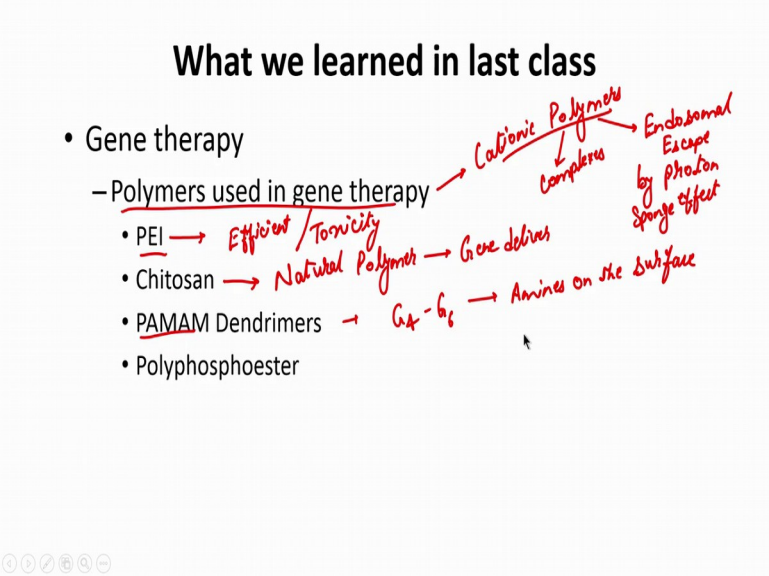
Drug Delivery Principles and Engineering
Prof. Rachit Agarwal
Department of BioSystems Science and Engineering
Indian Institute of Science, Bengaluru

Lecture – 55
Genes as Vaccines

Hello everyone, welcome to another lecture of Drug Delivery Engineering and Principles.

(Refer Slide Time: 00:33)



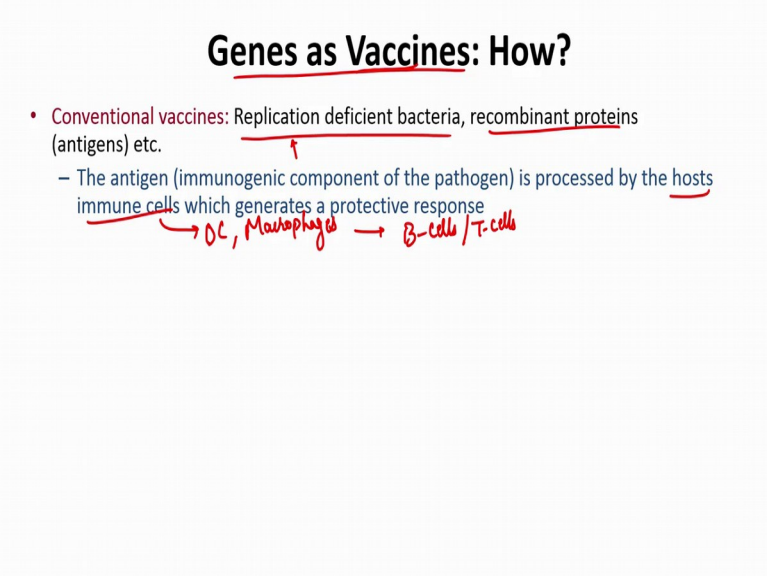
We have been talking about gene therapy at this point and we have looked in the last class as to what are the different polymers that are used in gene therapy. So, some of them that we discussed in quite a lot of detail is cationic polymers and the reason the cationic polymers are used is twofold. One is they can easily form complexes with your DNA and then the other is that they can cause the endosomal escape by proton sponge effect.

So, among these we discussed few of the specific ones, one is PEI which is polyethylene amine and what we found is, this is extremely efficient in compared to all other polymers. So, it is efficient; however, there are issues such as toxicity associated with it. So, you want to make sure that if you are using this you are using this at the right concentration and right amount. The other polymer we looked at is a natural polymer called chitosan and in this also we found that it is much more biocompatible and also can be used for gene delivery. So, it elevates some of the concerns of PEI in that regards and

then we talked about PAMAM dendrimers which I take into all the way to G4 to G6 generation to get several amines on their outer structure.

And, then that can be used to either conjugate or complex with the DNA and finally, we looked at one other type which is polyphosphoesters where, phosphoesters have been modified with amines. And, they are much more cytocompatible as compared to PEI or PLL, but then and we saw that they can also be use for gene transfection. So, we will take this gene therapy forward and we will look at it from a slightly different angle now.

