Drug Delivery Principles and Engineering
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Lecture – 62
Targeted Drug Delivery System Research Paper Discussion

Hello everyone, welcome to another lecture of Drug Delivery Engineering and Principles. We have been talking about some of the Targeted Drug deliveryry System in the last class. So, let us do a quick recap of that.

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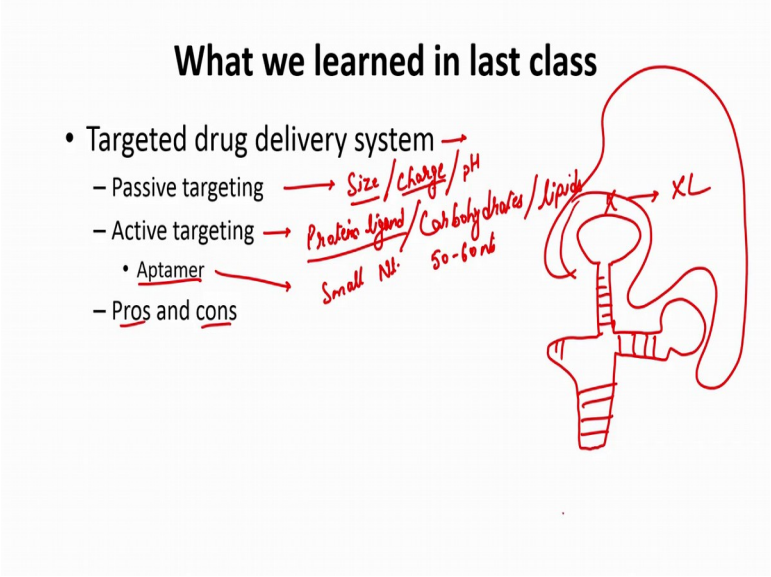

So, in the last class as I said we started talking about targeted drug delivery systems. So, these are systems that are targeted to a certain disease to a certain cell in a certain location in our body.

So, this targeting could be passive targeting, this could be on the basis of their size, their charge. So, we know that anything built above 6 nanometer, 10 nanometer is going to be retained in a body much longer because the kidney can not clear it. We know that EPR effect will cause accumulation of some of these particles between 50 to 200 nanometer range in the tumor, you know anything above 200 nanometer gets filtered out by the spleen. So, we want to target spleen maybe we want to inject something that is of that

size range of 200 to 500 nanometer and similarly to other organs as well lungs and liver also have a certain size ranges that get accumulated in those.

Similarly charge is another factor that can cause targeting to let us say a region which is quite a lot of ECM extracellular matrix which is maybe negatively or positively charged, this could be a property that is may be related to the pH of the local environment. So, all of these are sort of physical factors that can be modulated ,but are not extremely specific to a certain target.

And then we talked about active targeting, which typically involves conjugating some sort of protein, protein ligand, your carbohydrates, as well as lets say lipids and these are specific for a certain receptor. So, unlike this size charge in ph they are sort of nonspecific and different properties lead to their accumulation these are actually specific to a certain receptor. So, protein X is going to bind to it is ligand X ligand similarly carbohydrates and lipids so, that is why they are termed as active targeting.

One of the thing we discussed in a bit of detail, apart from other antibodies and these proteins that can be used is aptamers and these aptamers are nothing, but these are small nucleotide based molecules let us say 50 to 60 nucleotides and they attain a certain tertiary structure. So, maybe something like this is attained due to various bonding that is happening between these nucleotide sequence and maybe this has a binding site just by it is structure. It is tertiary structure to a protein and so, that way you can have a targeting. So, we discussed about aptamer, we discussed how we can find these targets as well as use them in vivo.

Then we discussed pros and cons of these targeted drug delivering system. So, pros are of course, that you make it specific for a certain target and cons are basically what is observed in the literature that not a whole lot of it is actually being used in the clinics. Because maybe the targeted drug delivery systems are not working as well maybe they get marks by the protein adsorbtion maybe they change the form of kinetics completely and it is not going to it is original target.

But whatever is the reason, they are not even the lot of research is being done on it substantially lower percentage of that goes into the clinic compared to non targeted systems ok.

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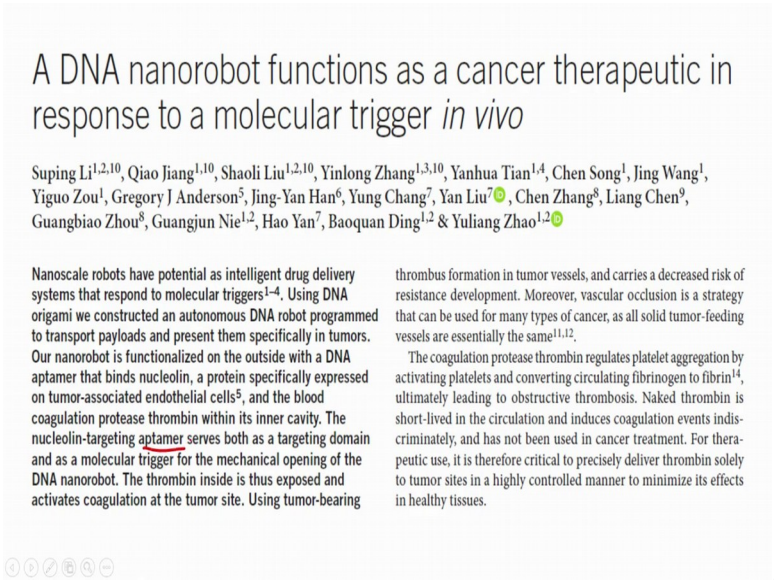

