Drug Delivery Principles and Engineering
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Lecture – 61
Targeted Drug Delivery System

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

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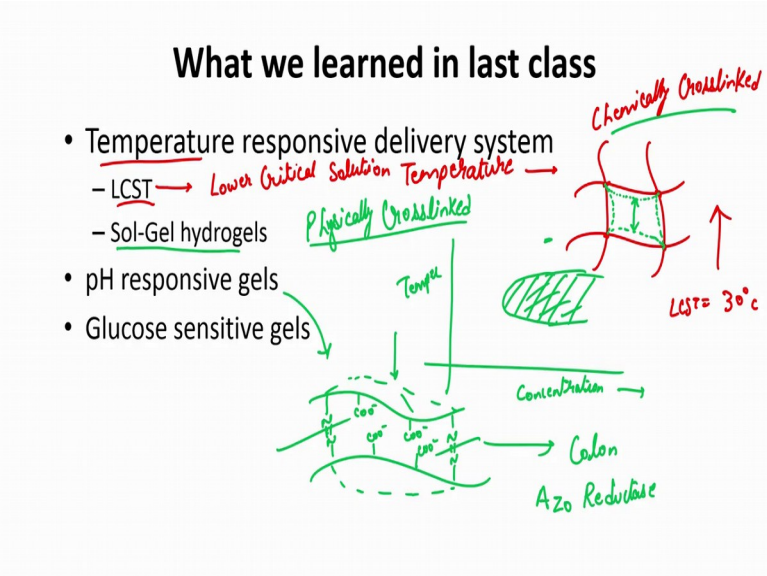

We have been discussing about how to go about and use responsive systems, so this is a quick recap of what we learned in the last class. In the last class we started our discussion on various responsive delivery systems particularly the temperature responsive delivery system and so what that entails. So, in temperature we first looked at LCST exhibiting polymers, so this is nothing but Lower Critical Solution Temperature.

And what this says is typically you will find that most things become more soluble as you heat them up whether it is some protein, whether it is some polymers. But, there are some unique type of polymers that actually show the reverse property where if you heat them up they become more hydrophobic and they actually become less soluble. So, such polymers are called LCST polymer and the temperature at which this switch between the properties happen is called LCST.

And what we found here that , if you let us say make a hydrogel out of such polymers which is let us say chemically cross linked. So, all of these places are fixed, but the polymer itself will change property as a temperature changes. So, let us say if we now heat it up above the LCST. So, for example, let us say the LCST for this particular polymer system is about 30 degree celsius. So, at room temperature it is fairly well soluble it is fairly hydrophilic. So, it will have a certain swelling ratio it will interact with water molecules around it and will swell.

But once you let us say go to 37 degree celsius which is a body temperature what will happen is this is going to become hydrophobic and it will not try to have interactions with water. So, it will then try to have interaction with other chains which are also hydrophobic. I n that case what will happen is these polymer chains will basically repel water and try to come close to each other.

Now, well the since they are cross linked at this at these points and they cannot leave those points, so basically there pore size is going to shrink. As a that is that is what the LCST is and we discussed how this can be done. Similar very similar thing a variation of this is sol-gel hydrogels which are nothing but instead of being chemically cross linked they are physically cross linked and that means, that now these chains can move around. But then if they are becoming hydrophobic they may not actually form any kind of gel , what will happen in these cases is if you have a polymer it is going to show a phasic diagram which is something like this where you have concentration on the x axis and the temperature on the y axis. So, only within this area will this be gel anything above or below it will not gel. And so, that is because the chains will be too soluble at a higher temperature, but then if the temperatures in this range they will have enough of hydrophobicity. So, actually does a physical crosslinked gel

Then we also talked about pH sponsored gel which we have been talking about throughout this course, but we looked at some more variations to that. So, how you can vary the pH and have more swelling or less swelling and depending on that another applications here we looked at an example where we had a colon specific delivery. So, we had polymer chains that were cross linked within Azo bond and these polymer chains themselves had lots of COOH group.

So, where this is the case what will happen is as it is travelling through let us say the stomach these polymer chains are non-ionic, because they are already protonated there carboxyl is not going to lose it is proton and they have a certain pore size. So, maybe your drug is in here which is encapsulated as they go to a higher pH in colon. Now, what is going to happen is these all are going to become charged, so all of these will become charged as a result then started to electrostatic repel each other and the pore size will increase.

So, this is basically going to become like this and not only that there is a lot of microbiota in our colon which has Azo reductase as an enzyme that is being produced and that is going to cleave these bonds. So, eventually you will have your drug release in colon instead of your stomach or intestine. Then we also looked at glucose sensitive gels and in these again there could be several variations, but one variation we looked at was a pore that was covered by polymers that were fairly water soluble, so they will extend and not let anything within this device.

