Drug Delivery Principles and Engineering
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Lecture: 29
Tissue Engineering – II

Hello everyone, welcome to another lecture for Drug Delivery Engineering and Principles. We are now discussing Tissue Engineering, let us go over what we discussed in the last class.

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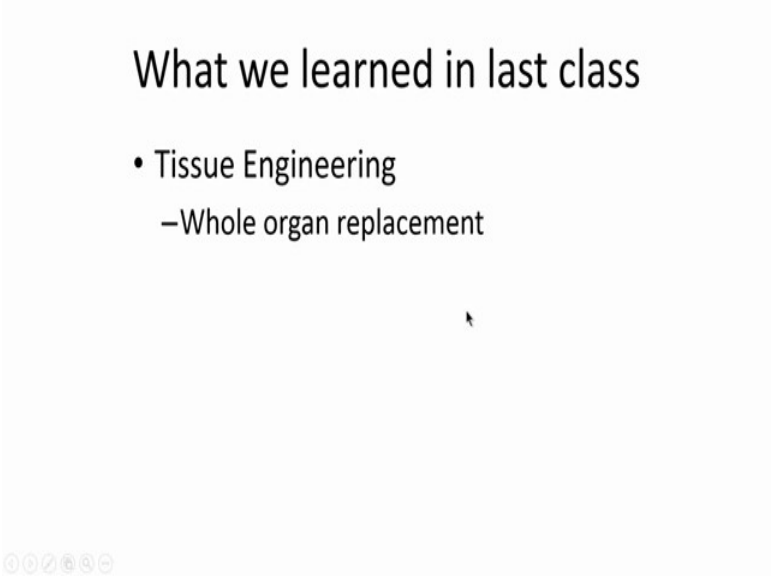

We introduced what tissue engineering is, why it is used, why it is so important? There are several applications – one is whole organ replacement. So, essentially if your organ is damaged or it is not functioning, with the help of tissue engineering, you can get replacement for that - either from other donors or by growing that tissue in the lab.

And so you can essentially do all of that. So, we discussed various pros and cons that are involved in getting these whole organ to be generated in the lab and we will continue the discussion further.

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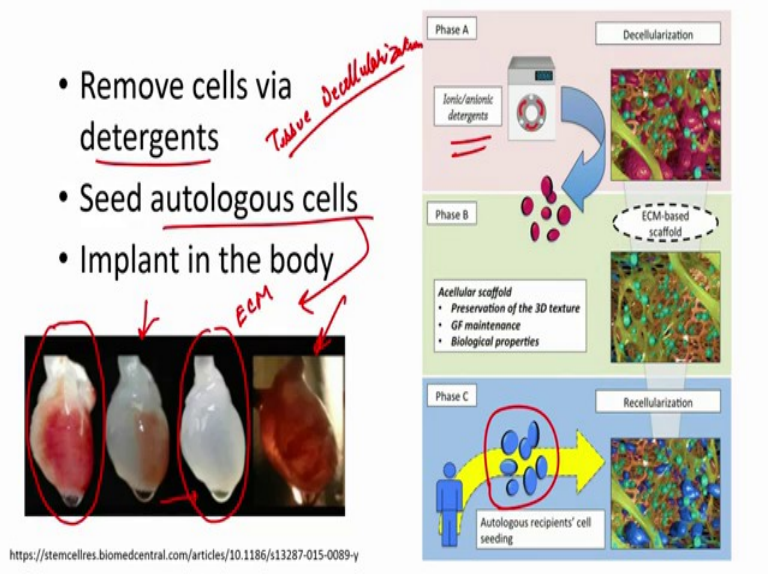

So, last time we talked about the tissue decellularization and what I discussed is, let us say if you get an organ from a donor or let us say from an animal, what you can do is you can treat it with reagents such as detergents and another cytolytic detergents which will essentially then remove all the cells, you are now starting to see all the pigment is gone.

So, all the blood and all these different cells that were present in this vicinity are gone. And eventually you are starting to get to a very clean organ that only contains essentially the ECM structure which is fairly conserved among different species and among different individuals.

And so at that point, you can then come in and seed autologous cells and by autologous I mean the cells from the same human and cell. Let us say if I need an implant for my liver or heart in this case; they will seed some of my own heart cells isolated from my body, which will then expand and populate it. And once it is populated, there are already blood vessels which are also getting populated with some endothelial cells as well and that then can be used to implant it back in my body.

So, here is typically how this goes. So, you have a phase A where you get a tissue; so you can see all these cells that are present in this tissue. You treat it with some sort of detergents and these detergents then remove all these cells. So, in this phase B only the ECM structure that is remaining, all these cells are gone.

And then in the phase C, you take the autologous cells from the patient who needs these tissues. And you seed them back, let them grow to a certain extent where they are sort of getting to some functional level and then you put it back in the human, where these things will then grow.

So, again this is a fairly lengthy process if somebody is coming with a heart issue and needs a heart immediately; you cannot really use it. But if it is a chronic problem, where the heart is slowly losing function and you know you have a few months; then you can try to go through this process and get the heart to be replaced. And again it depends on that tissue, it depends on the problem, but all of this can be then tackled.