(Refer Slide Time: 03:15)

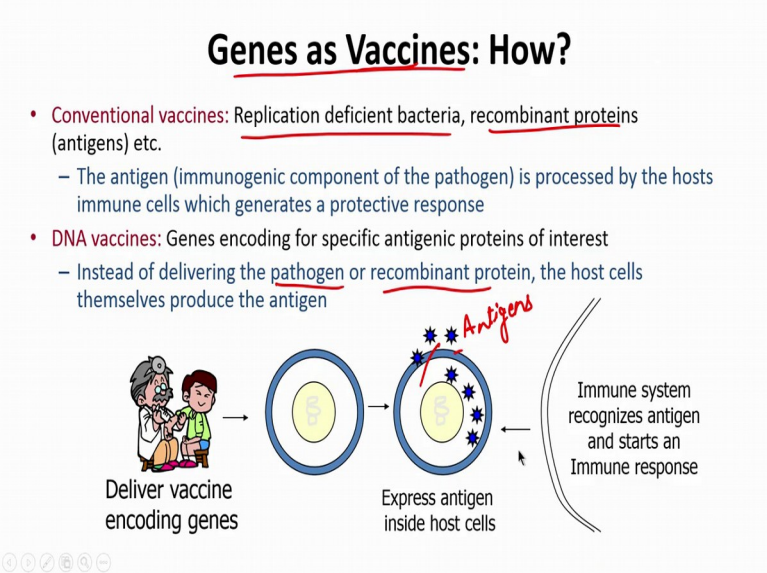


So, what about using genes as vaccines? So, so far we have been talking about preventing the immune system from and destroying any of this, taking away the product that we are injecting in our body. But what if we actually want to target immune system is there an application for that? And, another things we have talked about in previous module is vaccine. And so, how can we use genes for vaccines so, let us see. So, the conventional vaccines is you deliver any inactivated bacteria. So, it could be either replication and defective or you use some recombinant proteins, subunit vaccines that can be used.

So, these antigens which is the immunogenic component of the pathogen is then processed by the host cell and then its presented to the adaptive immune response. So, this is host immune cells here and mainly dendritic cells and macrophages and these then presented to B cells and T cells which then have a large repertoire of the receptors and

which ever does have binding to it starts to proliferate. And, then further refines its binding its a somatic hypermutation and then finally, result in generation of immunity. So, that is what the conventional vaccines were targeting.

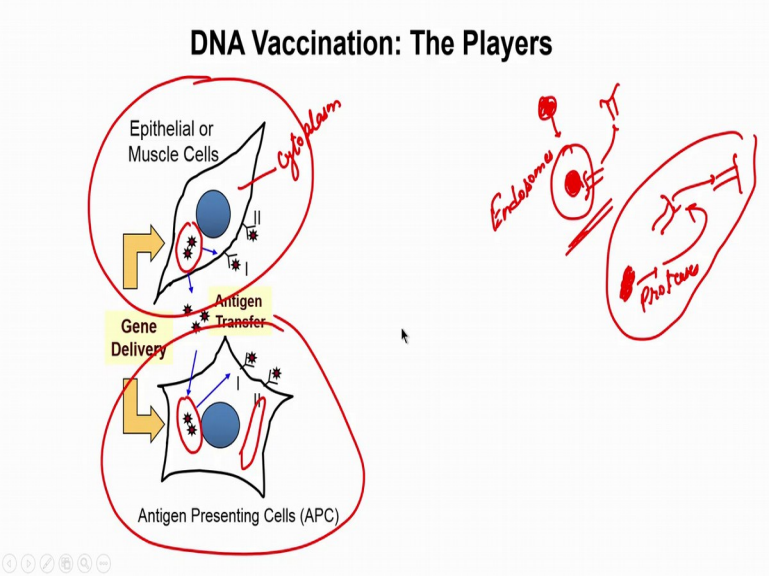
(Refer Slide Time: 04:45)



Now, when we talk about DNA vaccines or genes as vaccines, we are saying that let us instead of delivering the whole bacteria or the recombinant protein, why do not we deliver something that is a gene or a DNA and that codes for the antigen that you want. So now, instead of delivering the whole pathogen or a recombinant protein, you are saying that a host will them self produce this antigen. And, this antigen will stay there for quite a bit of while because the host cell as long as there is going to continually produce it. And, then your antigen, you do not have to deliver externally or multiple times in this case.

So, the whole concept is you go to the hospital, you get a vaccine which is nothing, but gene that is encoding an antigen. So, this could be in a particle form or your cells do get transfected with the gene, your cells once it is transfected they will then secrete out these antigens. So, these are all antigens either secreted or intracellular and then the immune system will come and recognize this as a pathogenic gene that is present and will mount an immune response.

(Refer Slide Time: 06:07)



So, let us look at some of the players. So, you are looking at some cell that is producing the antigen and then you are also looking at antigen presenting cells and that are going to process and present it to the immune system. So, you can deliver to both type of cells, it just depends on the application. Once it is there now your antigen is being produced in the cytoplasm. So, that is the that is the major critical thing here, that we are looking to get the antigen to cytoplasm. And, why would we want that? So, if you remember from our immune module we were saying that they could be two types of responses the class 1 and class 2; one is where you are taking things from outside.