A DNA nanorobot functions as a cancer therapeutic in response to a molecular trigger *in vivo*

Suping Li[1,2,10], Qiao Jiang[1,10], Shaoli Liu[1,2,10], Yinlong Zhang[1,3,10], Yanhua Tian[1,4], Chen Song[1], Jing Wang[1], Yiguo Zou[1], Gregory J Anderson[5], Jing-Yan Han[6], Yung Chang[7], Yan Liu[7], Chen Zhang[8], Liang Chen[9], Guangbiao Zhou[8], Guangjun Nie[1,2], Hao Yan[7], Baoquan Ding[1,2] & Yuliang Zhao[1,2]

Nanoscale robots have potential as intelligent drug delivery systems that respond to molecular triggers[1–4]. Using DNA origami we constructed an autonomous DNA robot programmed to transport payloads and present them specifically in tumors. Our nanorobot is functionalized on the outside with a DNA aptamer that binds nucleolin, a protein specifically expressed on tumor-associated endothelial cells[5], and the blood coagulation protease thrombin within its inner cavity. The nucleolin-targeting aptamer serves both as a targeting domain and as a molecular trigger for the mechanical opening of the DNA nanorobot. The thrombin inside is thus exposed and activates coagulation at the tumor site. Using tumor-bearing thrombus formation in tumor vessels, and carries a decreased risk of resistance development. Moreover, vascular occlusion is a strategy that can be used for many types of cancer, as all solid tumor-feeding vessels are essentially the same[11,12].

The coagulation protease thrombin regulates platelet aggregation by activating platelets and converting circulating fibrinogen to fibrin[14], ultimately leading to obstructive thrombosis. Naked thrombin is short-lived in the circulation and induces coagulation events indiscriminately, and has not been used in cancer treatment. For therapeutic use, it is therefore critical to precisely deliver thrombin solely to tumor sites in a highly controlled manner to minimize its effects in healthy tissues.

So, in this class, we will talk about a paper, we will discuss this paper which is titled a DNA nanorobot functions as a cancer therapeutic in response to molecular trigger in vivo. And this is a paper that looks at this DNA nanotechnology very similar to aptamer, but at a much complex and a much grander level and let us see what it does, it actually also uses aptamer and we will come to that in a moment.

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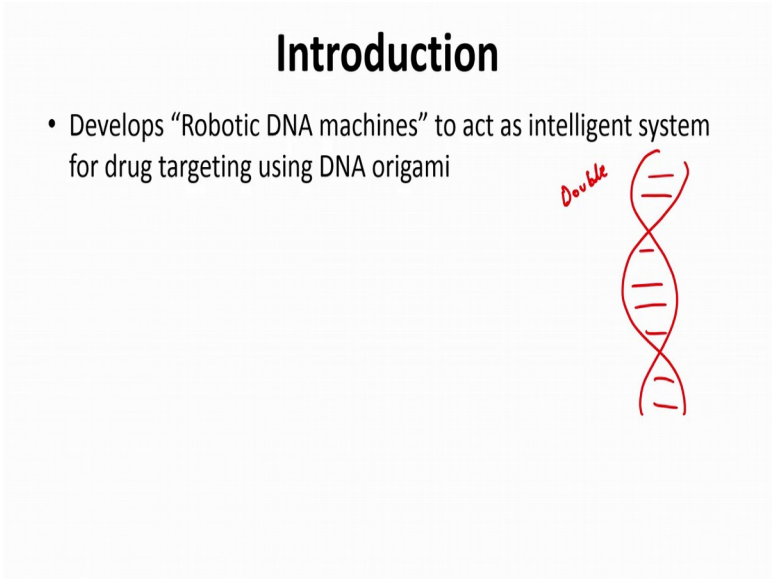

So, this paper is developing a "Robotic DNA nano machines" that act as intelligent system for drug targeting using DNA origami. So, what do you understand by DNA

origami, this is nothing but it is a process in which you try to make complex and diverge shape using DNA. So, so far we have talked about polymeric particles being used to make particles or it could be liposomes lipid bilayer that is present.

Now, we are saying why we only want to stick with synthetic polymers and these like these lipids, we will use DNA to make complex shapes. So, most of the time when you have seen DNA in the literature ,you will find this to be a double helical structure. This is a classic DNA that you will see in the field, but depending on what these nucleotides are so of course, this is double stranded. But let us say if we take single stranded DNA and modify it in such a way that it is pre-programmed to assemble in a certain orientation, then we can make various shapes out of it at least at the nano scale. So, that is what the DNA origami uses.