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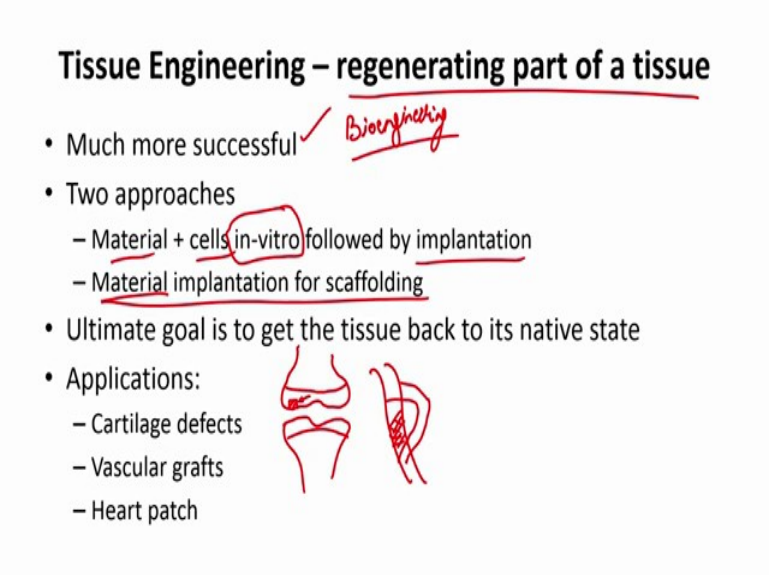

So, let us move on to the next part of the tissue engineering which is regenerating part of a tissue. So, again this is much more successful in terms of the bioengineering part of it. Again the organ donor is fairly successful as we discussed, but in terms of regenerating part of tissue and bioengineering strategies; when only a part of the tissue is required then bioengineering strategies are much more successful.

There are essentially two approaches one is you can take a material, you can take cells you can mix them in vitro and then you can follow it by the implantation. So, essentially you are providing the cells that are required, you are giving the material that is required, you even sometimes letting it grow in vitro or just mixing them in vitro. And then you put for implantation and then you hope that these cells and these materials will survive.

And then these cells will eventually start to do their function and you will get the response and the restoration of whatever function was lacking back in the body. The other approach is also just to use the material itself to provide scaffolding. A lot of the time the body has a lot of regeneration capacity. So, it will regenerate as long as it finds space and finds right environment.

So, in this case all you are doing is giving it a right environment. There is a whole library of these materials. Some materials are widely used for certain organs, other materials widely use for some other organs. So, all of this can be done and the right kind of material can be chosen for the right kind of tissue. And you get that in scaffolding and let the body heal itself and hopefully with this approach, you can get some function restoration after a few days, a few weeks and depends on the tissue again.

The whole ultimate goal here is to get the tissue back to its native state. So, I want whatever the problem was there in that tissue to get resolved and it should go back to what it was before any kind of disease or any kind of accident that had occurred. And one of the applications of this is cartilage defects. So, a lot of the time some patients may have defects in the cartilage. So, let us say if I draw the knee joint and this is the cartilage layer, in some cases sometimes what happens is a piece of the cartilage is damaged.

So, in those cases what you can do is you can come in with a scaffold, just fill this piece with it and let the other cells populate it or you can come with some of the expanded cells already in here; let it integrate with the rest of the tissue. The same thing happens with the vascular grafts, where you can have some part of your blood vessels getting damaged maybe it is a plaque, maybe it is something else.

And so if you have a vessel some part is blocked, you can take another vessel and just grafted such that it connects back to the in next tissue and you can just bypass this block that is present. So, and then there is heart patches if a part of the heart gets infarcted since the cells have died.

Some of the function is not present anymore; it is not beating in the correct rate. So you can come with a patch which you can put on the dead area. And let the cells from the vicinity populate it and essentially differentiate into heart muscles which can then cause a restoration of the pumping.

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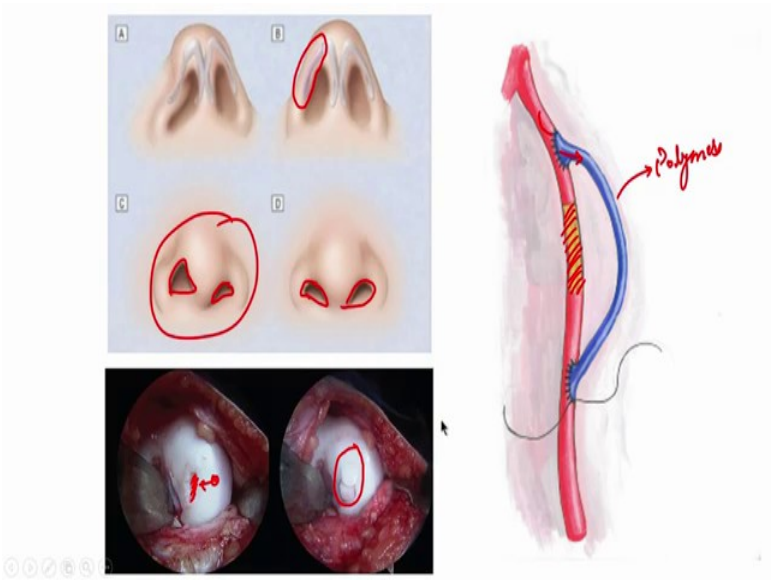

So here again, some examples. So in this case you will be seeing some defects; this is more on the cosmetic purposes and this can also affect breathing. This has been corrected by putting in an implant that, in this case, is structurally supporting so that this nasal opening is actually open and the air can go through.

