Medical Biomaterials
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Lecture- 18
Biological responses

Hello everyone. Welcome to the course on Medical Biomaterials. Today, we are going to talk about biological responses. So, when you implant a biomaterial or when a biomaterial comes in contact, what are the responses that are going to happen? So, may be this class and the next class is going to be little bit biology, those who feel that it is too much, may be can just look at it superficially, but I think students with the biotechnology background or medical microbiology background or medical biotechnology background should be able to appreciate this.

And it is very important that you also come to know- what are the various biological responses, mainly platelet activation, blood coagulation, inflammation, complement activation all these are very, very important, because you have a foreign body that is being placed inside the human system. And we cannot just ignore, an engineer cannot just ignore this aspect and believe that a biologist or biotechnologist will take care of that. So, we need to know little bit, but it is going to be little bit heavy in the area of biology now in this class as well as the next class.

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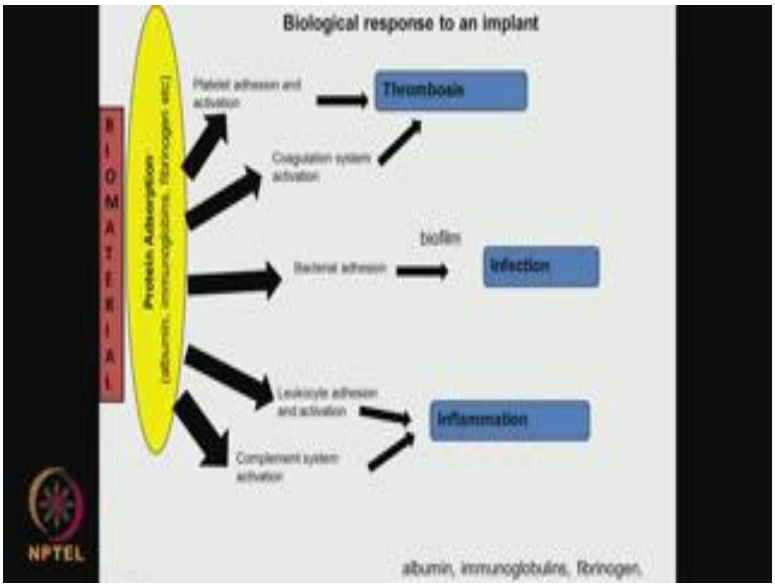

So, if we look at this slide I talked about in the previous class also. So, we have a biomaterial immediately proteins get absorbed, proteins like albumen immunoglobin, fibrinogen etcetera. This can have the platelet adhesion and activation this can also have effect of coagulation system activation, which can lead to thrombosis. Bacteria can absorb on top of the proteins some of the proteins enhance bacterial adhesion some of them do not, so there is a biofilm formation this leads to infection. We did talk about this aspect quite a lot in may be a few classes back if you remember.

Then we have these leukocyte adhesions, activation complement system activation leading to inflammation. So, all these are happening as soon as a biomaterial is placed. So, we need to understand little bit of this coagulation system platelet adhesion thrombosis as well as the aspect of inflammation.

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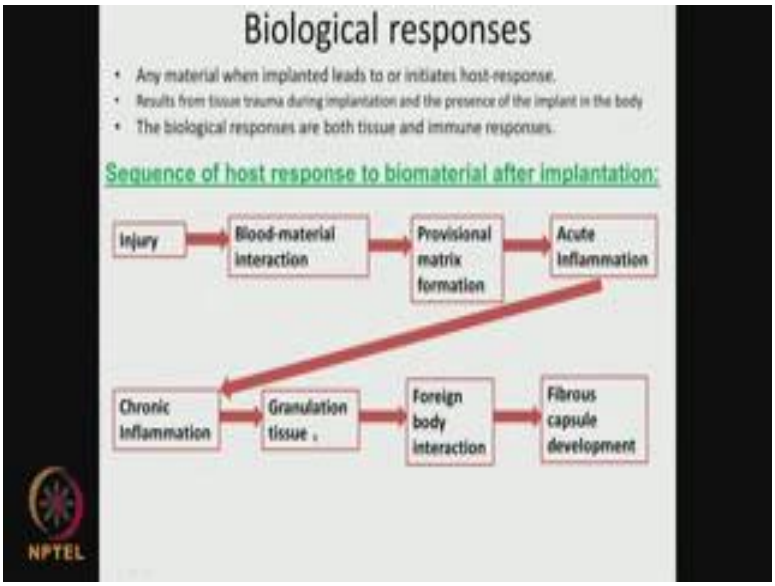

