Drug Delivery Principles and Engineering
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Lecture - 30
Tissue Engineering – III

Hello everyone, welcome to another lecture for Drug Delivery Engineering and Principles. I am Rachit and I am going to continue what we have been discussing. So, for the last few classes, we have now moved to a module which is on tissue engineering. Let us see what we learned in the last class. So, basically we talked about tissue engineering in the last two three classes about, why it is important and what it actually involves.

And so, it is essentially using material, cells, any engineering concept to restore the function of a tissue or regenerated tissue and similar applications. And among these there are several different classes of tissue engineering, we have discussed few in previous few classes. In the last class we talked about regenerating part of a tissue.

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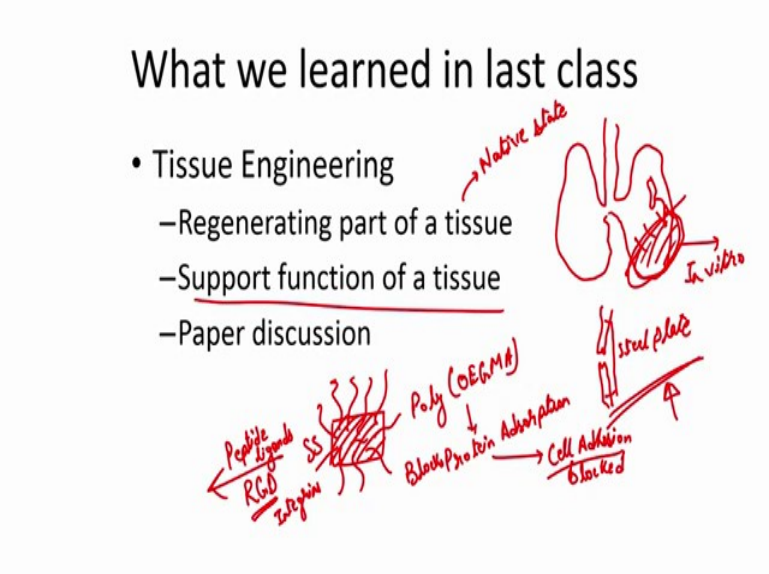

Which means, let us say, if some part of my lung is actually damaged and I want to regenerate this tissue. So, what are the different options that I have – I can generate this part in vitro, and then once the tissue has reached a certain function in vitro, I can put it back and suture it with the rest of the tissue to improve the function. Or what I can do is I can let the body take care of it. So, I can just put the scaffold with some cells. And let

those cells do the regeneration in that tissue itself, which typically is a lot better in terms of integration with the tissue.

And then the final thing that I can do is I can just put the matrix and allow these cells in the surrounding area, to migrate in and populate this with maybe the tissue stem cells or some other cells that can then restore and increase the function of the lung that was lost. And then the next thing we talked about was support function of a tissue. So, very similar to the previous case, but in the previous case our major goal is to get back to the native state.

So, as if the person was healthy and so, that includes both functions as well as how the tissue architecture is, but in terms of supporting a function of a tissue we do not really want to mimic the native state, all we are trying to do is support a function. So, this could involve a fracture in the long bone, lets say this is a fracture and I am unable to heal this immediately, but the person needs to walk.

So, what you can envision is, with a surgery this can be joined with let us say a steel plate. And all we are doing, I mean our steel plate is no way is going to mimic what was the native state, but it does allow the person to move around. So, the function is somewhat restored. And then eventual healing will happen and hopefully it goes back to the original state.

And then we had a paper discussion. In this paper discussion, we were talking about how you can modify a surface of stainless steel. And in this case we were looking at how the authors have modified the surface with a polymer which is poly oegma.

We have of course, discussed poly OEGMA before in a polymer drug conjugate classes, where we were saying this is an alternative to PEG, which has lesser immune response compared to PEG. And when you do that it prevents cell adsorption. So, once you do that first of all it prevents protein adsorption (blocks protein adsorption) and if there is no protein that is adsorbing on the surface and then we know that the cells actually mediate their attachment to the surface through these proteins, so, the cell adhesion is also blocked. And when the cell adhesion is blocked most of the tissue rejection or tissue walling off, fibrosis all of this happens through cell mediated pathways.

So, if the cell adhesion is blocked itself, then there is more chance that the implanted material will not be rejected. And then what the authors further did is then modified it with specific peptide ligands. So, in this particular example, they used molecule called RGD, which has a binding site for integrins on these cell receptors.

And once you bind through that, you signal through that, you can have the cell perform a certain function that is involved through that signaling. And that way you can actually even cause repair to happen even faster rate than what the native body would have done. So, these are just some of the strategies we discussed in that paper. So, to give you further example of how something like this can be used, what are the current problems, we are going to discuss another paper today.