This is more on the cosmetic purposes. So, what you can see is this is; this has been a bigger hole and the other nostril is much tinier. So, after a surgery with use of such materials like this, with structural support, you can get them to be more symmetric as well as easy in breathing as well. Then we already talked about the cartilage grafts as well; so in this case you can see this is an image of the knee cartilage from a patient. So, you can see that there is a hole, it is clearly in the cartilage; this is going to cause a lot of pain and a lot of issue while walking.

So, what is done is you can come in with a small graft you can place it in; this graft may be populated with cells by yourself or you can let the body cells from the surrounding to go into this area. And eventually this is going to help with the ease of pain as well as improved lifestyle. And this is the vascular graft I was talking about let us say this is a blood vessel and a part of the blood vessel is clogged.

Then you can take a vascular graft; this could be some polymers that could be used for this purpose. And you can sort of just bypass this area so that the blood can actually flow in and the downstream tissues and cells can survive.

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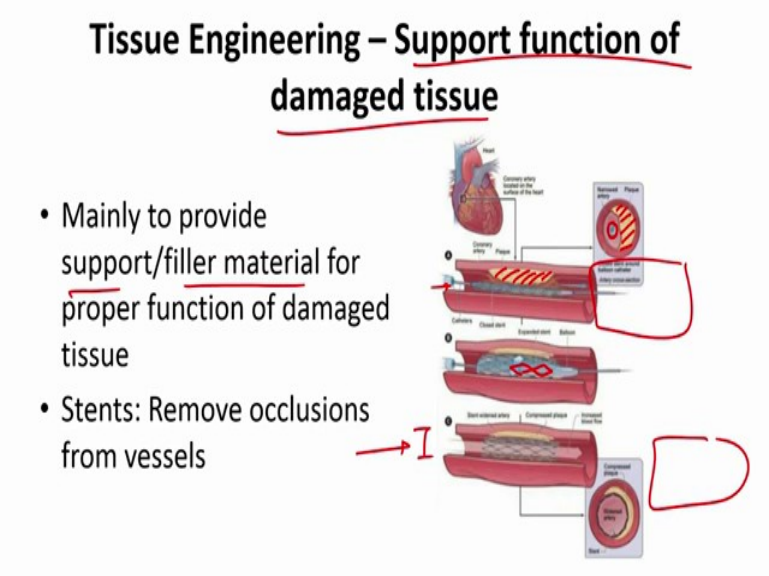

The next thing we are going to talk about is supporting the function of the damaged tissue. So, unlike in the previous case we are you trying to replace a part of the tissue and through scaffolding, our major thing was to sort of get that tissue back in its original state.

In this case we are not really concerned about that, what we are concerned about is the function should be restored. Whether it is restored by getting the tissue back in its original state or by some other means, for this application, it is ok if even if it does not look like what it looked like before the damage had happened. So, this is essentially mainly need to provide support or the filler material for proper function of the damaged tissue.

So, here is an example where we are seeing stents being used. So, this is a blood vessel which is getting clogged because of plaque formation. So, what is happening in the plaque formation as you can see here, that almost like 60-70 percent of the blood vessel is clogged; as this plaques grows more and more what will happen, is the cells that are present in this area are not getting enough blood supply that they were getting earlier.

Then these cells do not get blood supply, they do not do their function; if they do not do their function in case of heart especially, the heart will stop beating and then the moment the heart stops beating, the patient is in extreme danger.

So, to prevent that what is done is this stent is used. So, you have a catheter that is being threaded to that site, once it reaches the site where the plaque was, a balloon is used to expand a metal stent that is present. And as metal stent expands it pushes on the plaque and actually physically moves the plaque away.

So, what you can see is now my blood vessel is almost open and all these cells that are downstream can survive. So, that is what is typically done in cases, where people come in with problems of plaques. And this is actually very serious if you do not do this will more or less lead to a heart attack very soon, where a part of the heart just stops beating and the person is not getting enough blood supply throughout the body.