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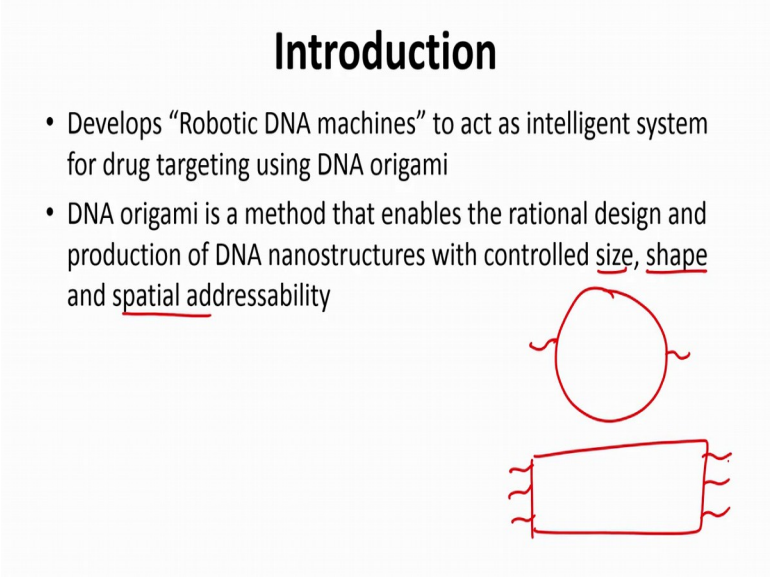

So, as I said DNA origami is a method that enables the rational design in the production of DNA nanostructures with control size shape and spatial addressability. So, you can make various sizes, you can make various shapes, as well as you can have lots of spatial cues on your thing so, not only you can make a sphere you can design it such a way that diametrically opposed it there are DNA overhang and similarly with various shapes also, you can design particles that maybe only on one side, you are having DNA overhang. So, you can give some anisotropy to the system as well.

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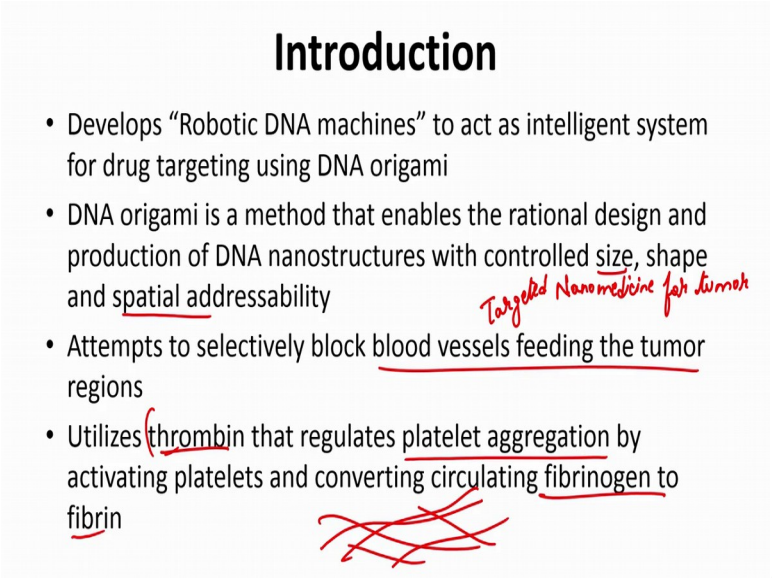

So, in this particular paper they are using this DNA origami to selectively block blood vessels that are feeding tumor regions. So, again this particular paper looks at targeted nano medicine for tumors and let us see let us see how they are doing this. And one other thing that it utilizes is thrombin that up regulates platelet aggregation by activating platelet us and converting circulating fibrinogen to fibrin.

So, we have already talked about thrombin and the blood clotting pathways when we were talking about blood response to materials and we discussed how thrombin which is an enzyme that converts fibrinogen to fibrin which basically forms these long chains of fibers and then they help in clotting the blood if there is any exposure or any injury to the blood. And so, they have used this thrombin to block these blood vessels that are feeding tumor regions and so, how are they able to make it. So, specific is what we will discuss in this paper.

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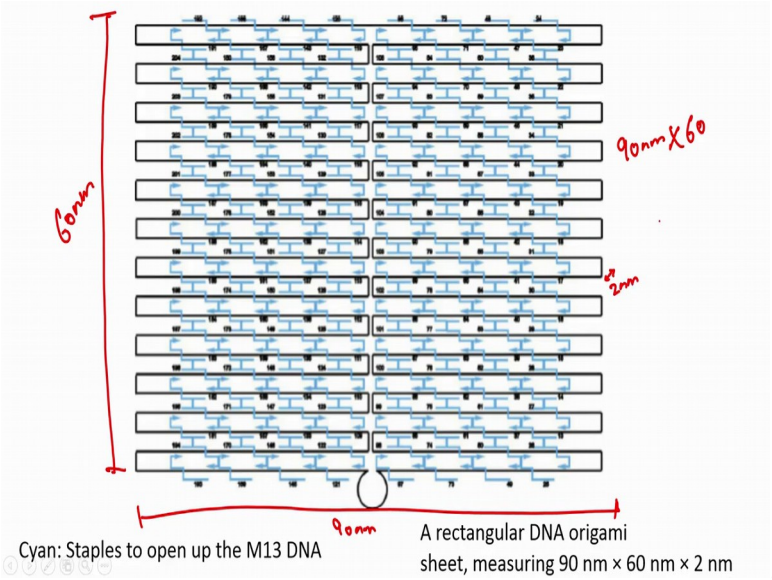

So, here is what they have done. So, they have used DNA origami to make this very large sheet and large is; obviously, a relative term, in this case the sheet is about 90 nanometer to by a 60 nano meter. So, this is 90, this is 60 and this is a sheet so, it is 2 dimensional, but it has a minimal thickness which is about 2 nanometer.