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And that paper is essentially titled simple coating of fabric fragment that enhances integration of screws that are used for osteoporosis.

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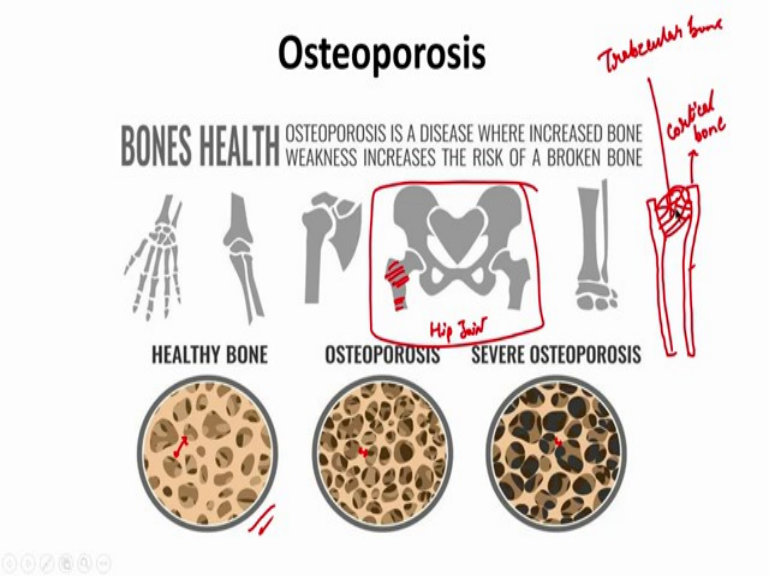

So, let us start off, first of all, what is osteoporosis? So, just a little bit of biological concepts here. So, what typically happens in a healthy human is there are bones and bones are typically developed to be fairly sturdy. And there are few bones, which are porous bones. So, especially this becomes important in cases of long bones. So, let us say if this is a hip joint. So, let us say this is a hip joint and all these bones that are connecting the different parts of the body, they are essentially divided into two types of bones - one is called the cortical bone. So, essentially you will have bone thicker near the joints as you can see its quite thick here, and then it sort of thins out.

And so, in this also there is sort of a wall of a bone. And this is called cortical bone. And then inside this cortical bone, you get this porous structure that you see here and so, essentially all kinds of pores are running through here, this is called trabecular bone. So, this trabecular bone is to support cortical bone, it supports movement in all three dimensions. Its not a heavy support as the cortical bone is, but still its a fairly good support especially at the joint areas.

So, what happens in osteoporosis is essentially the trabecular bone starts to sort of thin off and as you can see the bone between the pores is sort of decreasing as time is progressing. And eventually its becoming so thin that its not able to support the whole weight. And so, now this is weakening the as a result the support on the cortical bone is also weakening. And as the person ages and this is related to the aging quite a lot

especially in female patients. And what you will find is then they will be very susceptible to fractures. And typically this happens after the menopause, so, somewhere around age of 50 is when this becomes quite severe.

And so, basically a big problem that these folks will suffer from lot of loss in their bone strength and they will suffer lots of fractures as well. So, this is a problem.

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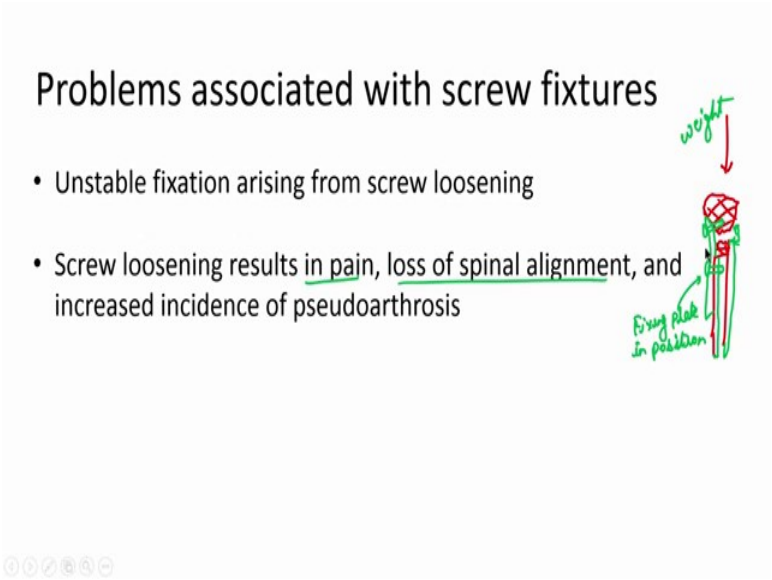

And so, what is currently done, as I briefly mentioned before, so, if you have a bone fracture especially on a weight bearing bone, then typically what is done is we put some metal plates. So, let us say if this is one of my long bone and it suffers a fracture, because this trabecular bone is weak. So, what is typically done is a metal plate is put in. So, obviously, at this point this bone cannot bear any weight since it is disjointed.