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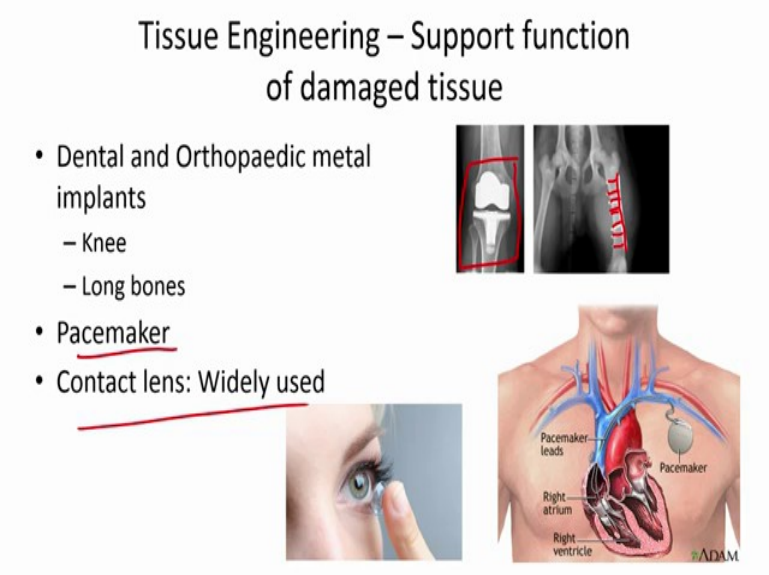

Another example of this is we briefly discussed anyways, which is the total knee replacement. So, in this case you can see an X-ray of a knee that has been replaced by metal. So, if the cartilage of the knee was completely damaged or some long bones have fracture and need structural support, you can see screws in metal plates being put into the patient body. And that will allow a patient to actually bear weight and walk around even though the bone is actually fractured and the bone can then heal over time.

And in this case, this is not going to get healed, you just replaced it completely with a metal joint and this will help the person to walk around again. Then pacemakers are used quite a bit, so sometimes the signaling is not proper and because of that the heart beating is very erratic. So, you can use a pacemaker which will give electrical signals in a

coordinated manner so that it beats synchronously and supply blood all over the body. And then contact lens that again very widely used, you probably know people who use or you yourself are using these contact lenses.

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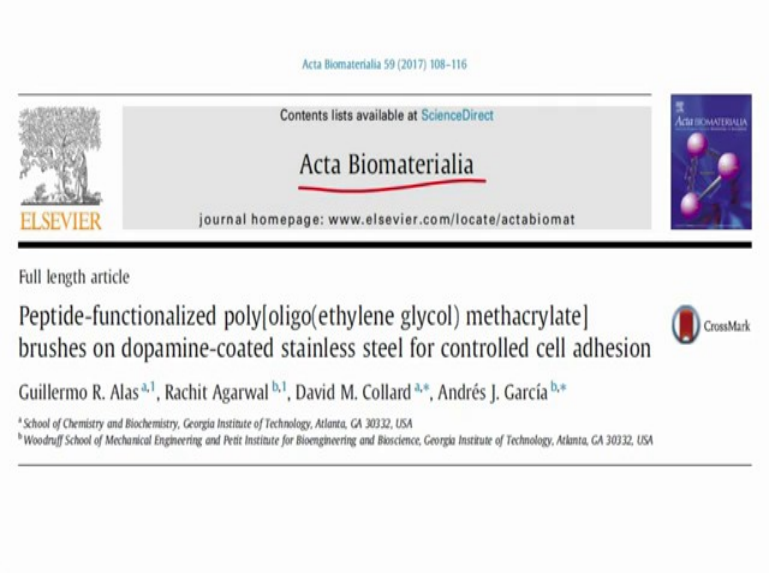

So, let us briefly discuss another paper as an example here. So, this paper is published in Acta Biomaterialia and it is using peptide functionalized brushes for controlled cell adhesion and so let us talk about why this is required.

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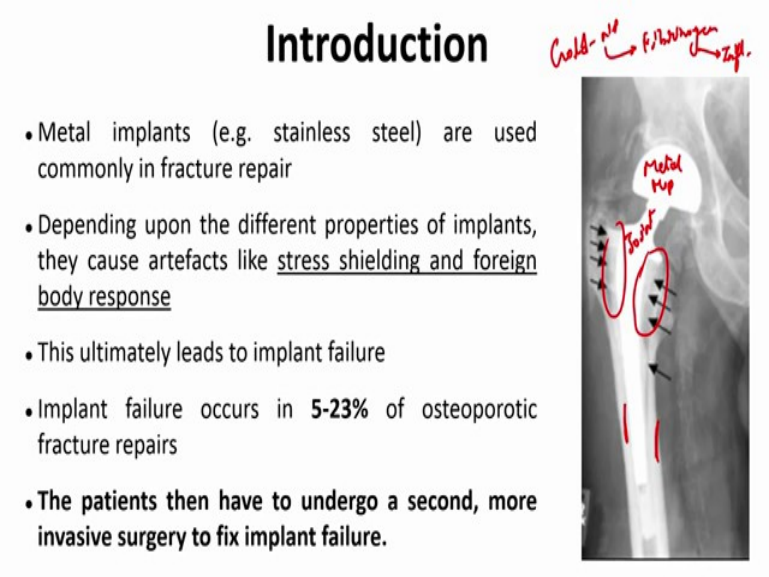

So, as an introduction what you see is let us say I have put in a metal hip joint. So, in this case this is a metal hip joint, but what is often seen is the body sometimes do not really like that there is a metal present in the vicinity.