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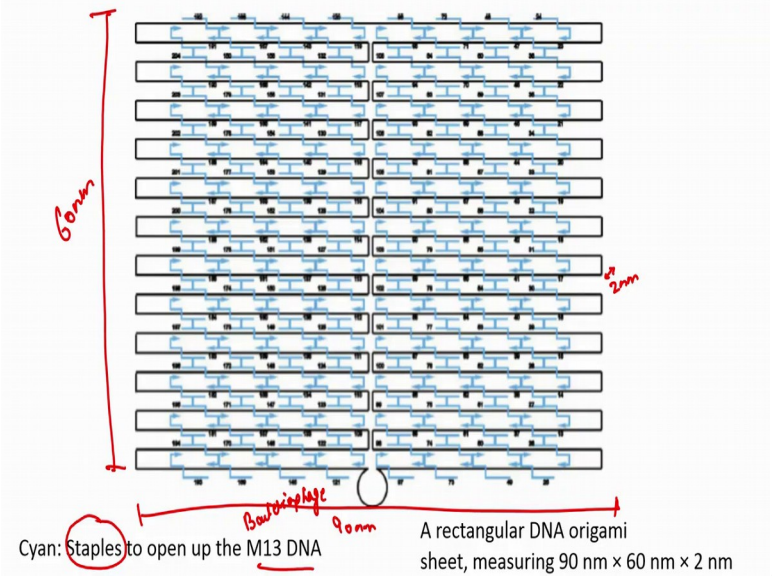

And what they have done is, they have also made these staples which are again made out of DNA itself to open up this m 13 DNA. So, this is M 13 which is a bacteriophage. This is the virus DNA and they have used these staples which have homology to this M 13

DNA and that has just opened it up and actually pinned it down as this sheet. So, that is the original structure that they started with.

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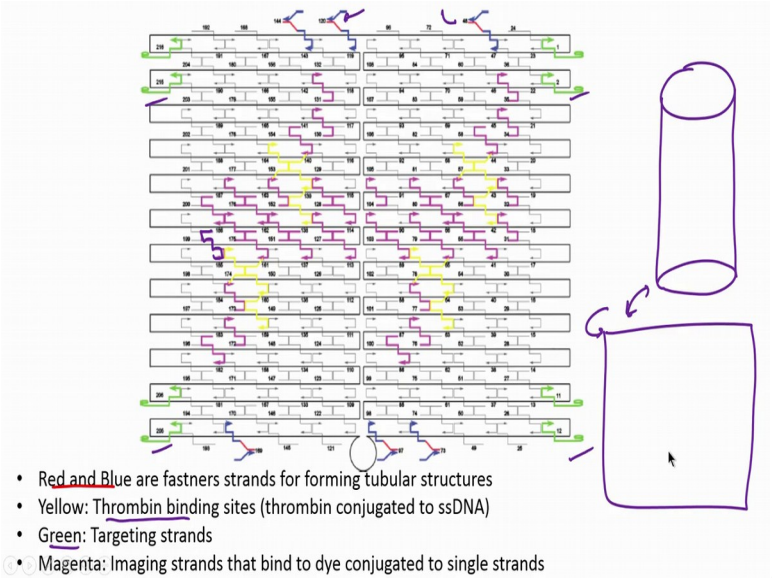

And then what they did is then they modified the structure further. So, again this is the whole sheet you can see all these staples here and there are also numbers to show where is what and what they have done is, they have further modified it by using these red and blue regions. So, let me just change the colour since the red and blue is already there so, maybe I will shift to purple.

So, what they have done is, they have used these red regions as fasteners for forming tubular structure and when I say tubular structure they are actually in their paper trying to roll the sheet up and form a tube. So, basically a structure like this is what they are aiming for. So, if you open it up it is going to become a sheet like this, but if you then roll it up it is going to turn into this tube and. So, they put these red and blue fasteners which have been used to form this tubular structures so, they again have some homology with each other.

The yellow is incorporated which has binding sites for thrombin. So, all these yellow sites that are present they have certain single strand DNA overhang and they have somehow modified thrombin in such a way that the thrombin can bind to these single stranded overhang of this yellow region.

Then what they have done is they have put some green strand as well. So, again these are all DNA sequences which are slightly modified with each other and have very different applications, but all of these green sequences these are there to act as targeting strands. So, they will bind to a certain target which they feel that they can tap into in the tumor in microenvironment.

And finally, the magenta is the imaging strands so, there because it is a research and they want to see what happens to the system. They have also added these magenta strands which are something that are bound to a dye and that can be used to track where this particular a nano sheet or nanotube is going .

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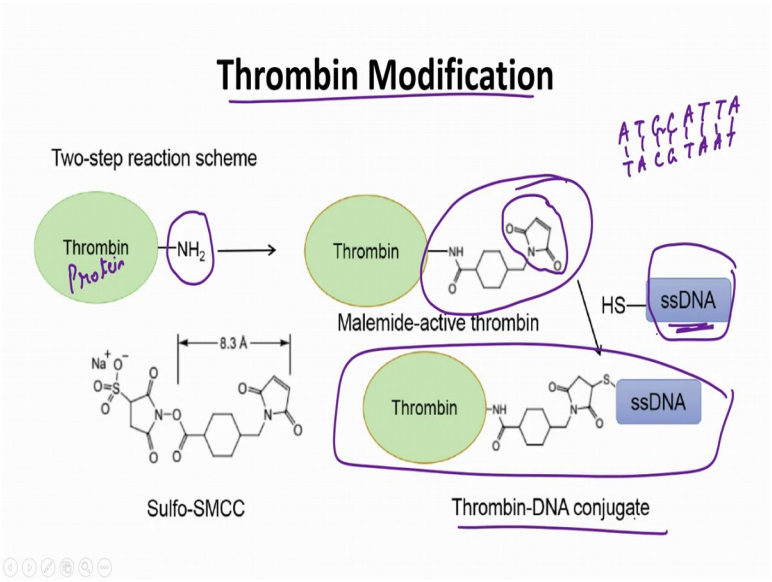