So, when any material when implanted leads to or initiates host response because that is a defense system, so many things can happen the host takes care; results from tissue trauma during implantation because when there is a surgery done opened and medical device is placed. So, there could be a trauma, and presence of the implant in the body. So, this foreign material is going to be present inside, it would be weeks, it could be years it could be for the rest of the life. So, there could be a tissue response, there could be immune response, because immune system in the human body is very, very important

it is our defense when there is a foreign body immediately the immune system takes over and tries to destroy this foreign body.

So, there is an injury because the physician or a surgeon has opened and placed biomaterial. So, there is an injury, the material there is blood material interaction, there is a matrix formation, acute inflammation taking place, chronic inflammation, then there is something called granulation tissue, foreign body interaction, fibrous capsule development. So, the whole fibrous tissue tries to encapsulate your entire biomaterial, so that us lot of defense things happening. So, you have inflammation taking place here the acute inflammation and the chronic inflammation, and then lot of immune response taking place here.

And here you could be having blood material interaction, platelet activation, and there could be thrombosis forming all those things can be happening here. So, we will look at each one little bit in more in detail.

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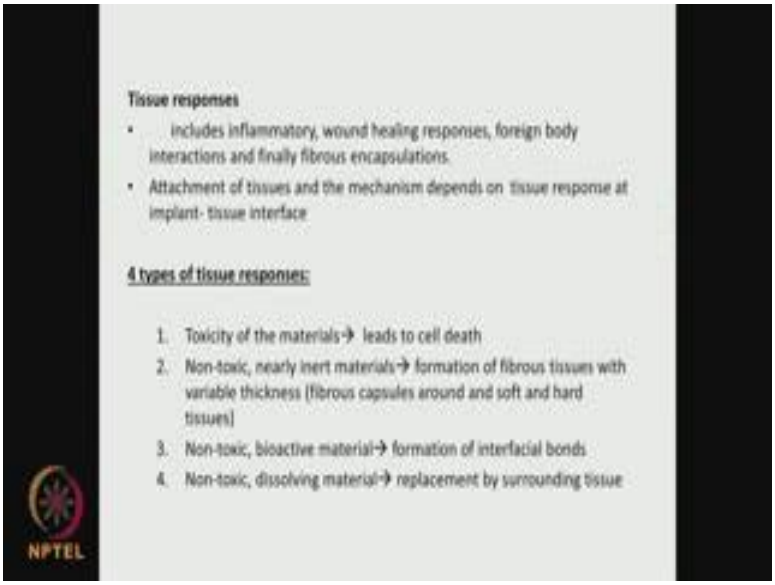

So, what are these tissue response, it includes inflammatory, would healing process, because the surgeon has opened created a wound and there is a foreign body. So, there is a wound healing response, foreign body interaction and finally, fibrous encapsulation like I showed you, there is a fibrous encapsulation that is taking place here. So, what are the four types of tissue response, toxicity of the material, because the material itself could be toxic which may be killing your cells, there could be some lichen. For example,

its known poly methyl acrylate is made up of acrylic acid, acrylic acid could be little bit toxic to the cells which may lead to cell death, polylactic acid is not toxic, but the lactic acid could produce some acidic response which could be leading to cell death; so toxicity of the material.

Non-toxic nearly inert material; so if you have material which are absolutely non-toxic then you are going to form fibrous tissues of various thicknesses. So, there is going to be fibrous capsules around this could be soft and hard tissues. Then non-toxic bioactive material formation of interfacial bonds, because the material itself is bioactive, so it interacts with the biological system, so there is a interfacial bonds are formed. Non-toxic dissolving material replacement by surrounding tissue because the material is getting dissolved; so we can have four types of material materials which gives you toxicity, there are material which are inert not toxicity, there are material which are non-toxic and they are bioactive, and there are material which are non toxic and they disappear.

So, all these leads to different types of responses as you can see on the right hand side. So, we need to understand each one them little bit in more detail.