So, what is typically done is a metal plate of various dimensions and various strengths can be put in and to hold the plate in place, screws are also put in. So, there will be some screws, they will be punched into the bone to hold this metal plate. Now because of this metal plate being present, it can actually support the weight. So, that is what is typically done; however, with this procedure there are few issues and that is been mentioned here. So, these screws are fixing the plate right, they are fixing the plate in position. So, what is found now is that this fixation becomes loose over time.

Maybe these screws are not interacting with the bone very well, this cortical bone that we have and they will get loose over time and that sort of causes this screw to come out and essentially result in failure of the fixation. And once that happens the patient is again in a lot of pain and cannot walk; so, that is a problem. There is a lot of pain, loss of spinal alignment is also seen that can cause even more pain.

And the bone can also start to resorb further. So, I mean in this case you want this bone to grow back, but now what is happening is because of all this movement, all this improper fixation, what you will see is that this screw is chipping off the bone, the bone is actually resorbing away from the screw and the plate. So, its a big problem in the field.

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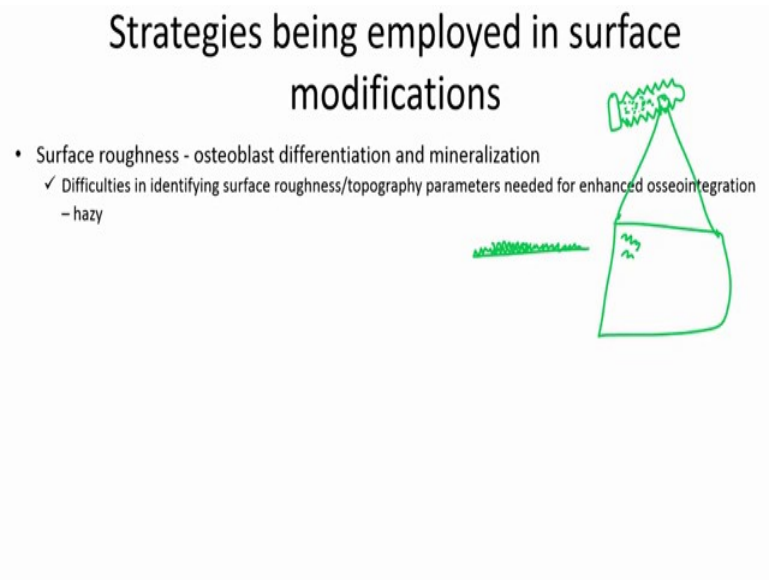

So, what you can do now to improve screw fixation. If we are able to prevent the screw from loosening, then we can alleviate some of these problems. Some of the strategies that are being applied is; first of all is to use surface roughness. So, a lot of the time these screws, if we zoom in let us say, if this is a screw, if you microscopically look at it, people are making it very porous.

So, now, if I zoom in to let us say this area, what I will see is there are lots of pores or maybe actually I will show a transactional view. So, if I show a transactional view there is lots and lots of small crevices and ridges. And what that allows is the screw can then bind to the bone very well, because the bone can actually then grow into these grooves

that are present on this screw. And it essentially gives a lot more surface area for the screw to hold onto the bone.

So, that is one strategy that is being used, but then the problem has been that even after so much of the research its not very clear as to what should be the surface roughness and topography that causes enhanced integration and whatever you typically see is fairly minor improvement, it is not a major improvement.

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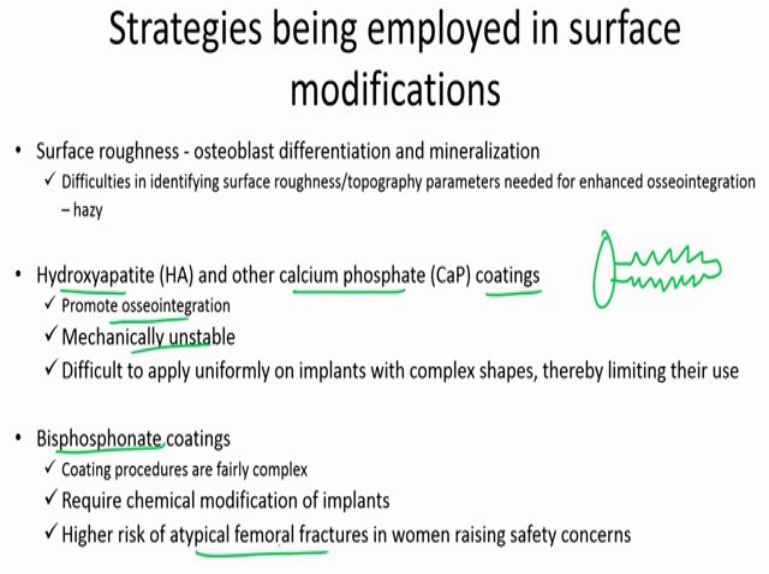