So, let us say this is a particle, this gets taken up into an endosome, gets degraded into the endosome, has whatever antigen it has, that is get presented then to the cell membrane. And, then another class is that somehow your antigen is able to go into the cytoplasm. Once it is in the cytoplasm then there is a different receptor that takes it up in the cytoplasm. Let us say here is your protein again gets degraded by the proteases, gets loaded onto these receptors that are expressed also and are in the cytoplasm and are going to the membrane and that way you will trigger a different type of immunity.

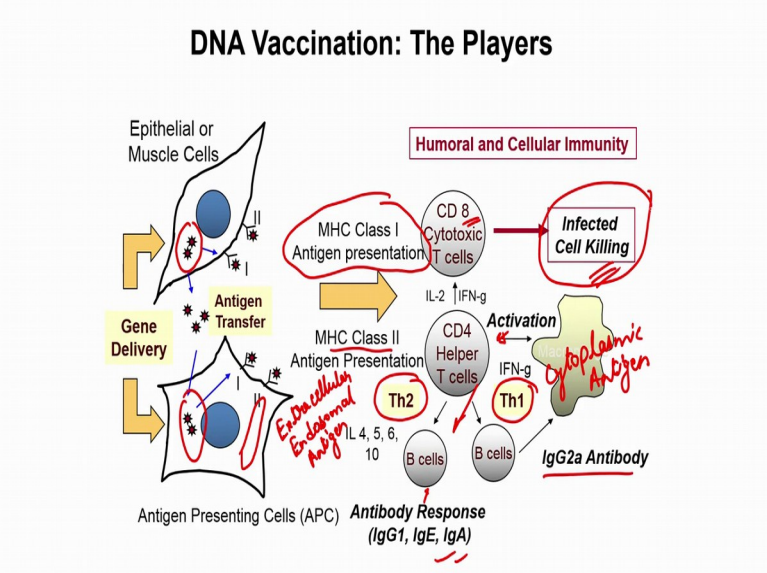
So, its it is fairly easy to get to this from an external system because, as I said most cells will try to take a particles or any external let us say pathogen that you are giving them. But, then it is very hard to go towards this route and that is because it is very hard to deliver these things to the cytoplasm. So, but then one thing that the gene does is if it is a

if its a gene that is present inside a cell and the cell is expressing that gene, then those gene will result in RNA that is could not go into the cytoplasm and then cause the production in the cytoplasm.

So, that is the critical step here and that is why gene or DNA vaccination is something that has got hold off because, that is one easy way that you can get quite a lot of your protein in the cytoplasm; get it through the pathway, the class 1 pathway here and that way you can then trigger the immunity for intracellular pathogens right. So, if it is a pathogen that is intracellular you expect the pathogen to have all its protein, all its antigen in the cytoplasm and that is what you are trying to mimic here.

So, once this is then if its if it is not the immune cell, that you have transfected you can have these antigens go and transfer to the immune cells.

(Refer Slide Time: 09:27)

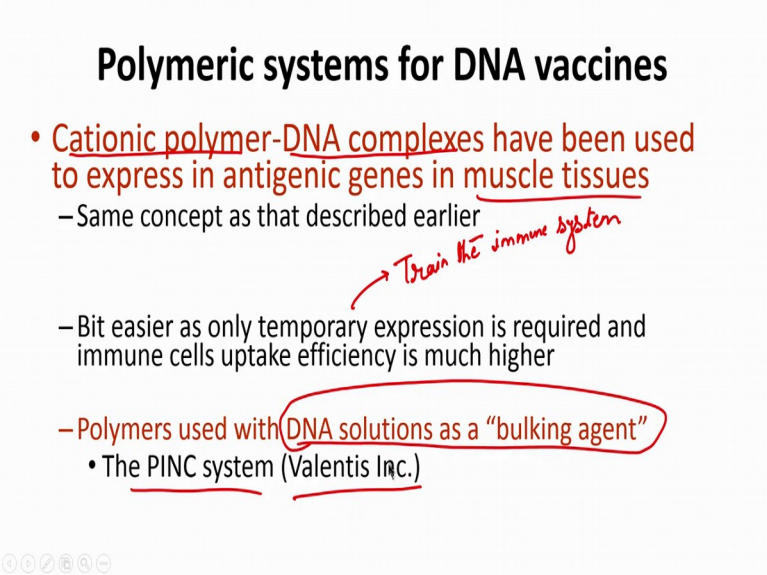


So, you can get both the humoral as well as the cellular immunity rights, if the if the presentation is through the MHC class I as you just mentioned you will go ahead and get to CD8 cytotoxic T cell response. If the antigen is to the class II which means through the endosomal route you will get the CD4 helper cell as well as the B cell response. So, CD4 helper cells will go towards the Th2, will activate the B cells you will get a strong antibody response. You can also go Th1 and you get a different type of antibody. So, these are two different types, so T helper cells 1 and T helper cells 2.