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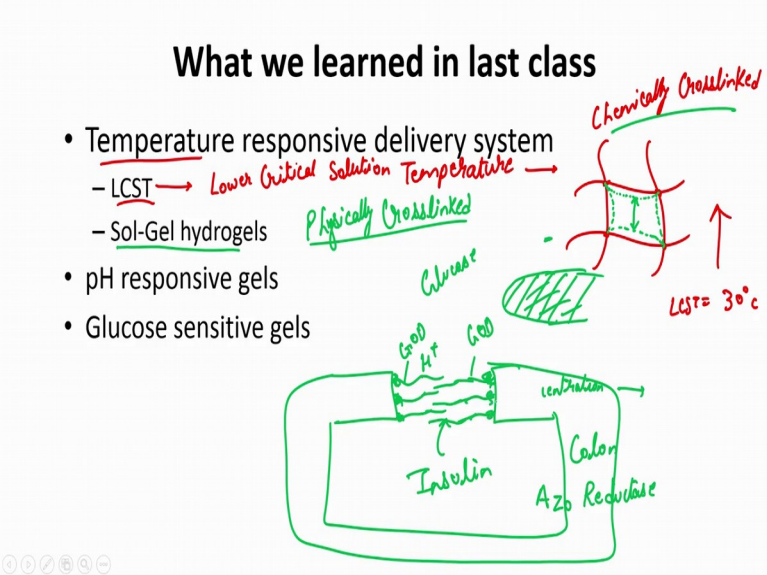

So, let us say if your insulin is here the insulin cannot go there, because these polymers are physically blocking these pores. But then lot of enzyme called GOD: glucose oxidase was stacked to these polymers and whenever enough glucose is present in the surrounding. So, let us say glucose comes this is going to react with this glucose oxidase and produce H plus ions causing a local drop in the pH. These things will change

property with the local drop of pH and will shrink down and essentially become , can find to the surface rather than spreading out, hence opening the pour for the insulin to come out.

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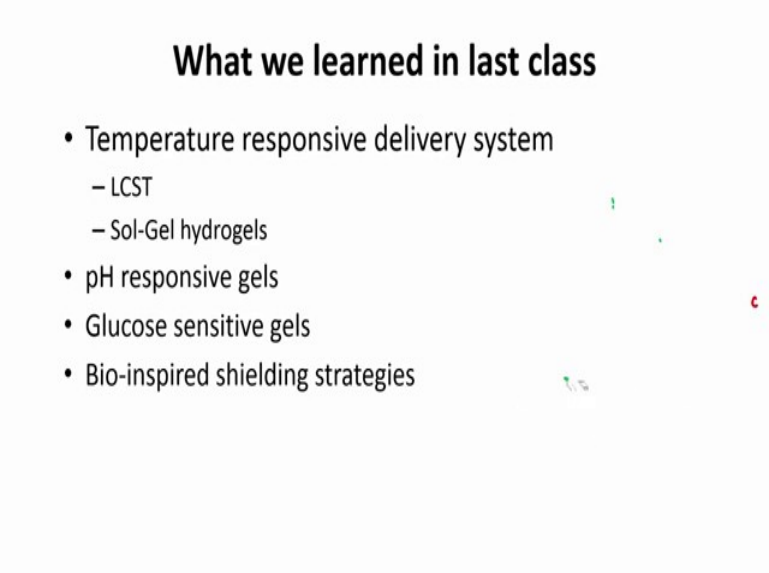

So, these are some of the systems and we discussed in the last class, and then towards the very end we learned about some bio shielding strategies. And so, those were more that let us say we have particles and we do not want our biological system our immune system to detect them. Then what is a way that we can prevent that detection and there are several things that we can do as we have already discussed throughout this course and this is just sort of a repeat of that.

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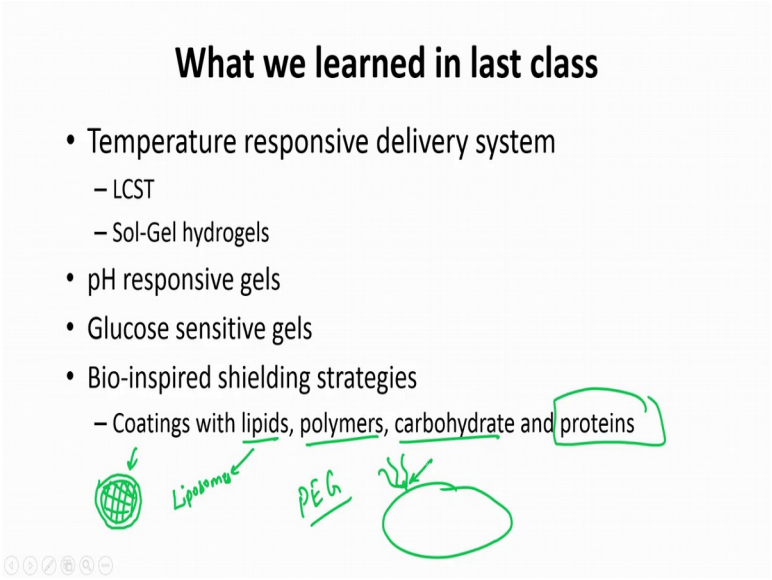

So, one thing that we can do is coat them with lipids. This is something very similar to your liposomes which I have actually made out of a lipid, but even take your polymeric particle. And then you can coat it with this lipid layer which could be made from these lipids and derived from the human sources. And, that will mean that the body will only see this and think that this is something of a self particle similarly you can coat it with polymers.