So, let us see how thrombin gets attached to the sheet now. So, what they have done is, they have taken thrombin. It is a protein and protein contains lots of amino acids which have amines present on them. So, they have taken this amine they have modified it where the malemide groups.

So, they have added a malemide group as you can see here and here is a malemide group and we have talked about how malemide group is extremely efficient in binding to your cysteine or thiol molecules and they have taken single stranded DNA which has homology to the last single stranded DNA that they were binding to. So, again this single stranded DNA has complimentary sites so, that it can bind to these yellow sites here.

So, again it is all DNA, it is all DNA based interactions. So, maybe and just to give an example maybe this sequence is ATGC, ATGC, ATTA let us say and we know that A binds to T. So, maybe that yellow sequence is nothing, but TACGTAAT. So, what will happen is because they are complementary, they will tend to anneal and bind and so, now, they have taken the single stranded DNA they have modified the thrombin with the single stranded DNA. So, they are getting a thiol DNA conjugated now it is a single stranded and that is how they have gone about doing this modification.

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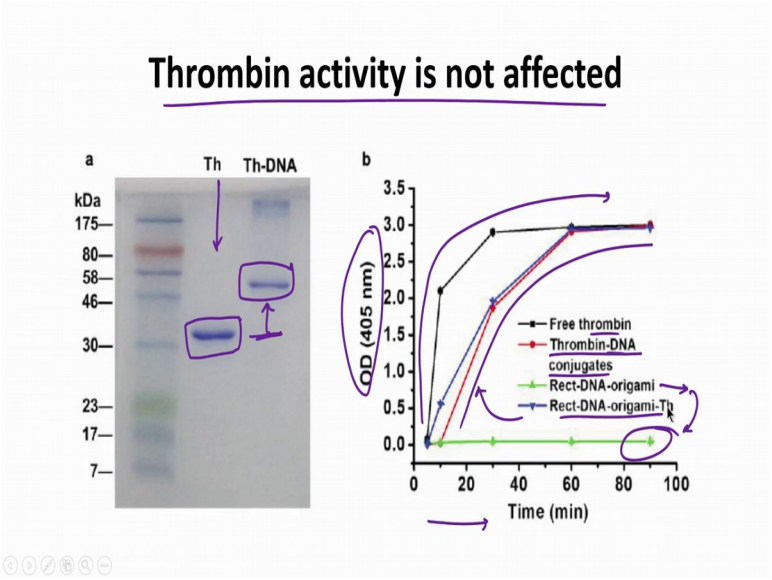

So, here is their initial data. So, they are saying first of all a thrombin was successfully modified with the single stranded DNA. So, here is the page gel. So, the protein is running and remember the bigger the protein the slower it will run in the page gel and so, this is where the natural thrombin will come at about 35 KDA.

But once they modified it with the DNA you will see that it has shifted there is no bind in this region, but it has shifted up and it has now increased its molecular weight by about 10 to 20 KDA because that DNA sequence has been attached to it and again then they are saying now we have modified it is thrombin still active. So, that is what they are measuring here so, they are saying that this is a free thrombin activity. So, they are using some metric using OD to determine thrombin function.

And when they modified it with this thrombin DNA conjugates they actually see the activity slightly reduced, but still fairly active. So, it is some blood clot that they are

trying to see in overtime. So, even though it may not be as efficient as free thrombin it still works and that is what they have used. If they only use the thrombin which is not modified with the DNA they do not see any activity at all; if they only use the DNA sheet they do not see any activity at all. But otherwise they keep seeing activity either in free thrombin or in thrombin DNA conjugate thrombin conjugated to their particulate system or sheet.

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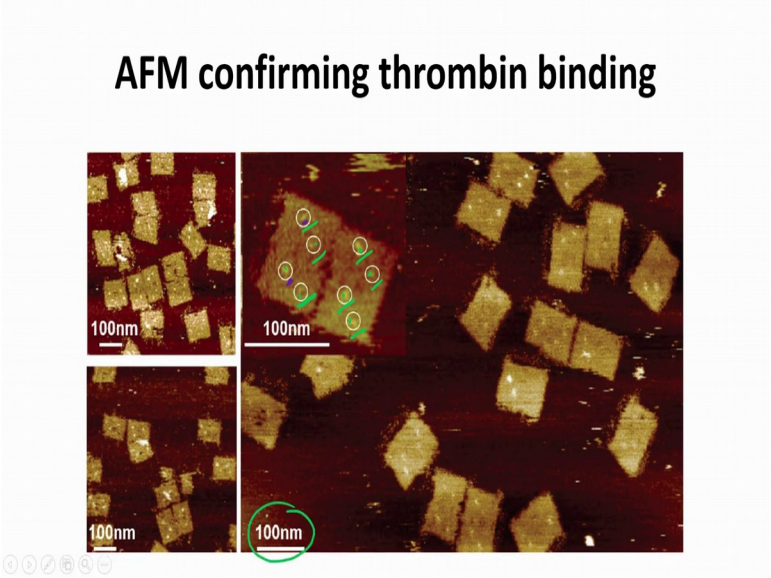

So, here is an AFM data showing the ds indeed they are able to modify their sheets such that the thrombin is binding to it. So, you can see these are sheets these are DNA sheets that are op that are open and when they have modified it, they see that there is a height difference that they can observe. So, all of this they are showing so exactly at the place that was designed in the original structure, they can see that their thrombin is binding at those sites and you can see the scale bar here as well.