So, depending on which one you are activating. So, one gets activated through an external antigen, one gets activated through a cytoplasmic antigen. So, this is for the cytoplasmic antigen and this one is for the in general I mean; obviously, there is a little bit of crosstalk in all of this. But, this is what the general accepted norm is then this is for the extra cellular or endosomal pathogen or antigen. So, depending on all this you will get different types of response. And, basically you will need the activation of your immune cells the CD8 will directly kill the infected cells.

So, if it is more cytoplasmic this is where and this will go, leads to the death of the cell. So, one thing to note here is that eventually all of your cells that are producing this antigen may also die. And so, it is not going to be throughout your life, but it is going to sustain for a little bit till the immune system is actually become active against that antigen. And, that is all you want from a vaccine anyways to for immune system to be able to recognize it, store it in its memory bank and be able to kill it next time it sees it.

(Refer Slide Time: 11:55)

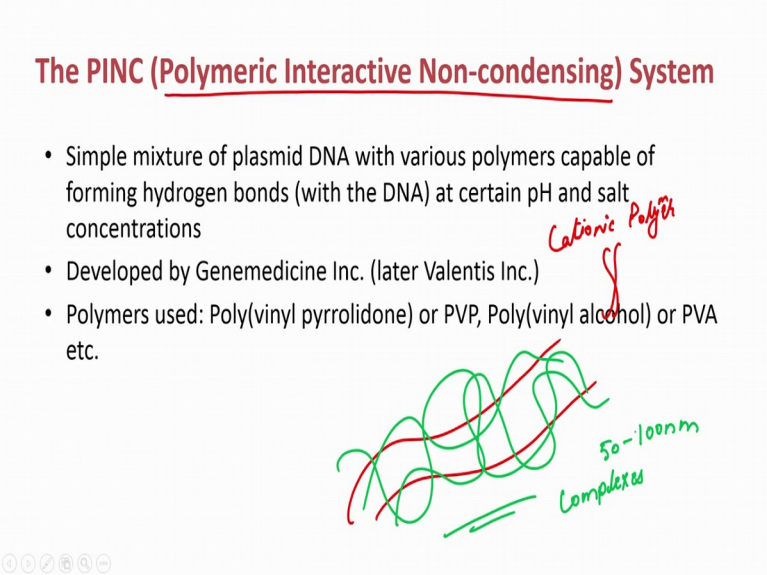


So, what about polymeric systems that we can use for such DNA vaccines. So, again very similar to what we already discussed in the last class. So, these are going to be cationic polymer, you are going to make the DNA complexes with this cationic polymer. And, then you can use this to express genes in any target tissue, muscle tissues a very similar concept to what was described in the previous class. It is bit easier because, in

this case you are only as I said you are only looking for expression, so that it last for let us say 20 days or a month.

And, then you do not really want it to express you actually would like it not to express at that point unlike in the previous gene therapy, where you in some circumstances you want the expression to be there for life. In this case you only want it to be enough so, that its able to train the immune system and once the immune system is trained you do not really want the expression to be there anymore. And, there are other systems as well polymeric systems that you can use, one of them is a PINC system developed by this company. And, in this the polymers are acting as a bulking agent rather than as making complexes and all and those can also be used for such application.

(Refer Slide Time: 13:31)



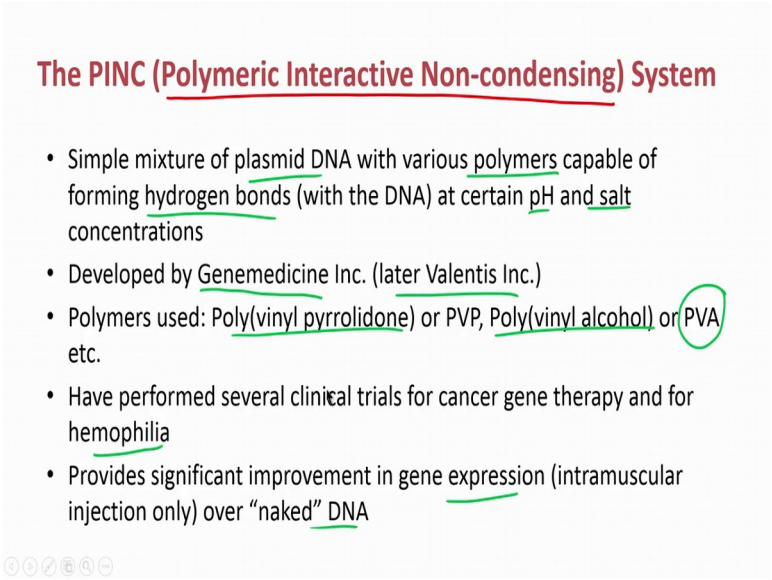
So, let us talk about this PINC system. So, PINC is nothing, but a Polymeric Interactive Non-condensing system. So, so far what we had talked about was a cationic polymer forming complexes with your DNA. So, let us say this was your polymer, in this case what you were getting is the DNA complexes which were entwined in all this. And, resulting in a particle which is anywhere between 50 to 100 nanometer, or at least that was a target, but in this system you are not looking to get these complexes.