So, this is few things we talked about those PEG is one of the good polymers that people have been using although we also talked about there are some antibodies that are being detected now against PEG. Similar to the PEG we can use carbohydrates, so this is again similar to what normal cells are our cells have lots of proteins and these proteins are all heavily glycosylated.

So, they also add to the shielding layer, so you can we can use something similar strategy on a particles. And then finally, we can coat them with proteins that are also present on the cell surface to sort of give a signal that this is a self protein or this is a self material.

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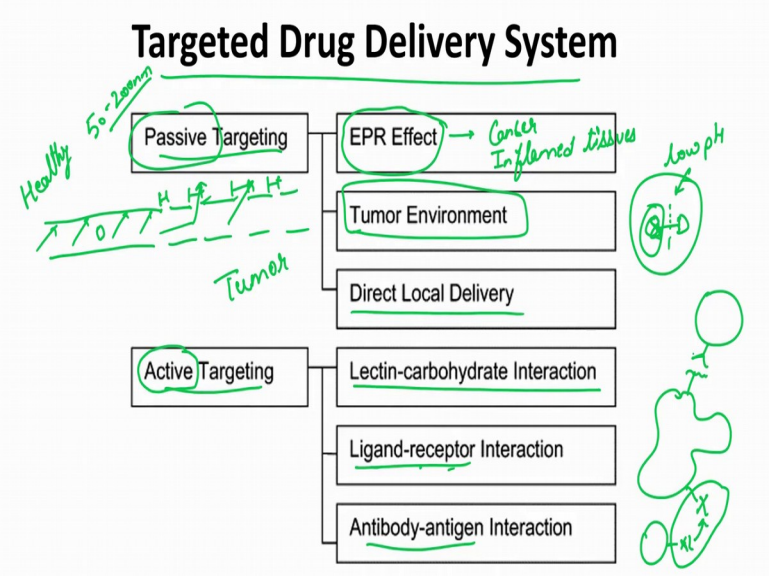

So, we will continue the discussion today in this class and talk about targeted drug delivery system. So, the targeted delivery system can now a divided into two categories, one is passive targeting another is active targeting. So, passive targeting is what we have primarily talked about through this course now EPR effect was one that most targeting to cancer as well as inflamed issues. And the major rationale behind that was that the blood vessels that are present in this inflamed or cancerous tissue are much leakier than the healthy tissues.

So, let us say this is a healthy tissue, and this is a tumor ,then in these regions the blood vessels are leaky just because either they are immature or this lot of inflammation that is causing this drop in the vascular network. So, what you can do is you can make particles that are big enough to come out through these pores. And in that case they will keep on circulating through the healthy vessels because they cannot go through, but as soon as they reach cancerous vessels they can come out.

So, something like a particle which is 50 to 200 nanometer is targeted to the cancer; however, this is a passive targeting because at this point you are not really forming any active born it is not very tumor specific, it is any tissue that is leaky in it is blood vessel will cause accumulation of these particles. So, this is what we call as specific targeting, other things that tumor environment again. So, you can have let us say your drugs or pro drugs which are targeted to let us say a local pH or something like that

So, that is again, let us say if I have a pro drug. Maybe I have this drug which conjugated by some labile bond to this heavy molecule that is protecting this drug from it is activity as well as it is degradation. But then this cleaves at a low pH and we know that tumor environments also have a low pH just because they have a very high metabolic rate. And so, this drug will accumulate everywhere, but in the tumor environment it may break, it may also break in some of other environments. And so, this is how drug is inherently passively targeted to these tumor regions or any region which is low in pH

T hese are all good, but these are again all passive targeting, there is no active targeting involved and I am; obviously, one other method is to use a direct local delivery. So, if it is for lung you directly give it to inhalation if it is directly you want to deliver it to the tumor and you have a big tumor you can directly injected into the tumor itself. So, that is also categorize in the passive targeting.

So, let us look at what is active targeting and as you can already see in the slide, so we have this is a specific to a direct interaction. So, let us say there is a receptor ligand, so we know may be a tumour cell because of it is high metabolic rate and because of whatever mutation they might be expresses a protein x on the surface which is present only in the tumor or at least in much higher amounts in tumor. Then if I then put my particle with the ligand of x, then this is actively target to the tumor because now this is going to go and bind to the tumor cells and the tumor microenvironments.