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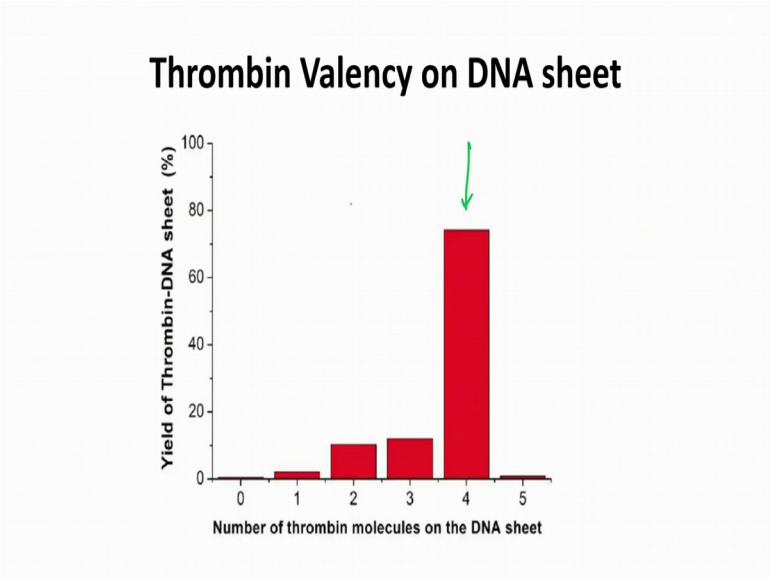

So, it is fairly small and then they have also then figured out how much thrombin is binding to per DNA sheet and they are finding typically about 4 molecules of thrombin are binding to 1 DNA sheet. So, as you can see here about 4 molecules of thrombin are binding to 1 DNA sheet on an average.

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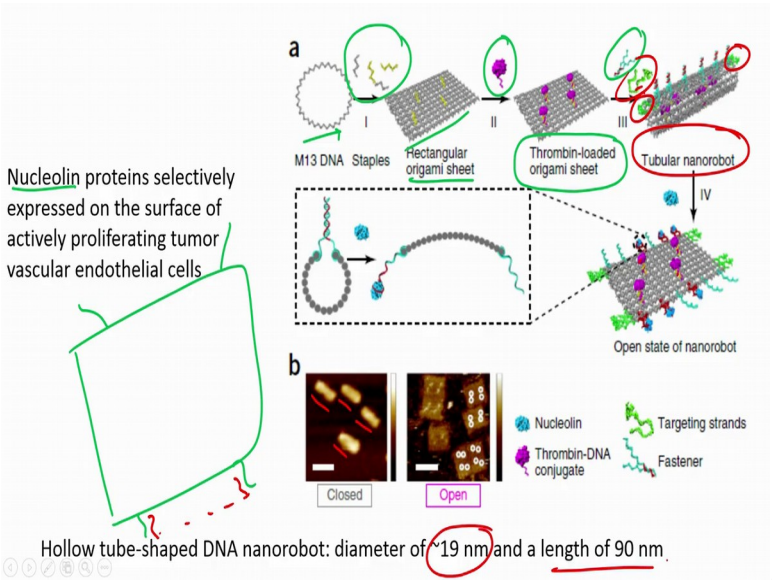

Then what they are saying is nucleolin proteins are selectively expressed on a proliferating endothelial cell. So, how are they going to differentiate between an endothelial cell that is a healthy versus the one that is in the tumor region. So, for that

they are saying that nucleolin is a protein that is known to be expressed in proliferating endothelial cells and the proliferating endothelial cells you will only find if there is an injury or if there is a tumor which is causing that new blood vessels to form and so, that is what they have done here.

So, here they are showing that so, here this is the whole system. So, here is your M 15 DNA you add your stables it opens up, this is a rectangular sheet you come with thrombin also, that is complementary to some of these staples that are at bit a site. So, the thrombin gets loaded then you come and with some more staples and that are now binding to those other staples that I said is used for fastening. So, since those staples are now complementary to 2 of those staple so, if I let us say draw a sheet and let us say I am saying I come up with another DNA structure that has homology for this as well as this region.

So, what will happen is, this will try to fold the sheet and that is what is happening here and then eventually there are also nucleolin based aptamers that they have used, which are fastening this in this particular structure. So, when they do find a nucleolin they will open up, because nucleolin is higher affinity for these fasteners and then is when the sheet opens up and exposes thrombin to which then caused the blood clot.

So, that is the whole system so, you can clearly see here they have been able to show that these sheets do roll up into these tubes and these things have a diameter of about 90 nanometer and the length is the same as the original sheet which is 90 nanometer.