The other strategy that has been used is to use a few molecules such as hydroxyapatite and calcium phosphate, these are essentially something that are natively present in the bone, the bone actually likes the surface. And they have been shown to actually promote osseointegration; get the mechanical instability to go away, but then the problem has been that, even with these strategies there is still instability that is present. And they are sort of difficult to apply, they are very complex these coatings are not trivial it requires quite a lot of work to basically code it uniformly. And especially in a shape like a screw, where you have all these kinds of ridges that are playing present, it becomes very hard to get a uniform coating even in these ridges and all so, which is limiting their use.

And then another coating that is very rarely used bisphosphonates; bisphosphonates are a class of compounds that have shown quite a bit of promise with the bone fixation and but the problem is that the coating procedures again have fairly complex quite a lot of chemistry that is required you have to modify the implants chemically in that causes

them to have different properties maybe different oxidation different amount of things that are leeching out.

And there have been some risks with the bisphosphate, themselves induce some a typical femoral fractures in women; especially this is used in clinics quite a lot. And its been she had been seen as some femoral fractures do a start happening in women. Although albeit at a low percentage, but it is a problem. So, these are some of the shortcomings of these strategies.

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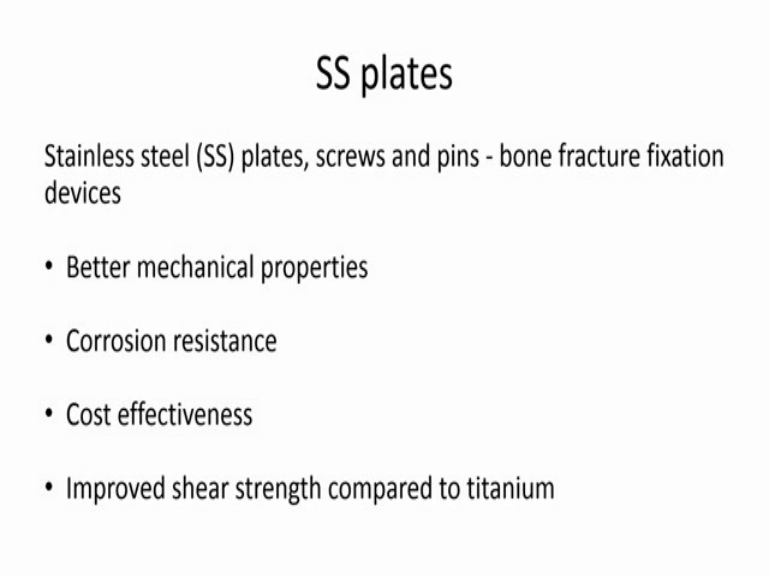

So, then one of the strategy that, this particular paper is shown is to use stainless steel plates and develop a coating over it. We will talk about the coating, but let us talk about why these stainless steel plates are being used. So, stainless steel again is one of the major materials that is used for bone fixation. And there is several reasons for it, I mean as you probably are aware of, stainless steel is a fairly strong material, it has a lot of good mechanical properties.

It is able to bear weight over quite a long period of time. It does not really corrode much. So, that is a good thing. It is fairly cheap. So, the implants are not very expensive. There are also implants from titanium, but those tend to be fairly expensive. And then they have improved shear strength compared to the titanium as well. So, I mean again titanium is again a great material to use. So, is stainless steel, but in this paper they focus more on stainless steel. So, we will talk about that here.

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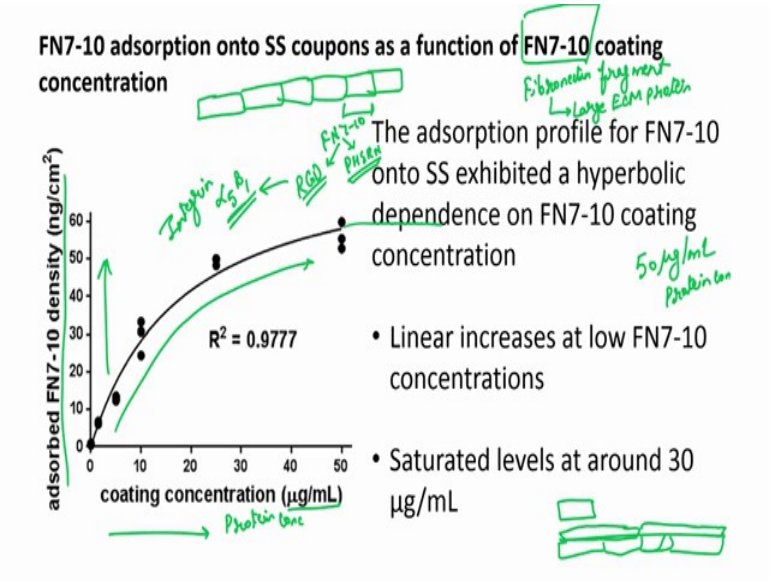