So, that is active targeting, so it could be on the basis of ligand and receptor. So, this particular example , this could also be that a certain lectin or a carbohydrate is present in the tumor. And, you conjugate your particle with the carbohydrate or the lectin to be able to target it to that tumor cell or it could be any other cell, it does not have to be tumor, but it could be other disease as well and then finally, it could be an antibody antigen interaction.

So, let us say we know that there is an indigent this is very similar to the ligand receptor, but more specific in terms of it is function and role. Let us say if they we know that there is an antigen that is present on the tumor regions or that is present on let us say in a bacterial cell, or in mammalian infected cell. We can then take our particle put these antibodies onto that and these antibodies will then go and bind to this and that will be more active targeting. So, that is the major difference between passive and active.

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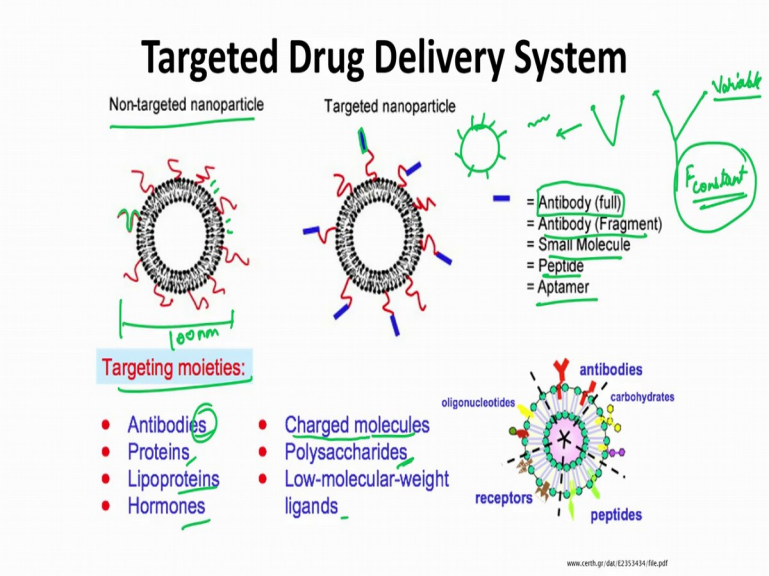

So, there are lots and lots of targeted drug delivering systems that have been tried. So, this is just an example with liposome, but this could be true with any type of particle. So, here is just a normal non targeted particle it is a liposome in this case it is speculated to have an on circulation maybe it is diameter is about 100 nano meter. And, that is what allows it to accumulate in some regions maybe you have certain charge on this as well maybe this is negatively charged maybe this positively charged just depends on what your application and target is.

And, but if you are looking at targeted nano particle what you will do is you can then modify this targeted nano particle in such a way that the exterior surface whether it is the peg chains or is just a particle with the exterior is modified with some target or some ligand that is specific for the disease you are looking at. So, again there are several targeting moieties that one can go through ,n there are antibodies you can use the full antibody.

So, again antibody is also divided into an Fc region, and the variable region; Fc region is basically the constant region. So, which means that this is fairly similar in most types of antibodies, and then variable region is what allows it to bind to different targets. So, this is fairly variable and triple (Refer Time: 15:30) different targets.

So, sometimes since mostly for most targeting specific applications you may only need these regions. So, people are able to even separate out these segments to reduce the

antibody size and not have anything else around, so, you can use antibody fragments as well you can use any small molecule.

So, maybe some cells are heavy in the requirement for glucose. So, you can even put glucose on your particle and then because more and more receptors on this side do bind to it, you can use a small peptide again very similar concept. So, why reduce it to this why cannot we just reduce it to whatever the moiety of the peptide that is present on these surfaces that are responsible for it is recognition. And then there is also another thing called aptamer and we will talk about aptamer in next couple of slides.

So, all of these are targeting moieties that have fairly used. So, antibodies, proteins which are again antibody is the type of protein, you have lipoproteins, hormones, you can use charged molecules. And if you use charged molecule this is technically not an active targeting this is a passive targeting, you can use poly specific polysaccharides some low molecular weight ligands. And again as it is shown pictorially here you can mix and match them you can see in particle again we put with 2 or 3 ligands to confirm much more targeting to such particles.