So, these are complexes; in the PINC system you do not want complexes, what you are doing is using polymer as more of a bulking agent. So, it is just a simple mixture of your plasmid DNA with various PINC polymers and this is these polymers are capable of

forming hydrogen bonds. So, these are weak bonds at a certain pH and salt concentration. And, as the pH and the salt concentration will change these hydrogen bond will also cease to exist.

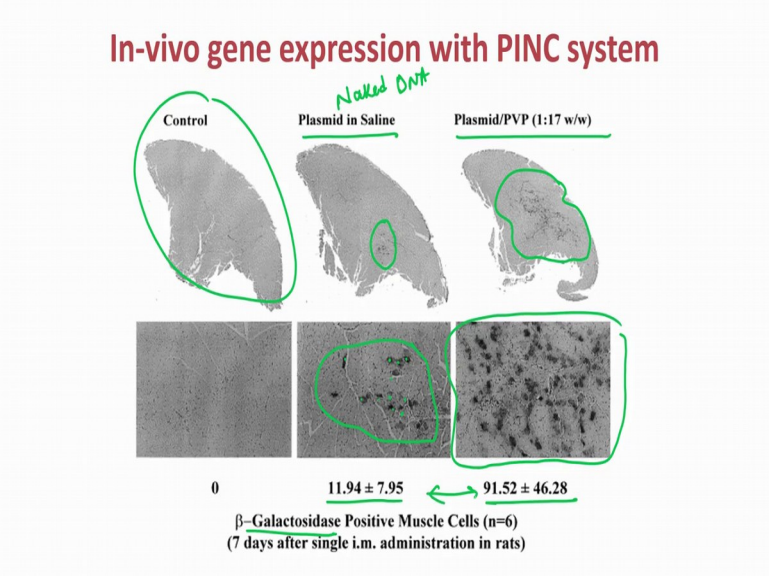
So, as I said this is a developed by this company called Gene medicine which was later known as Valentis and the polymers ever used here was polyvinyl pyrrolidone, polyvinyl alcohol or PVA. So, these polymers were widely used for this and what they were doing is they are not heavily ionically charged, but they can form hydrogen bonds with your DNA molecule.

(Refer Slide Time: 15:17)



And have performed actually several clinical trials for cancer gene therapy with this as well as for hemophilia and they do provide significant improvement in gene expression over the naked DNA. However, not as much as some of the other complexes that can provide and so, it is not being used quite a lot in research phase at this point, but they do have a good history of use in several clinical trials.

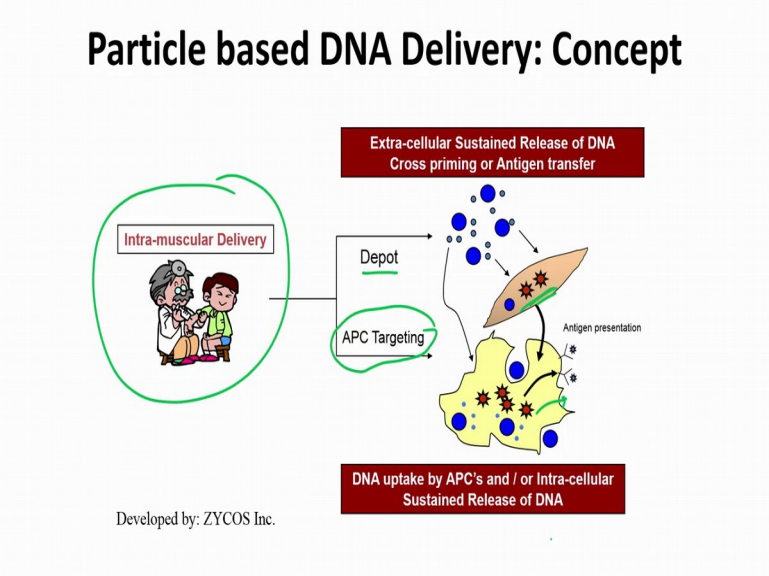
(Refer Slide Time: 15:47)



So, here is some example. So, here you have a control muscle that you are looking at in this case you are delivering beta galactosidase gene and so this is just if you deliver naked DNA. So, plasmid in saline and you are looking for beta galactosidase positives muscle cell using some enzyme. So, that is going to cause these black precipitates to be present. So, you see that if you are using just saline you do get some regions where you do see this and you can zoom into that. And, you can see and there are some cells that have taken up this and actually expressing this enzyme.

However, when you use your this PINC system with the plasmid and PVP at a certain ratio you find that quite a lot of this is actually being expressed in these muscle cells. And the reason for that is PINC system is first of all acting the bulking agent, so maybe it will still trigger some endocytosis and it also protects the DNA little bit from the external environment and this is for the quantified. So, you almost see a 10-fold increase compared to just the naked DNA.