Because once this metal put, there are all kinds of proteins adsorbed to it and we already learned what happens if not the right kind of protein to absorb to it, they expose some sites which may cause inflammation. So, we know that from the gold nano-particle which was absorbing fibrinogen and then was causing inflammation. So, if something like that happens then this implant will eventually get rejected because what you are starting to see here is; there is not enough integration of this hip bone.

So, this is your normal bone and what you are seeing here - so this is the typical density of the normal bone, but as you see here the density of the bone is actually decreasing. So, because of the inflammation the bone is not really lagging this hip joint, the metal joint that you put in, and its slowly degrading it out.

And so that is a serious issue because if all the bone that is present in the surrounding goes away, then there is nothing to stabilize the metal joint. So, you put there in the metal joint stabilize the bone, but what you are eventually doing is causing more damage to the bone. So, it can happen in few cases and we want to prevent that.

So, again these metal implants are used commonly for fracture repair; depending on the different properties, they can cause artefacts, they could be foreign body response, they could be stress shielding maybe and the mechanical properties do not match up; there are some issues. So, this will eventually to implant failure and now the patient will have to go back to the hospital, they will have to take this metal out they will have to put a new metal and that can also still lead to the same problem.

And then this failure rate is actually quite high. In fact, in some of the osteoporotic is it can be as high as 23 percent; so, basically everyone in fourth patient will have this implant failure and will have to come back for the surgery. And as I said the patients will have to undergo a second and a more invasive surgery because now not only you have to put a new thing, but you have to first remove the original thing that was put in. And so it is very invasive, very costly very painful; so it is something that needs to be avoided.

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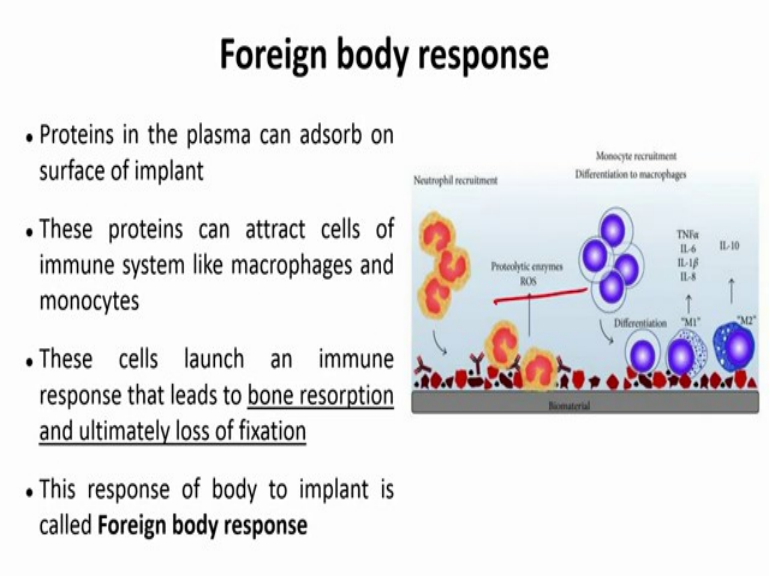

And again just a quick thing on the foreign body response. You can have all kinds of proteins getting deposited. And if they are triggering inflammation then these things will attract macrophages and monocytes from the immune system. This is going to launch a whole immune response all kinds of cytokines, all kinds of enzymes being released and eventually the bone around it because of all this inflammation will dissolve. So, this is essentially called a foreign body response.

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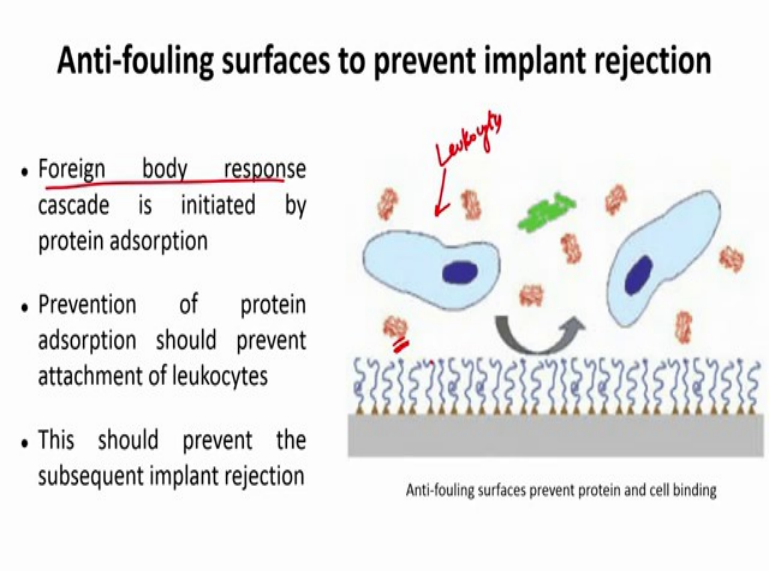

In this case what the authors are trying to do is to prevent protein adsorption completely. So, as we have discussed there are a few strategies where you can prevent protein adsorption; hydrophilic surfaces are better.