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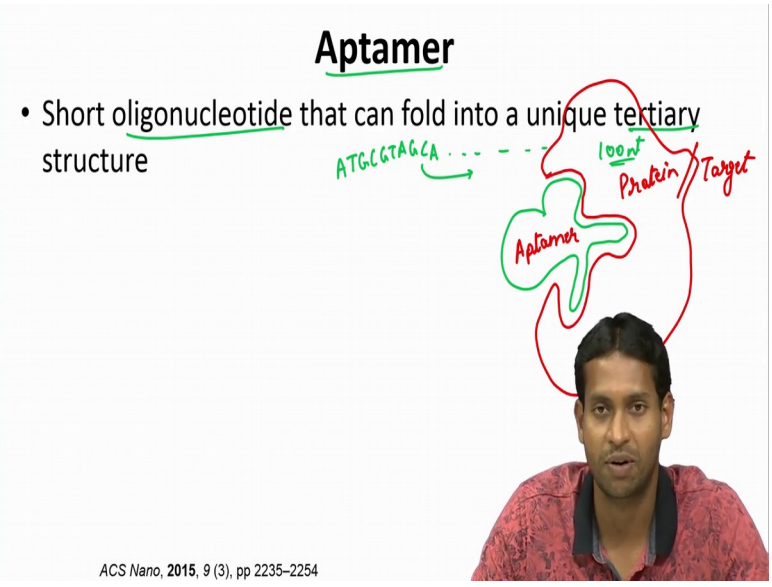

So, as I said aptamer is another class that I will just describe it is not probably as widely known as some of the others. And what does an aptamer? Aptamer is nothing, but is in short oligonucleotide that can fold into a unique tertiary structure. So, we know what oligo nucleotides are.

So, these are just sequences of your nucleotides ATGC and if I arrange them in a certain order such that we have several of them and let us say if this sequence continues to 10 nucleotides. Then this structure itself can roll around and try to minimize the energy and in that process it can form a 3 dimensional tertiary structure which could look like something like this right. And then in many other shapes I mean this is just a random shape that I have drawn.

Now, again this is fairly small and we are talking about 100 nucleotides only, so it is not a huge protein like structure it is a small protein like structure and maybe there is a protein that is complimentary do this. So, maybe I have a protein that looks like this and if that is the case and the aptamer has the sequence complementary or the structure complementary to this protein binding side then what will happen is this aptamer is going to go ahead and bind to it.

So, here is your aptamer is maybe a protein and it may not be protein, it could also be lipid it could also be something else carbohydrates it just depends on where you get a hit, but something like that will then have affinity for your target or I should say protein slash target. So, and this is what it relies on and now the field of DNA synthesis is actually a progressed very rapidly and people have been able to bring the cause down significantly as well as have the throughput very high.

So, if I want a sequence which is 100 nucleotide it is not a problem these days is fairly cheap as well as you can have quite a lot of amount being produced in a very in a very small time. So, it is fairly feasible to make lots of aptamers and lots of types of aptamer. So, let us see how then these help.

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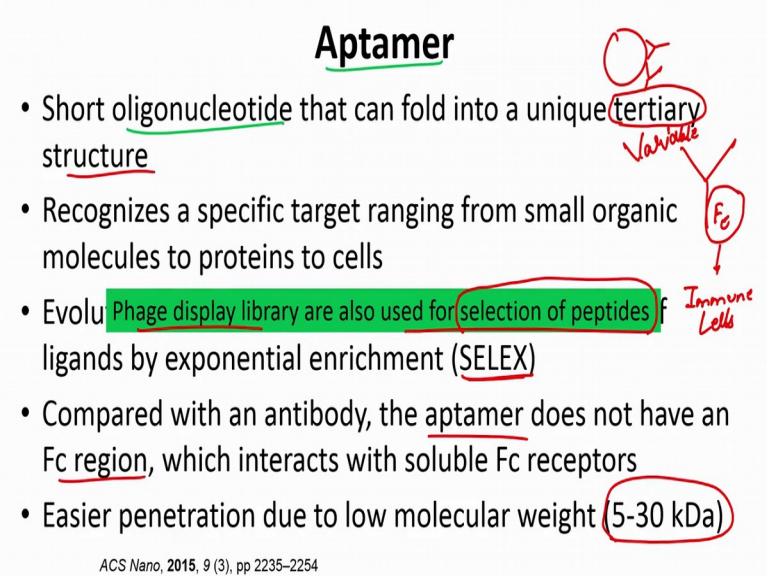

So, as I said they will recognize a specific target ranging from small organic molecules to proteins to even cells sometimes we do not even know what the target is. But, but we know that it binds to certain cell where exactly it is bind to the certain cell may not be known.

And so, on the basis of that people have now come up with a protocol and a method which is called SELEX and this is nothing, but it is a systematic evolution of the ligands by exponential enrichment. And it is an evolutional selection method and we will we will describe it in a moment as to what it is. And one of the advantage with the aptamer compared to an antibody, let us say we do find an aptamer and we will go into how we find an aptamer. But let us say we do find an aptamer which is able to bind to a certain target that you are interested in.