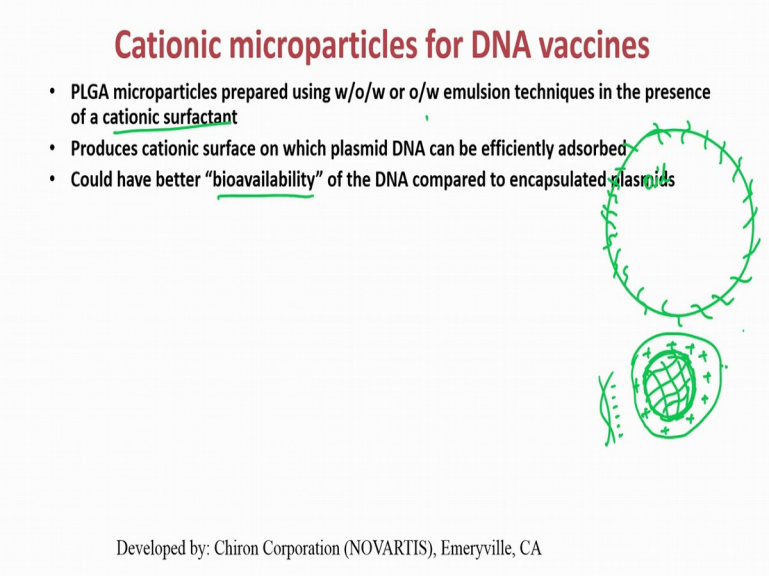
(Refer Slide Time: 17:07)



So, what about using particles for this DNA delivery for vaccines? So, again it is very similar system that we have talked about. So, you are using some intramuscular delivery. So, you go to the clinic get that, you can then either form a depot somewhere where the immune system can come in and start taking up these particles or releasing these DNA. Or, you can directly have it such that they go inside these APC's - Antigen Presenting Cell.

So, what you see here is that, you are now getting your antigen in these local cells and this is being expressed in cytoplasm or in the endosomes and getting presented out for rest of the immune system to take care of .

(Refer Slide Time: 18:05)



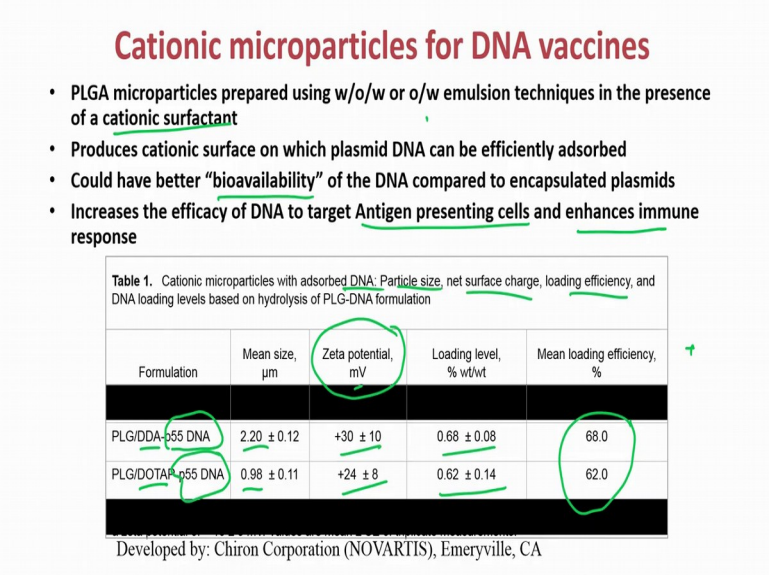
So, again we have talked about PINC system and the cationic micro particles for normal gene therapy. In this case we are also going to look at for DNA vaccines. So, PLGA particles again I used for this, you can make PLGA particles either by double emulsion or by single emulsion oil and water, and you can add some cationic surfactant. So, that what will what will that do? If you have surfactant it is going to, let us say this was a droplet let us say oil in water. And, if you have surfactants here what you will find, if the surfactant since its amphiphilic it is going to line up at that interface.

And, because it is lining up at the interface when this oil evaporates in the particle forms the eventual particle will also have the surfactant layer on that surface and if the surfactants are cationic, then you end up being with a cationic polymer. And obviously, the PLGA chains are here. So, this is now positively charged, so if you mix it with the DNA what will happen is the DNA, since is negatively charged will ionically interact and deposit on the surface. And, then you can now use this particle to deliver it to various cells based on the size as well as it may also show endosomal escape because, the presence of these cationic surfactant and you can then use this for gene therapy for vaccines.

So, as I said this produces cationic surface on which plasmid DNA can be efficiently adsorbed. So, this process here this could have a better viability of the DNA compared to the bioavailability of the DNA compared to the encapsulated plasmid. So, because at this

point it is easier for the DNA to come out immediately right because, otherwise what will happen is you would have to wait for the particle to degrade before it comes out. But, if it is on the surface the moment the dielectric outside changes or little bit of the particle degrades, the DNA will come out.

