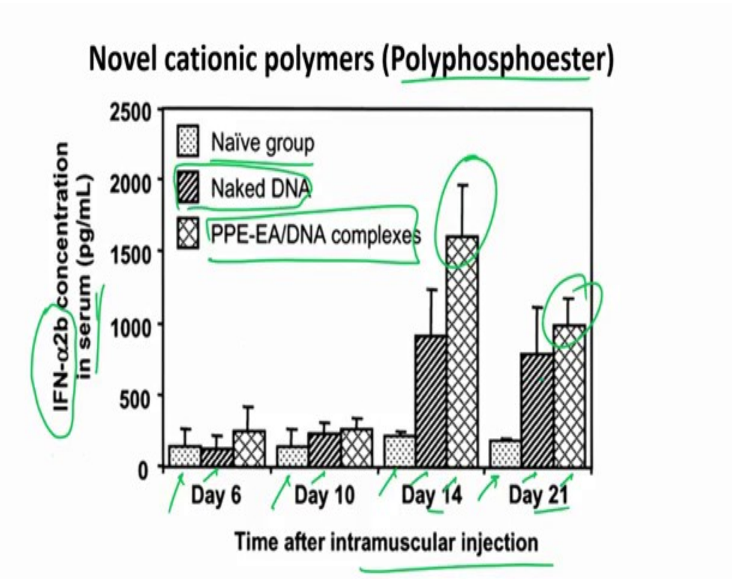

And then they did experiments to see if they can increase the INF alpha concentration by delivery of an INF alpha coding gene and. So, they measured this concentration in serum and what they see is, in the naive group which has not been given any treatment, even after several days of course, you do not expect any change in the INF alpha concentration, in the group that was given a naked DNA. So, without any polymer you do not really see much enhancement. So, it is almost at day 14 and day 21 you do get some more signal, but it is still not as high, but when you give the complexes you see quite a bit enhancement in signal protein at day 14 and 21 even more than your naked DNA.

So, this is after intramuscular injection and they are measuring the concentration in the serum. So, again if this has to work it has to go through all those barriers and only then this protein can be produced and enhanced. So, again you can see that using polymers help in enhancement of the signal, but then one thing to note of course, is even though it has low toxicity the polyphosphoester do not really work as much as PEI, but then at least you are not worrying about the safety issues with that we will stop here and will continue rest in the next class.

Thank you.