So, then it offers lots of advantages over antibody first is aptamer does not have any Fc region. So, as I described that anti bodies and large molecules and that have an Fc region in a variable region, the basic function of the antibody is to be like a tag for the immune system. So, if an antibody is binding to a certain region or certain protein that acts as the tag for the immune system to remove that because that is something that is considered as foreign. And the way the body does that is through this Fc region, this Fc region has lots of receptors on your immune cells and once these immune cells bind to this antibody through the Fc region, and they will clear it off.

So, the last thing that you want is your therapy that let us say you put you are putting a particle and you have put an antibody that you are delivering it with it to get detected by the immune system and gets cleared off then you are losing lot of you drug lot of a particle formulation. So, that is one advantage first, the second is actually it is quite a low molecular weight. So, we are talking about 5 to 10 kDa, because of which that diffusion limitations are much lower, so remember bigger the size lesser is the diffusion.

So, this can diffuse this can go through the small gaps it can find anywhere and it will have a much larger range of a penetration into various types of organs and cells. And so, in that regard this is much better compared to let us say a 100 kDa, 200 kDa protein molecule like anti body and that will face this challenge. And I just want to point out that this selex method is actually very similar to the phage display library which is used for selection of some peptides.

So, in general when we are talking about aptamer you can actually compare them with small peptides. So, small peptides can also have affinity against a particular target, because of the same region they may acquire a tertiary structure or they may go at a secondary structure that may have affinity either by the structure or just by the interaction of the peptides with another amino acids on new target.

So both of them are similar in terms of their sizes as well as use for targeting. But; obviously, their backbones are very different and aptamer do attain a lot of higher tertiary restructure compared to peptide which usually are linear or secondly structure in nature.

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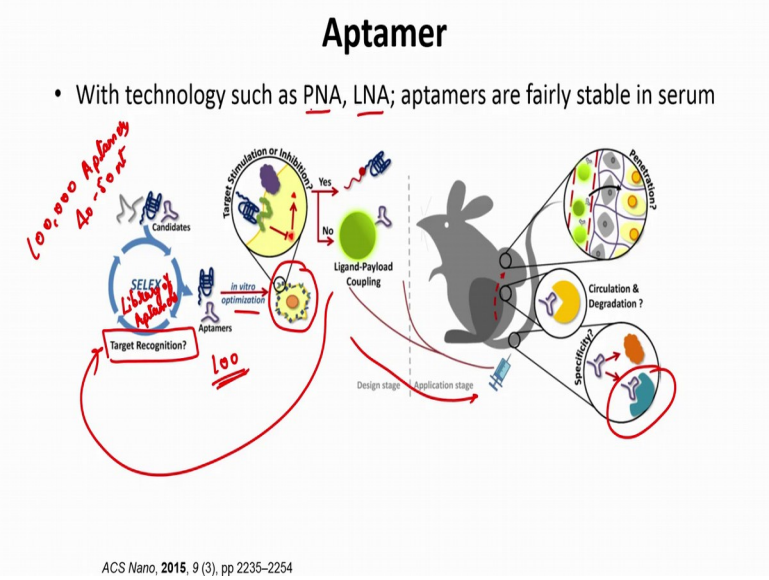

So, let us see how does the SELEX work, so here is a selex. So, what you can do is you can have a library of lots and lots of these aptamers in here. So, let us say this is a library of aptamers, and so you then put your target in that library and screen if anything is binding to your target. So, let us say you started with 100,000 aptamers all different maybe 40mer s or 50 nucleotide long in various permutation combination. And then from there you end up getting maybe let us say 10 of them that that is showing some binding to your target.

And you can test that through various methods you can run it through a phage gel, and if you see a shift in the molecule and shift in where the protein is coming you can say that that protein is now bigger because it is born to some other sequence in this case the aptamer Or, you can stain for nuclear nucleotides on your targeted cell after washing and that may show some affinity there then you do the in vitro optimization. So, you can take this hundred or maybe this is your 10 you can do some binding affinity to figure out which is binding in it a much higher affinity, and you can take them and do your in vitro as says

So, you can see whether you cannot target your payload to yourself what is the mechanism of action if it does target, if the target stimulation or emission whatever you are trying to achieve is happening. Then you can basically take it to your in vivo stage or if it does not happen you can go back to here you can try to screen with some other

library. Once that is the case or if it is happening, but if you are not happy with the binding affinity you can then take that sequence that you know is working in some small amount and you can try to then mutate it rationally one by one to get up much higher affinity.

And then you can go in vivo you can see whether this works or not whether it is not binding to any non-specifically to any other targets which may then cause toxicity and if all of this works then you will essentially have a therapy that this will targeted. And I I also want to point out that the DNA technology has progressed quite a bit and we have now been able to made modifications in your nucleotides.

So, here we have LNA which is a locked nucleic acid or peptide nucleic acid and these are just some variations to nucleotides and they are actually fairly stable in serum. So, even if we inject this in serum you find that they do not degrade they are well tolerated in the body So, this is one way the phage library is very similar also, where you are screening for peptides, instead of selex library, you will have a phage library.