(Refer Slide Time: 20:33)



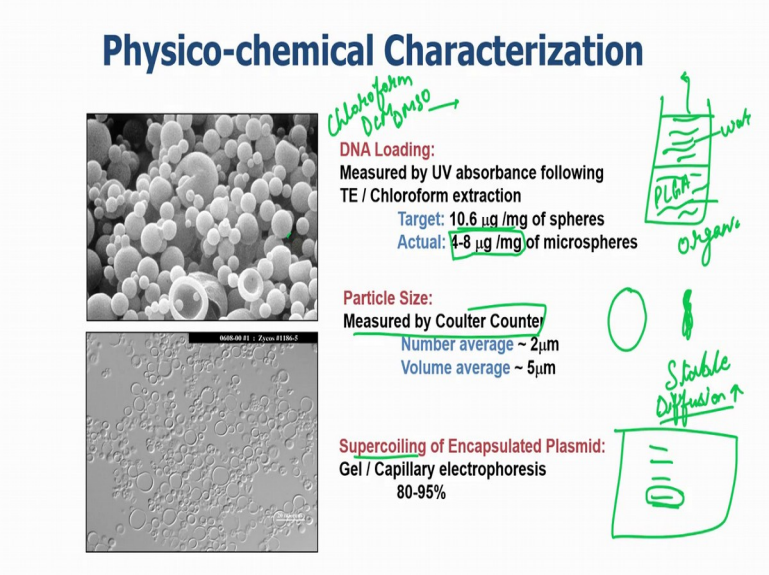
And, increases the efficacy of the DNA to target the antigen presenting cell and that is because now you have put it in a particle formulation. So, these APC's are very good in taking up anything foreign and especially particulate formulation. So, this is going to enhance the immune response and here is just some example again from some literature.

So, you are looking at cationic micro particles with absorbed DNA and at this point you are looking at various things such as particle size, the net surface charge, how much DNA you can load onto that and quantification of that. So, you can see, so in this case they have used two different cationic surfactants GDA and DOTAP, these are lipids and then they have mixed it with the p55 DNA.

So, what they see is they get different sizes. So, one is about 2.2 micron and another is 0.98 micron, you can run a technique called zeta potential. It is a technique to measure the surface charge of the particle and you see that indeed because of the presence of these cationic surfactant you do get fairly high milli volt surface charge. And, then you can then load the DNA and use fine actually you can load quite a bit of it. So, you can

load about 0.68 percent DNA on the surface and get fairly high loading efficiency as well. So, these systems can then be used further to enhance the DNA vaccines.

(Refer Slide Time: 22:11)



So, once you form these particles you can do some physico chemical characterization. So, you can do a simple SEM image and get the sizes and the morphology of this, you can do microscopy just normal light microscopy to further get the sizes. Then you can measure the DNA loading. How you can measure the DNA loading? You can dissolve these particles; so, all of these particles are soluble in let us say chloroform since PLGA is soluble in chloroform or DCM, DMSO also.

So, you can then solubilize it and maybe have some portion. So, what you can do is you can take it in a tube with DCM in water and once you do that you can put the particles and shake it. So, what will happen let us say if this is the organic phase and this is the water phase, then what will happen the PLGA which was in a particular form we will just open up in the organic phase and all the polymers will be present here whereas, DNA is a fairly hydrophilic molecule. So, it will localize in the water phase and then you can test this out with various absorbance assays.

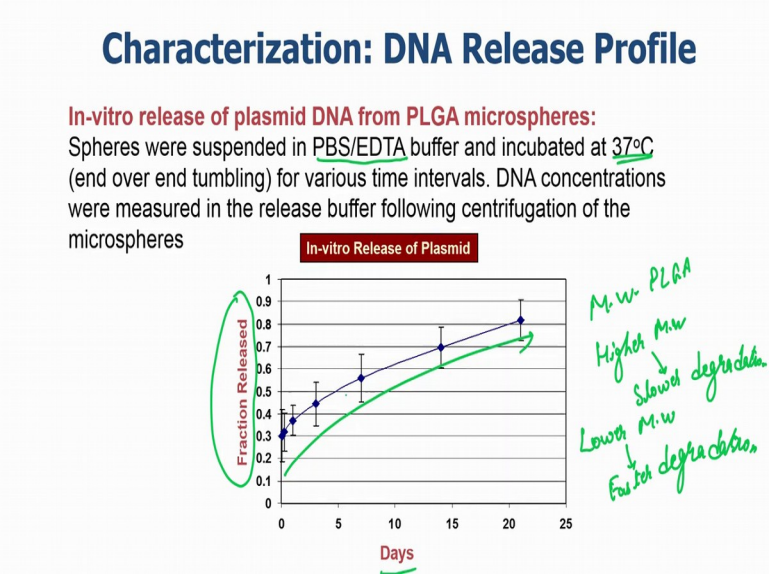
So, DNA has absorbance at a certain wavelength. So, then you can take it out and then you can estimate how much you have been able to load on the onto the particles. So, in this case ,they were targeting 10.6 they got about actually 4 to 8, but that will help you in designing how much those you want to give to the animal and the patients and depending

on how much loading you have. Particle size you can measure either by these techniques or there are other techniques as well such as coulter counter which is a light based technique. And, then you can you can see whether your plasmid is actually in a super coiled phase.