And so, the authors came up with an idea to use a small fragment of a protein, which is called fibronectin fragment. So, fibronectin some of you may know is a large ECM protein. And it is somewhat of a beaded protein. So, there is several domains in fibronectin that are connected together and so one of the fragment they are focusing on is the fibronectin 7 to 10 and the reason they are focusing on that is that fragment itself contains a couple of major sites.

One is then RGD site the same peptide that we talked about in the last class. And then there is also a synergy site called PHSRN. And what this site does, it promotes a particular integrin to bind to this RGD. So, RGD again is this little bit of promiscuous integrin binding ligand, it can bind to two or three different types, but this PHSRN promotes the binding of this RGD through an integrin on the cell which is called alpha 5 beta 1.

Now this alpha 5 beta 1 integrin has been shown that, if the cells are getting signals through this particular integrin it actually promotes bone formation. So, that is the whole concept that these authors have used here that can they develop some simple protein adsorption coating onto these material to which when these particular fragments adsorb, they will cause signaling through alpha 5 beta 1.

And that signaling will promote bone formation rather than bone desorption at the site. So, that is what they have done. So, first thing they did they read is to quantify, whether

these proteins can actually adsorb on the stainless steel coupons and if so, then to quantify that. So, what they saw that absorption profile of fibronectin exhibited a hyperbolic dependence on the concentration. So, this is again very similar to what we discussed in the protein adsorption class.

So, as you are increasing the concentration of your protein, this is protein concentration in micrograms per mL, the adsorbed density is increasing. And why is this increasing and its the same reason, because if you have a surface and let us say the protein is shaped let us say like this. When it adsorbs and there is not much protein around it. So, other proteins will take time to diffuse through the medium and the surface. It has time to expand on the surface and occupy a lot more surface. So, maybe for this given area only two proteins are able to adsorb on it, whereas, if you have lots of protein concentration in the surrounding, the diffusion time is less, then this protein cannot expand as much and maybe you can have 4 proteins adsorbing. So, that is why you see that as your concentration of the protein in the solution is increasing the initial concentration, the adsorption amount is also going up. So, very classic, like we had already discussed in the protein adsorption and then about at 30 micrograms per mL to 40 micrograms per mL you start getting a saturated concentration. After that, the diffusion of the protein to the surface is no longer the limiting step and at that point it does not really increase its sort of plateaus out. (Refer Time: 20:04)

And so, that is what is typically seen. So, then from this steady the authors decided to go ahead with the saturating concentration. So, for future studies, they always used 50 micrograms per mL of protein concentration to coat on the surface.

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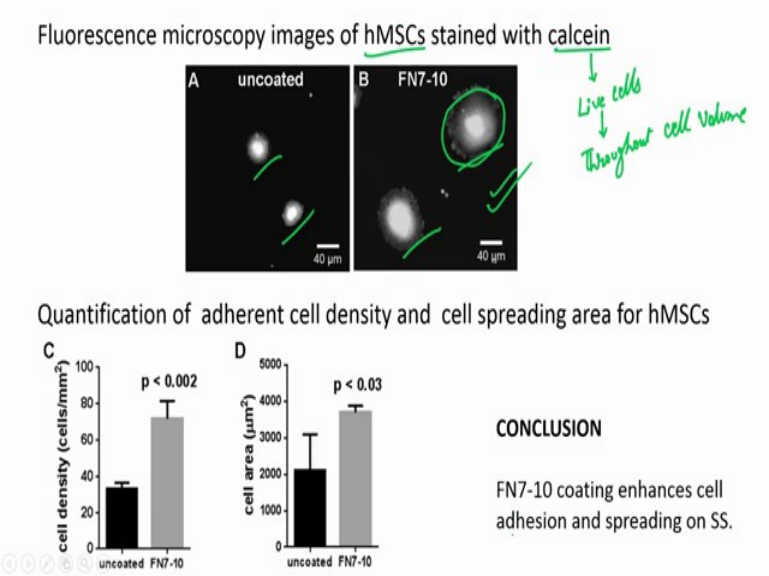

Then they basically looked at how the cells behave on a coated versus an uncoated stainless steel surface. So, here they have seeded human mesenchymal stem cells, these are stem cells that are also found in bone. And these are some of the cells that are responsible for new bone formation. So, as well as along with osteoblasts and things like that these differentiate to bone cells.