So, what is done is if you do not even have a protein adsorption, then this foreign body response cascade cannot be initiated. So, prevention of protein adsorption will also lead to prevention of the attachment of immune cells. So, all these leukocytes will not be able to bind to it because there is no protein to it.

So, I mean the leukocytes cannot really bind to these hydrophilic chains and they need some sort of protein to bind to. But if the protein is not present then you will not have these immune cells coming in and so this will prevent subsequent implant rejection. So, this is basically the whole concept of this paper.

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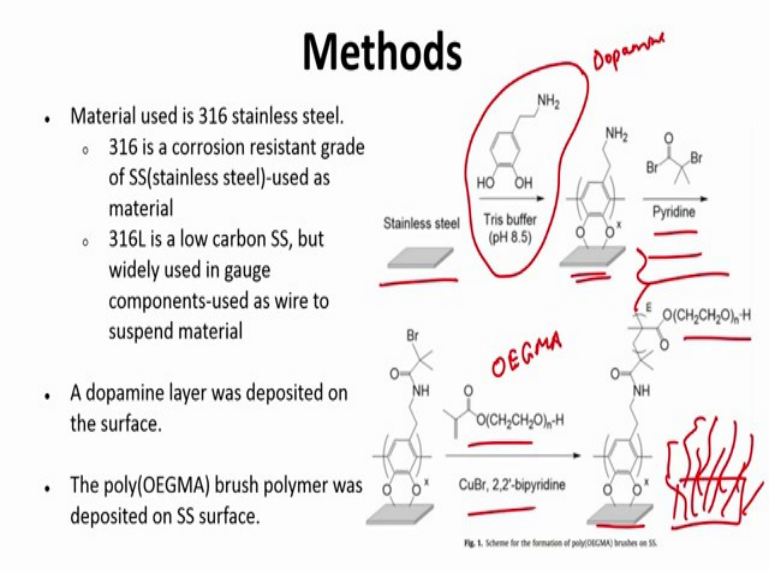

So, what then they have done is they have showed that they can take some stainless steel slides. They can do some chemistry on it. So, in this case they have added a compound called dopamine; they coated this onto the stainless steel.

They have then activated it with some initiator and then in presence of certain catalyst; they have reacted it with OEGMA. These are nothing, but small polymer chains we discussed about OEGMA in our polymer drug conjugates. So, what they are now doing

is they growing OEGMA which is essentially this growing chain with a peg pendant right.

So, this is going to continue and then the (Refer Time: 18:42) pendants are going to be there; so, it is going a make it very hydrophilic. And as we already know this is this act as a windshield wiper to make sure that none of the proteins are able to interact with the surface. So, this surface will essentially, if I zoom in, then they are essentially lots and lots of polymer chains that are here and they will not going a let the protein to absorb to it.

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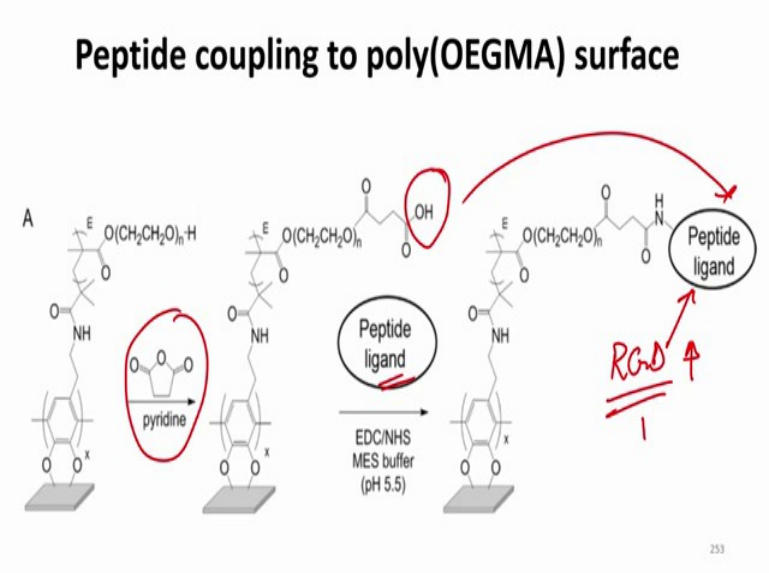

And then what they can do further is; let us say if I want to get even better a signaling from the cells, let us say I prevent the immune cells for coming in but what about the bone cells? I do want the bone cells to come in and sort of deposit more bone in the surrounding. So, then what I can do is or what these authors have done is then they have reacted it further to add peptide ligands.

So, they have used all kinds of chemistries here, but essentially what they have done is; they have modified this hydroxyl group and to add peptide ligands. And then these peptides depending on what you are using something like RGD; which is commonly used, you can then mediate what kind of signaling happens to the cell. So, you can give a signaling that causes the upregulation of bone deposition and interaction with the cell.