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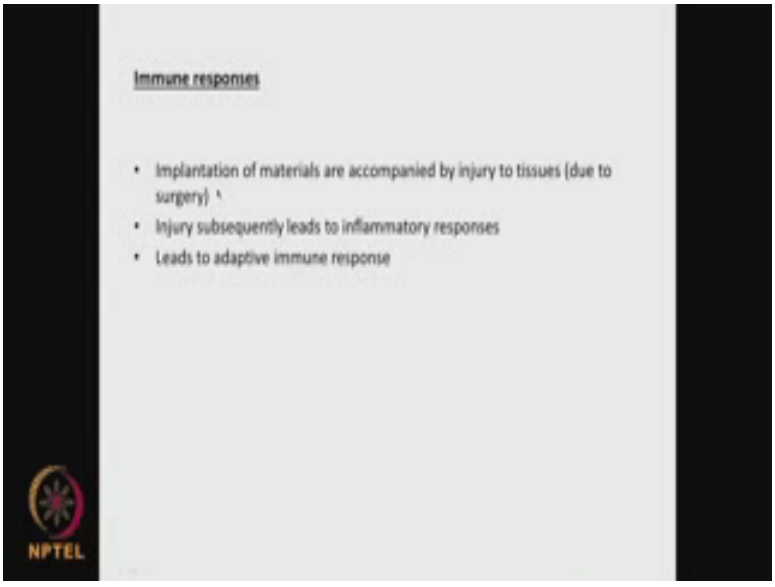

So, you are implanting a material. So, there is injury to tissues due to surgery injury subsequently. So, all this leads to inflammation and so there is going to be adaptive immune response because of all these.

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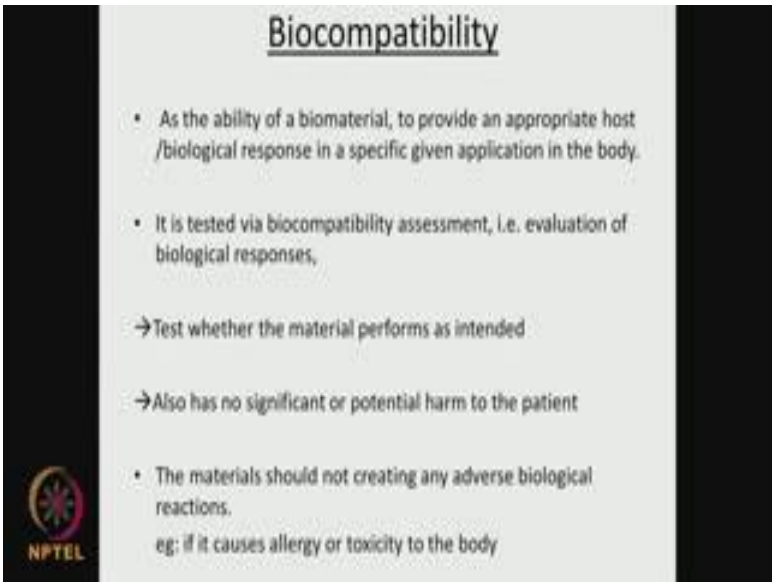

So, what is this biocompatibility, very, very beginning in the beginning I said all biomaterials have to be biocompatible. So, this is the ability of a biomaterial to provide an appropriate host or biological response for a given application in the body. There are many ways by which we can do biocompatibility assessment, I did talk about it in the tools we used different types of animal cells, and see the cell death, we looked at cell proliferation cell adhesion and we also looked at whether there is a membrane damage and so on actually. Test whether the material performs as intended.

Also has no significant or potential harm to the patient. The material should not be creating any adverse biological reaction there should not be any allergy coming into, for example, one material might not cause allergy to me, whereas the same material may be causing allergy to somebody else because of metal related allergy or polymer related allergy and so on. So, there should not be allergy there should not be toxicity to the body.