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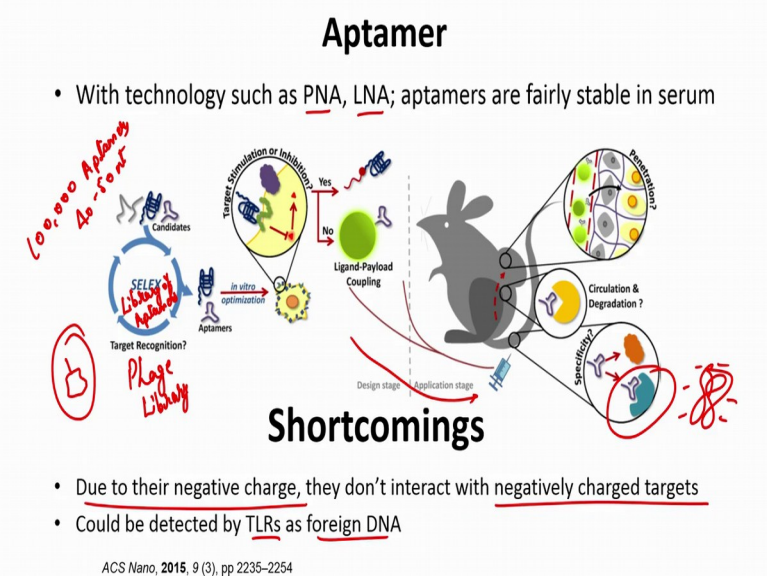

And, it is called phage library because we use bacteriophages to which are small viruses of bacteria. So, let us say this is a bacteriophage and this will display peptides and you see where these peptides are binding using this phage and a very similar method will eventually give you a target through peptides also.

So, what are some of the shortcomings of aptamer, first is of course, that they are negatively charged these are eventually nucleotides and, so the chances of them interacting with your negatively charged targets. So, let us say my protein is negatively charged, so if I have a protein that is bearing negative charge the chances of these aptamer is going to bind to this protein is very low.

So, now, you are basically reduced your targets to half essentially, because if you assume it is all random distribution then nearly half of your routine cannot be a target of this. And then the other problem is that it is actually a foreign DNA because these are DNA that is not what the body is used to. So, it can get recognized by the TLRs or like receptors of the immune system and that may still cause some inflammation as well as rapid clearance.

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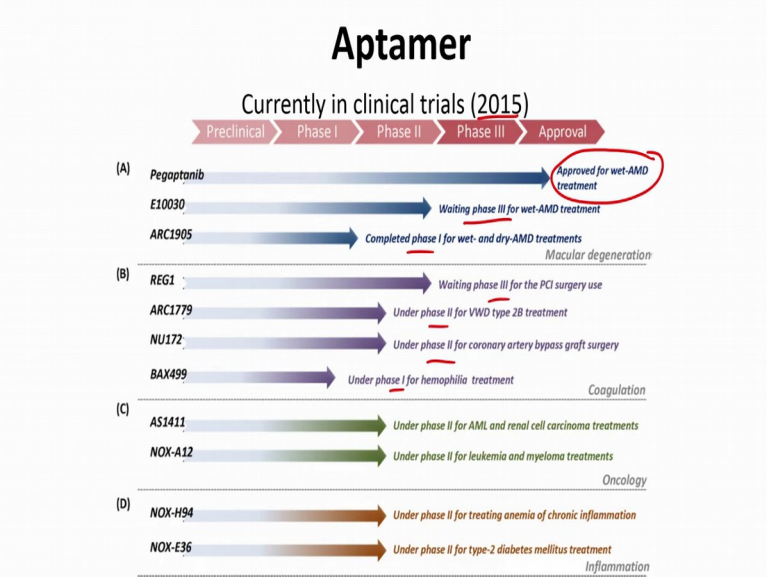

And then lots of aptamers have actually gone through the clinical trials this is this is a 2015 data some of them have actually been approved for certain treatments, lot of them you see is actually in quite a advanced clinical trials. So, there is quite a lot of enthusiasm with the use of aptamers is targeting moieties, and along with everything else there are antibodies which are also in clinics it is a targeting moieties, peptides all of these are fairly widely used. And we will talk about the processes to get clinical approval in our nano toxicology in a part of this course.