So, what they have done is they stained these cells with calcein, which is a dye that stains live cells, throughout the cytoplasm or in fact, throughout the cell body. And what you see is if they do not have any coating the cells stick to the surface, they do like it and they have a certain spread area, but when they have coat the surface, you see the cells really like it and they are really spreading on the surface quite a bit.

That is further quantified here, that an uncoated surface the cell density is lower. So, if you seed equal number of cells you find more cells on this surface and not only that the cell area is also higher, cell spreading is much higher on these surfaces compared to the uncoated surfaces. So, the conclusion here is that the fibronectin coating will enhance cell adhesion and spreading on stainless steel surface.

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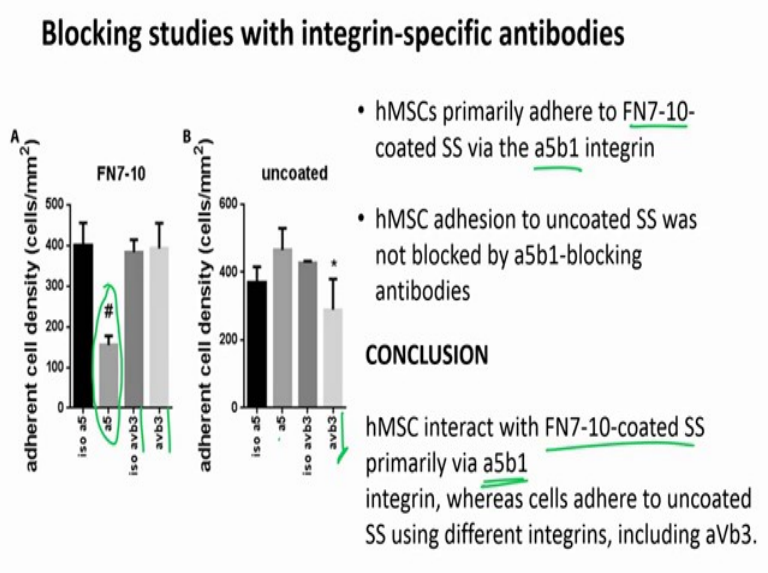

Then they figured out, whether the actual signaling that they were saying that the fibronectin 7 to 10 is signaling through alpha 5 beta 1 whether that signaling is actually happening or not. So, what they did is they blocked the cells with an alpha 5 antibody and what is happening now is that antibody is blocking the binding of the alpha 5 integrin to your fibronectin coated surfaces. And because of that you actually see that the cell density has decreased.

So, they have tried blocking with the other integrins as well and they do not really show any effect, but only when they have alpha 5 then they start seeing some decrease in the cell density. And on uncoated surfaces they do not really see that. In fact, when they block some other integrin, whose ligand is present quite abundant amount in the serum, they start seeing some drop, but not much significance is here. So, the conclusion is that yes, these cells interact with the fibronectin coating surface through alpha 5 beta 1 integrin, which is what the authors wanted as it is being shown to then promote bone formation.

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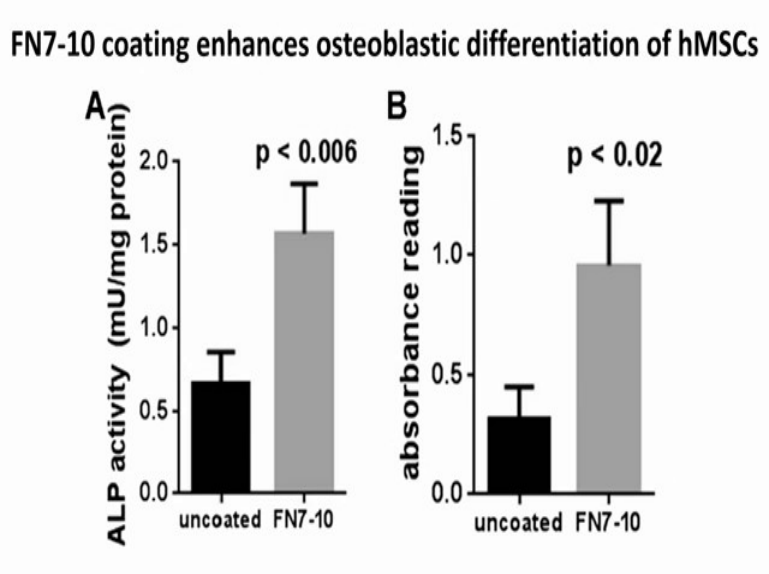

Then they looked at whether these cells; these human mesenchymal stem cells are actually differentiating into bone cells and whether they are producing markers for bones. So, ALP is one on the markers, its called alkaline phosphatase as one of the enzymes that is used for bone formation. So, again they see that, if you have uncoated surfaces you get little amount of ALP activity. Whereas, if you have coated surfaces you have much higher ALP activity and this is further quantified here.