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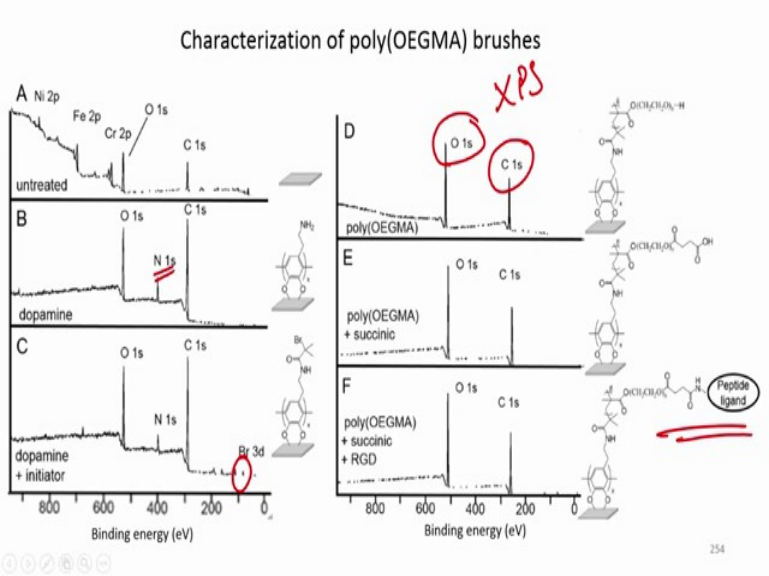

So, that is the whole concept; so here are some of the results. So, they have done some characterization of these surfaces. So, what they find is that the modifications happen; this is an XPS image to find what elements are there. And so what they do find that, yes, compared to the untreated you see a very different profile where dopamine has come in, a lot of nitrogen has come in on the dopamine.

And then you have added an initiator where a bromine peak showed up and then as we further went ahead; we are seeing that once the polymer was put in the polymer is mainly composed of the oxygen and carbon. So, that is what you predominantly see on the surface and then you can further modify it and get to your peptide ligands; which are again all carbon and oxygen based. So, you do not really see much change in that scenario.

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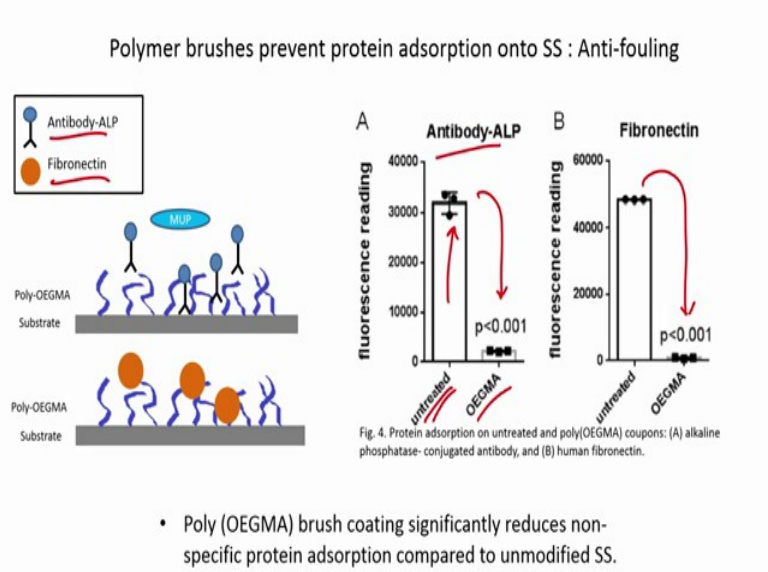

So, now that they have done it; they tested it out whether actually the protein adsorption is reduced. So, they have used two proteins here, one is an antibody which is something that activates immune response and then another is fibronectin which is again something that can cause binding of a lot of cells to these surfaces. It is an ECM protein.

So, what they find is on an untreated surface you get a very high reading of your antibody on the surface whereas, once you modified with the polymer this is vastly reduced. The same thing, there is almost none adsorption on fibronectin as well. So, essentially what they are saying is a poly OEGMA brush coating significantly reduces the nonspecific protein adsorption, when they compared it to the unmodified stainless steel.

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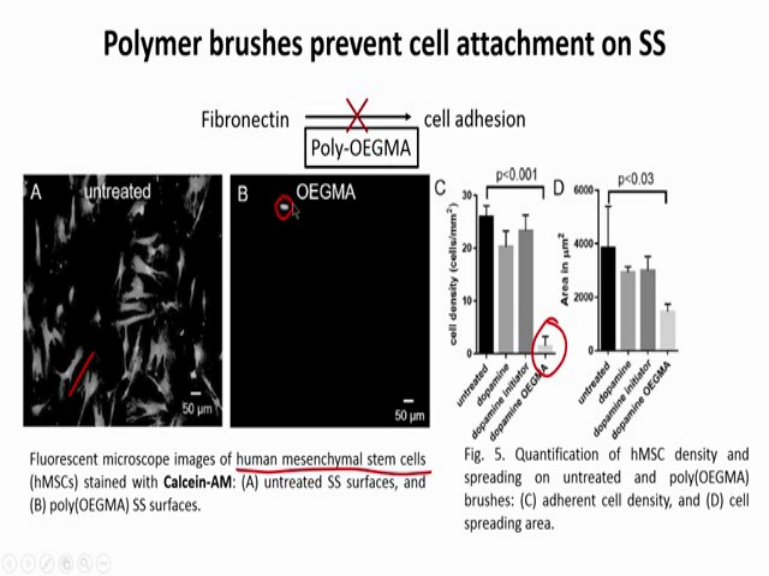

So, essentially fibronectin causes cell adhesion. There is poly-OEGMA, there is no fibronectin, and there is no cell adhesion that is going to happen and that is what you see here.