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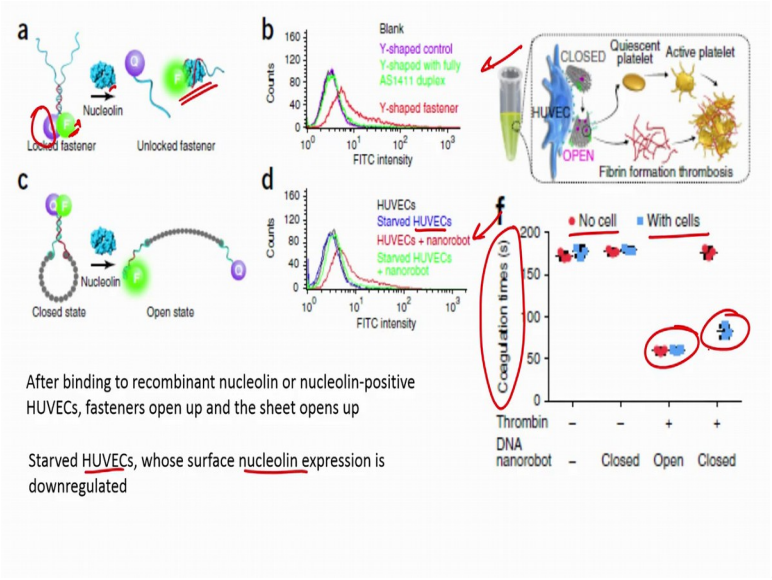

And again this is a more data on the nucleolin. So, they are showing that these tags nucleolin tags have been conjugated with the fluorophore and the quencher. And in presence of nucleolin they show that actually the fastener that was used to roll up the sheet, leaves it is original DNA strand and actually binds to the nucleolin causing the DNA sheet to open and this is further quantified here using some fret analysis. And then they also show that these are HUVECs which are endothelial cell and so they are a good model to study the system they starved HUVECs sort of down regulate the expression of nucleolin.

So, if you have starved you wax they do not open this up, but if you have a normal HUVEC they have nucleolin present and then they can open these sheets up. And again this is some more characterization so, only in presence of cells you see that the conjugation time has decreased and in presence of no cells you do not see that. And so, only in presence of cell as well as thrombin, you see that the conjugation time the coagulation time which is the blood clotting time has decreased and which is what they want.

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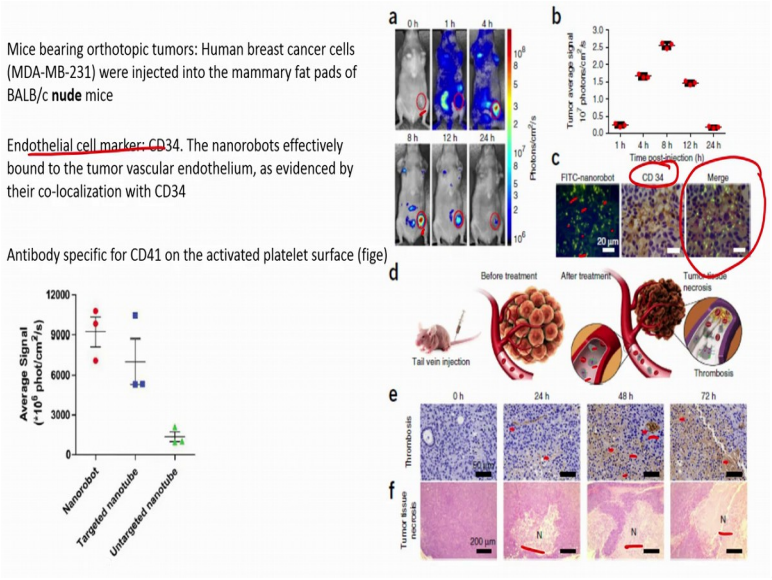

And then they went ahead and used a mouse model in this case an orthotopic tumor model and here they are showing that this tumor model is successfully grown first and then once you inject these therapies they are causing this blood clots. So, you can see now that all this is a necrotic region this is histology showing that these vessels that were feeding this tumor vessels have been blocked by their nano robot or in this case the DNA sheets.

And they have used various kinds of staining so, in this case they have used an endothelial cell marker which is CD 34 stain here and these nano robots effectively bind to these endothelial as evidenced by the co localization. So, you can see CD 34 , all of the stain is CD 34 and all of this stain is the nano robots in green and you can see that they are merging very well so; that means, that these particles are going and binding to those the cells that are feeding these tumor regions.

You can use an antibody specific for CD 41 on the activated platelet surface. So, in this case the figure E is showing that. So, they are showing that they have activated platelet us surface as well as the histology and then they have quantified the signal. So, they are saying that only in presence of targeted nanotube you get this specific result.