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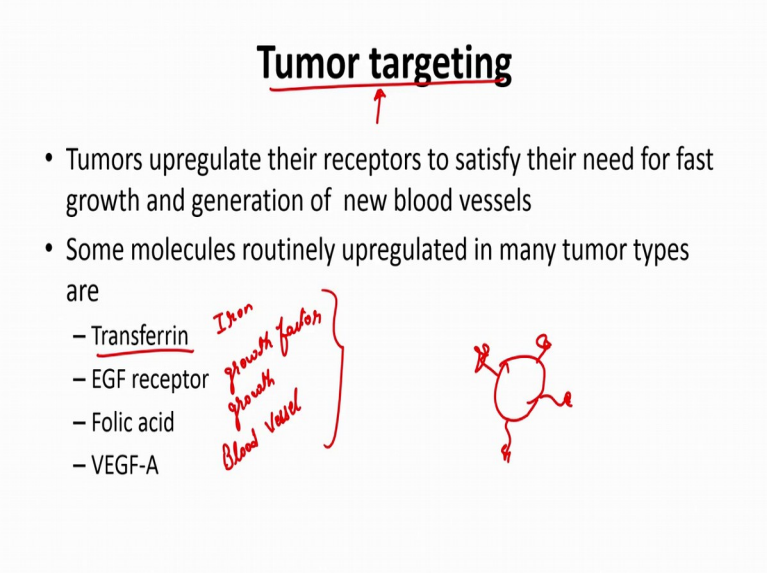

And just very quickly tumour is again one thing that is widely used for targeting basically most of the therapies in research are looking at tumor because it is a major problem. And tumors do up regulate lots of receptors, because they need quite a bit of nutrients for the fast growth and generation of new blood vessels. So, as a result there are several molecules that are routinely up regulated and used for tumor targeting.

So, transferrin is one which is in protein that shuttles iron, you have EGF receptor which is a growth factor receptor, similarly folic acid is another one that is required for growth, VEGF is for blood vessel growth. So, all of these as you can see are quite obvious that tumor may need these two proliferate and grow in size.

And so, because of this you can then design your particles with these targets displayed that are going to go bind specifically to the tumor cell and get internalized, but not to your healthy cells. So, that way you can then have more specific result compared to what you will get without targeting.

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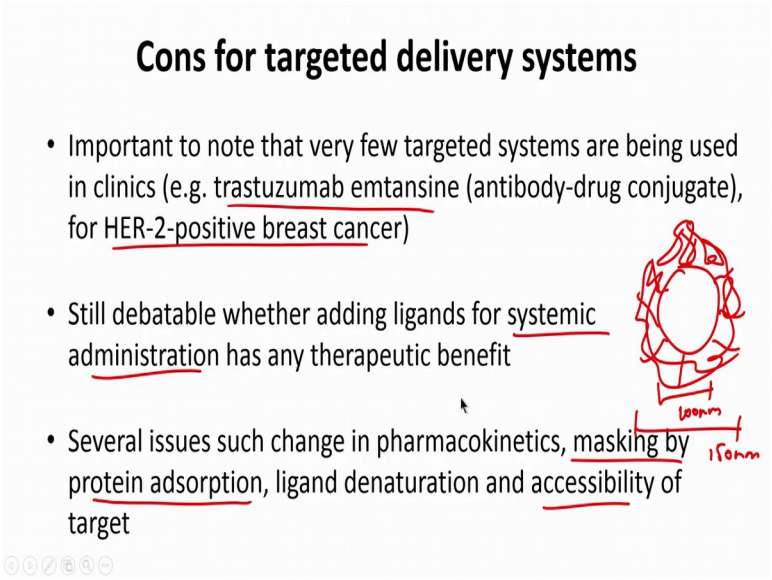

However, there are some cons of targeted delivery systems and some of them are first of all that it is important to note that very few targeted little systems are actually being used in clinic. So, just an example of a target drug delivery we use and again there several of them, but just an example is this particular antibody drug conjugate which is used for breast cancer. But there are not many if you look in the research phage lot of people are using these targeting moieties, but not all of them are being used in the clinics. And the major reason it is still debatable whether adding these ligands actually results in any therapeutic administration or therapeutic benefit when you are giving it systemically.

So, it is not entirely clear there are several issues such as once you add a targeting moiety on your particles you have now increased the size. So, maybe earlier the size was about 100 nanometer, now the sizes become 150 nanometers. So, that causes change in pharmacokinetics you can mask these peptides can get masked by protein adsorption. So, as I said all foreign material that you are going to inject in the body will come in contact with your proteins that are present and they may adsorb on the surface and eventually none of these targeting moieties are actually available.

They may change the type of proteins are absorbing, they may even denature during their journey throughout the body. And their accessibility is also a question mark, so that is why there is lots of cons for targeted delivery systems.

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And this is just a laundry list I just wanted to give it to you there are lots and lots of clinically approved drug delivery systems, again these this is probably not the exhaustive list it is from 2018. So, probably more and more have come out and have been approved, but as you can see even after all this we have been able to as a whole field being able to in actually influence quite a lot of peoples life. Because we have so many systems that are coming into the market which are showing better replicated in the feed drug that is why they are approved and, so this is just a laundry list I wanted to give it to you ok.

So, I think we will stop here and we will continue this further in the next class.

Thank you.