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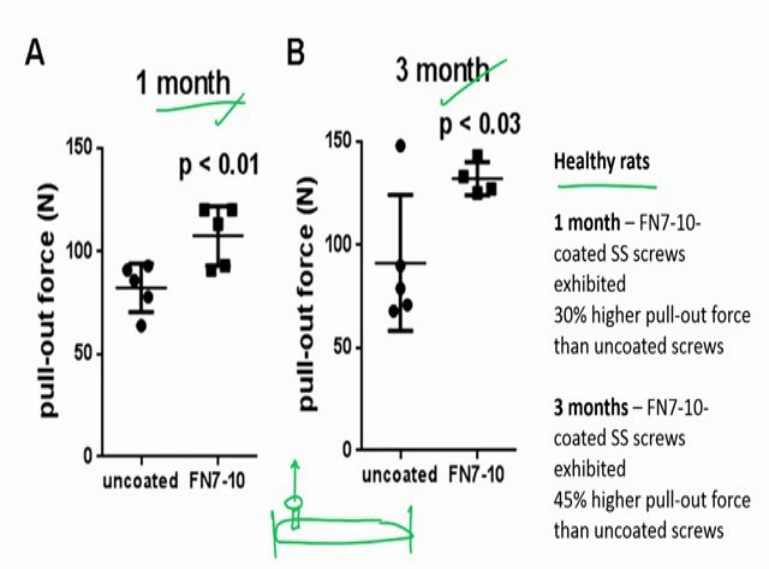

Then they actually used it in a rat model. So, first of all they use healthy rats and showed that, if they put a screw in the bone, in that trabecular area of the bone. Then what happens after let us say 1 month, when you take that bone out and try to pull the screw off. So, what they are measuring now is, let us see if this is a bone, you have put in a screw here and now what are you doing is pulling it out while holding the bone while fixing the bone.

So, this force that you are sort of observing in pulling this out will give you sort of how adhesive this screw is now on the bone. And what they do fine is as significant increases at both 1 month and 3 month, which suggests that actually the coatings do promote bone adhesion of these screws.

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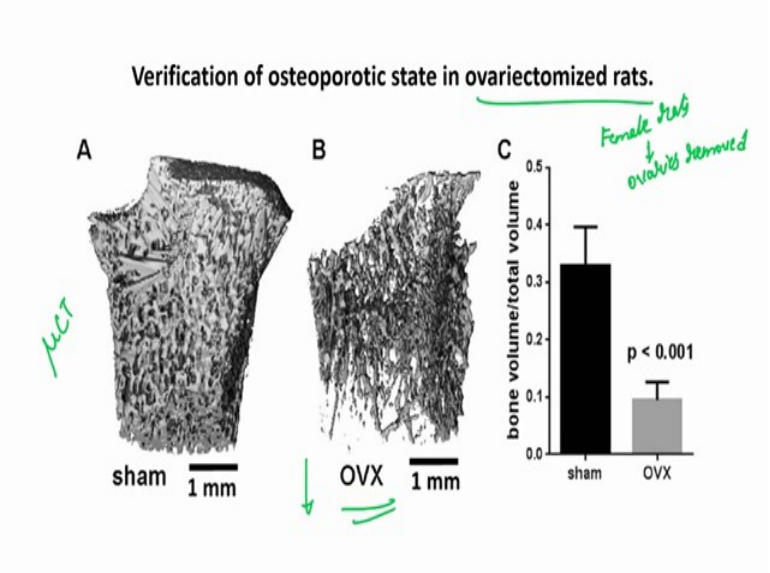

And then they used an ovariectomized rat model which is essentially these female rats. Their ovaries are removed and then they are allowed to move around for couple of months, what that does is because the ovaries are removed some of the enzymes and some of the hormones that are needed for the bone formation especially the trabecular bone formation are gone.