So, what that means, is instead of having a plasmid just like this its super coiled. So, at the super coiled phase it is much more stable and it is much more smaller. So, this diffusion is higher and see you can test that whether your plasmid is getting to the super coiled phase because, of all the synthesis process or not by running a gel electrophoresis.

So, if you run it you will see different bands at different places and since I said the diffusion and the movement of the super coiled is going to be higher. So, it is going to go further down compared to your plasmid that you can known as a control. So, this is just some characterization techniques; we have talked about some of these already during our particle module in this course.

(Refer Slide Time: 24:57)



And then you can see how it releases DNA overtime. So, you can take these PLGA microspheres loaded with the plasmid DNA. You can suspended in some physiological ionic concentration of PBS or EDTA and then incubate at 37 degree Celsius and let it sit there for some time. And, then what you can do from time to time you can collect the supernatant, see how much of the DNA is present in that supernatant. So, that is going to

tell you that how much of DNA has been released out from these particles and that is what they have done here.

So, this is the fraction of the total that was released. So, they first calculate the total by the method I just described and then you can collect the supernatant from the released PLGA in saline and what you see is over time you get a fairly steady release of your DNA. And so, depending on if you wanted to be faster release, what you can do? You can then change the molecular weight of your PLGA; so, higher the molecular weight, would lead to slower degradation.

So, depending on your application let us say if you want this to go on for months you will increase the molecular weight. And, similarly the lower molecular weight PLGA will go towards faster degradation. So, if you want this DNA to release much much faster you can then significantly reduce the molecular weight and that will cause the DNA to come out much quickly and then here is the immune response data. So, again this is what you are trying to see is that whether this enhances the immune response.

(Refer Slide Time: 26:51)

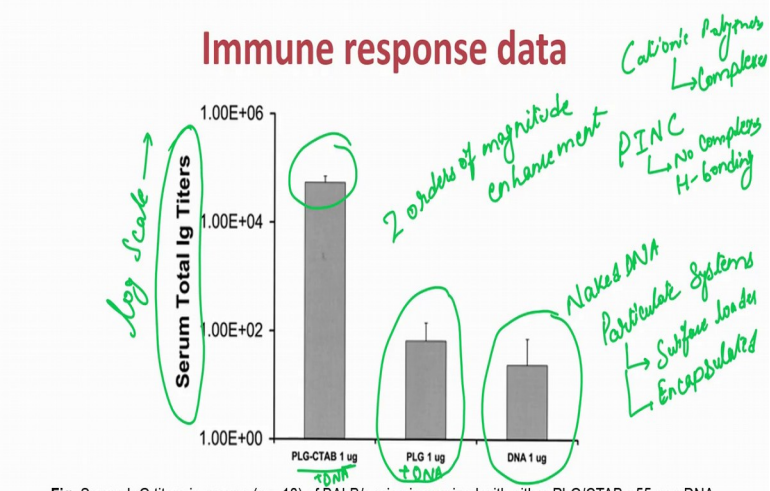


Fig. Serum IgG titers in groups (n = 10) of BALB/c mice immunized with either PLG/CTAB-p55 gag DNA, blank PLG microparticles + DNA, or DNA alone at 1 μg dose. Antibody titers are geometric mean titers ± SE at 2 weeks post-second immunization (week 6) time point. The response from the PLG/CTAB-p55 DNA was significantly higher (P < 0.05) than other groups.

So, if you give just the DNA by itself. So, this is again naked DNA, you see some amount of antibodies being produced against your antigen that is being coded by this DNA. If you give just the PLGA particle without the cationic surfactant; so, this is PLGA plus DNA, you see a little bit enhancement, but not really even close to what you want. I mean remember this is log scale and if you put this cationic surfactant and then

load then you can load a lot more DNA and you can see it also reserves in almost 2 orders of magnitude enhancement in your antibody.

So, that is what is more desirable. So, this is for the same amount of DNA that was given which is 1 microgram. So, this is how you can use it through particles as well. So, we have now talked about three systems, we have talked about cationic polymers, these form complexes. We have talked about PINC system, which is a non-condensing based system and these do not form complexes. This is mainly hydrogen bonding and then we have talked about this particulate system; so, surface loading or encapsulation. So, these can be PLGA based or some other polymer, but they are either surface loaded or encapsulated.

And, all three systems can be used and depending on what your application is and what your requirements are, you can use any of these three systems for your delivery. We will stop here and we will continue rest in the next class.