So, these are untreated surfaces they are cells; so in this case they have used a human mesenchymal stem cells. And what they find is on untreated surfaces, the human mesenchymal stem cells are growing well, they are spreading, but then as polyOEGMA is put in; there is a vast reduction in the amount of cell density that is present on this, hardly any cell that even see. And even the little bit of cells are there the area is also very low. So, not a whole lot of area, that means the cell is not spreading; it does not like the surface.

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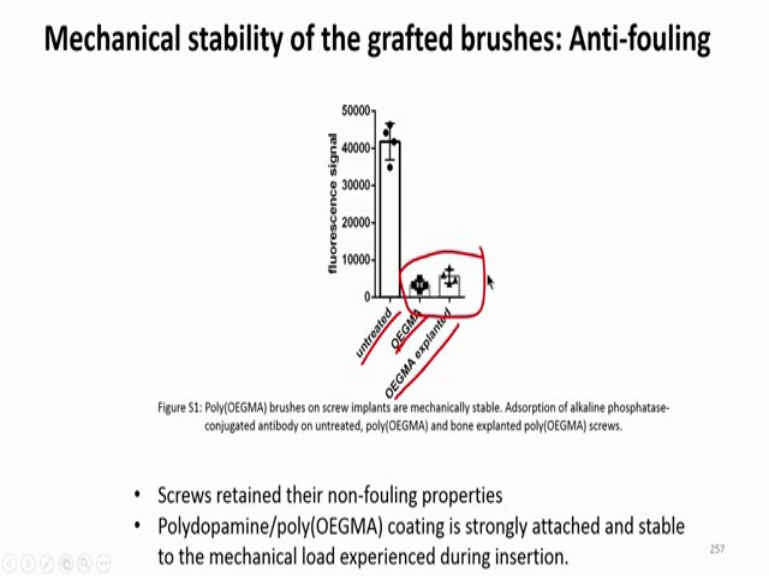

Since it is going to use for bone and let us say bone screws, bone metals, there is going to be a lot of mechanical stress on it. So, then they tested whether these coatings survive in this. So, what they did is they used untreated surfaces and the OEGMA surfaces and then what they did is they also used a surface which they implanted in a cadaver and explanted it. Just to mimic some of the mechanical forces that this surgery will cause on these metals. So, there could be all kind of screwing and all kinds of pushing and pulling across this. And what they find is these coatings do survive, they do not see any difference between the original OEGMA and the OEGMA that went through the surgery. So these coatings are fairly stable. So, in this case they were using screws.

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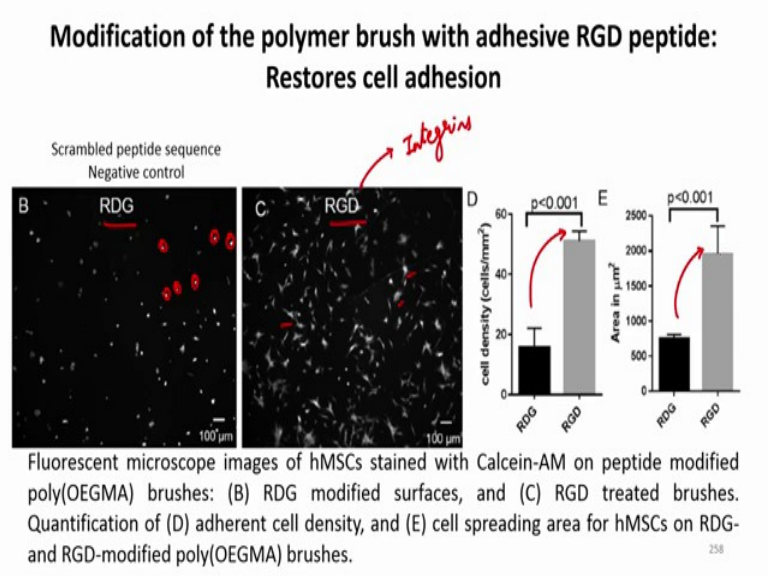

And then they went ahead and further modified it with the adhesive ligands. So, in this case they used a scramble peptide control which is RDG to which the cells are not going to interact to. And RGD which binds to the cell through integrins and what they find on RDG surface again you see less number of cells as well as more rounded cells with a very little area.

So, the cells are not liking the surface, but you can modify it with RGD and you can start seeing spreading of the cells everywhere. So, that is a good indication as to the cells are now starting to like the surface this is more quantified here. So, the cell density has increased; as well as the area of the cells that are attaching has also spread areas also increase, suggesting that the cells have now started to like the surface ok. We will stop right here and we will continue rest in the next class.