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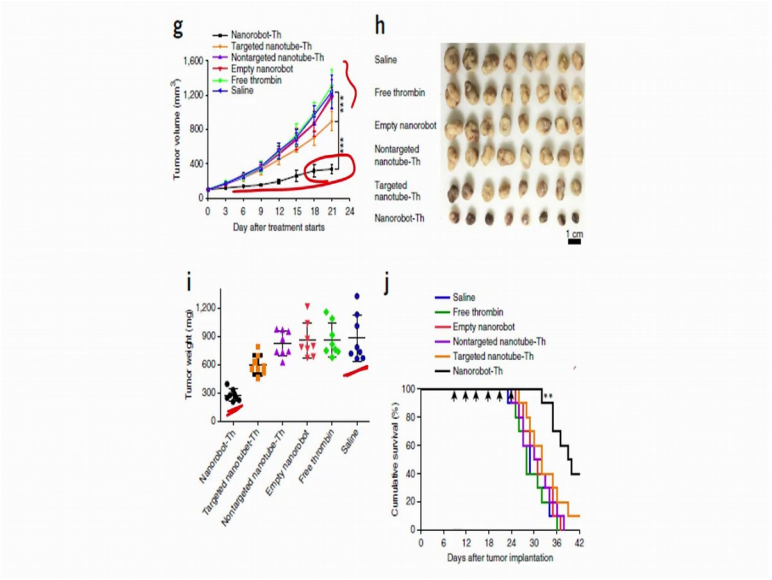

And then finally, they have looked at the tumor volume. So, you can see that the tumor volume when they have delivered their therapy is much lower compared to any other combinations they try to deliver. Same with the tumor weight it is much lower in case of the therapy versus just pbs or other controls and the mice also survive for much longer.

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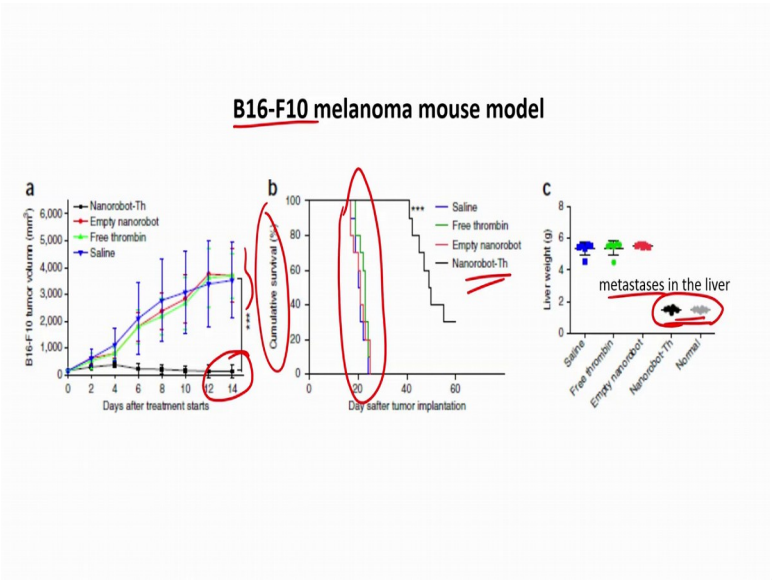

And then finally, in a B 16 - F 10 melanoma mouse model. So, these were all done with the nude mouse this is always a question whether how good these nude mouse are in terms of representative of what happens in humans. So, they have used a melanoma

mouse model itself and they actually get even impressive result where they find that when they use the therapy the tumor volume is almost none and anything else that they try does not really do any effect on the tumor volume itself.

And finally, the survival of the mouse is much better. So, most of these mice if you give traditional therapy or no therapy they die by 20 to 23 days, but if you have these nano robots that are being delivered they live twice or much more longer and then the metastasis which is basically the spread of the tumor is also very low when we have and these particles being delivered.

So, in this case they are just measuring it by liver weight and they find this there is no change in the liver indicating that metastasis has not happened in the liver.

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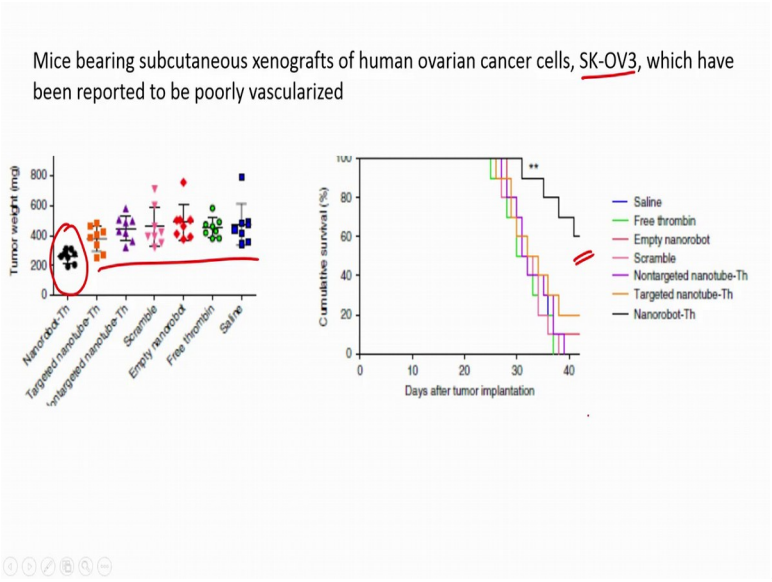

And so, then they have done then they went ahead and use the mouse models with human cancer cells. So, these are cancer cells that are actually poorly vascularized they have been known in the literature. So, this SK-OV 3 yeah ovarian cancer cell line has been reported in the literature to be very poorly vascularised.

So, they said let us make it more challenging how about we deliver it to a tumor which has less vessels and again in that same thing they also observe that even though they say the amount is not as pronounced,the tumor volume is much lower when they are delivering their therapy compared to the other groups and it also leads to enhanced

survival in humans. So, we will stop here and we will continue this further in the next class.

Thank you.