And so, you can see a difference, this is basically a micro CT. Showing the trabecular bone mesh network on these rats and you can see that the amount of bone present in the ovariectomized animal is much lower, than in the sham animals and this is again

quantified here. So, this is the bone volume in the trabecular region. So, essentially mimicking what happens in humans in cases of osteoporosis

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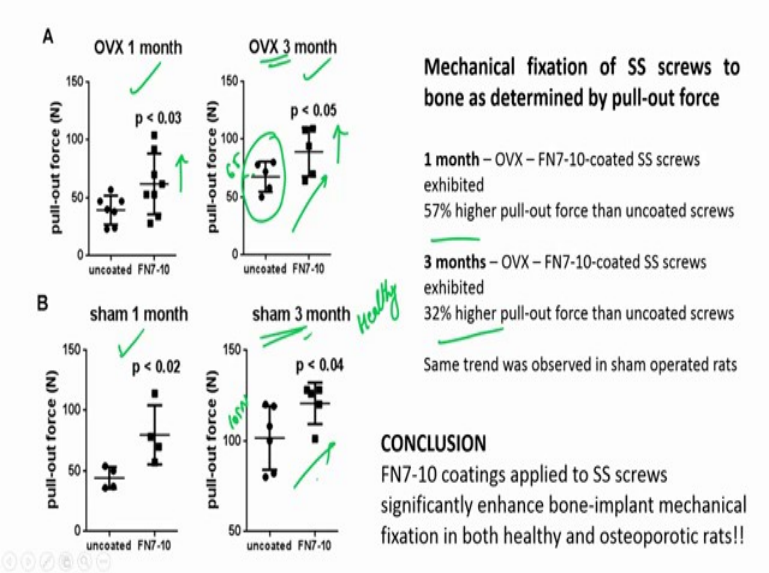

And so, in these you can call them diseased animals. In these diseased animals they did the same testing with the bone pullout. And what the observe is fairly very similar to what they saw in the healthy animals, that you know ovariectomized animals you see at 1 month and at 3 month. The forces are higher to pull this out and the same can be seen in sham as well as the ovariectomized animal.

So, shams are essentially rats that have not gone into surgery. So, they are age matched and they are housed together. So, these sorts of animals mimic everything except the surgery. So, you can potentially call them healthy, they have gone through a surgery, but they have not really had any ovaries removed. So, what do you typically see is almost 57 percent and 32 percent higher pullout forces at 1 month and 3 men respectively when you compare to the uncoated screw.

So, what this tells you is that these coatings are fairly easy to use, because all you have to do is essentially just dip these screws into these coatings. There is no problem in terms of coating complex shape, because the liquid will penetrate all kinds of shapes. And not only that, they further show that this actually results in a higher pull out force.

So, notice how in ovariectomized animals at 3 months versus the sham at 3 months, you see that the pull out forces are very different. So, an uncoated screw here, we are talking about close to about 65 Newtons whereas, this is almost 100-105 Newtons. It shows you where the bone is damaged in the ovariectomized one. So, even in the disease model they are able to show an increase in the pull out forces as compared to the sham animals.

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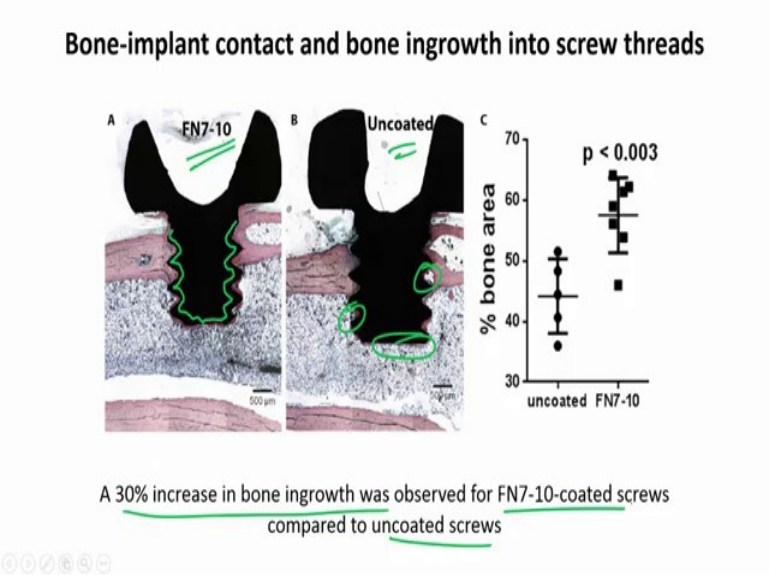

And then finally, they sacrifice these animals took the bone out stained the bones and did a histology. So, this is a histological image, so, here you have a fibronectin coated screw and an uncoated screw. And what you can appreciate here is look how well this screw is adhered to the bone. Look at this all this pink staining, which is clearly showing that nearly all part of the screw has bone over it.

Whereas, if you compare it with an uncoated you see these areas where there is really no bone present showing that the bone does like these coated screws and does form on that surfaces compared to the uncoated screw. So, if you just measure this contact area you find that 30 percent increase in the bone ingrowth compared to the uncoated screw in fibronectin 7 to 10 coated screws. So, we will stop here and we will continue further in the next class; see you then.

Thank you.