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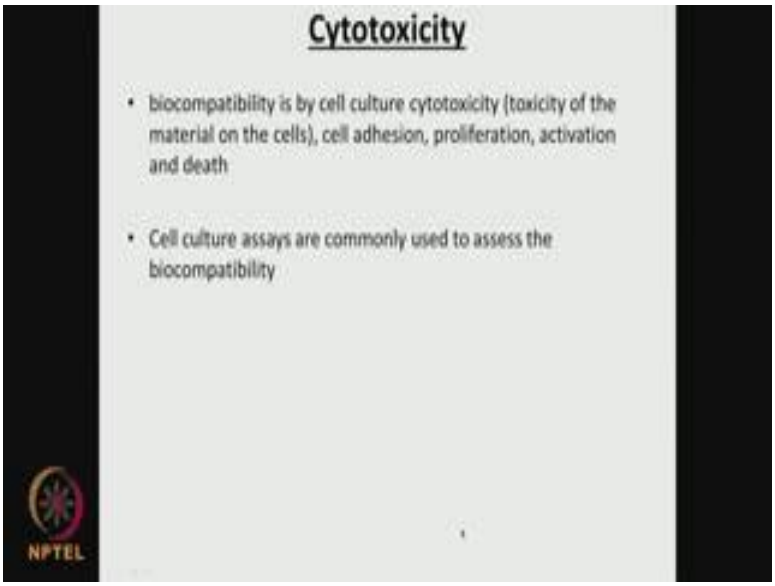

Then cytotoxicity is biocompatibility is by the cell culture cytotoxicity. So, what we do is we test the cells and see whether the cells are able to live or not get killed and we look at the proliferation, cell adhesion, proliferation, activation and death. So, these studies help you to understand whether the material is cytotoxic or the leeching the lichens from the biomaterial also cytotoxic. Generally this is done cell culture assays we did talk about it couple of classes back if you remember.

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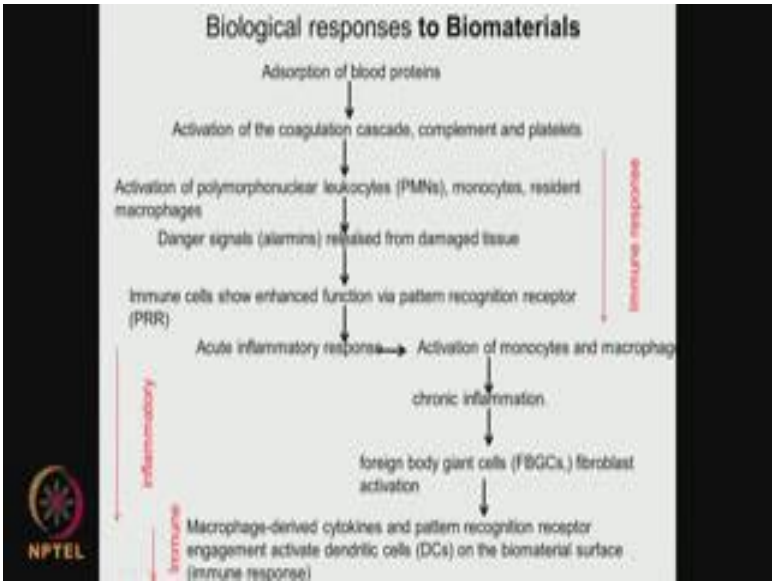

So, further into biological response to biomaterials, so initially as I said proteins get absorbed, then there is an activation of the coagulation, cascade activation of the complement activation of the platelet. So, blood contains lot of these. Then activation of polymorphonuclear leukocytes, it is called PMN, monocytes, resident macrophages. Then there is a danger signal released from damaged tissues. Then immune cells so enhanced function via pattern recognition receptor they are called PRR - pattern recognition.

So, all these are called immune response. So, activation of the coagulation cascade related to the blood. So, the blood tries to coagulate complement platelet, activation of PMN that is polymorphonuclear leukocytes monocytes resident macrophages that is your defense mechanism immune. Danger signals are released from the damaged tissues. So, the immune cells show enhance function via pattern recognition that is the immune.

Now we have the inflammatory response. So, there is an acute inflammation. So, it leads to activation of monocytes and macrophages, chronic inflammation, so foreign body giant cells fibroblast activation, so all lot of foreign body giant cells are formed. Again goes into immune response macrophage derived cytokines and pattern recognition receptors, engagement activate dendritic cells that are DC on the biomaterial surface. So, all these are formed. They are trying to engulf the biomaterial that is the immune system. So, as soon as the blood proteins get absorbed, so we have the immune response, we have the inflammation response, so many things start happening here.

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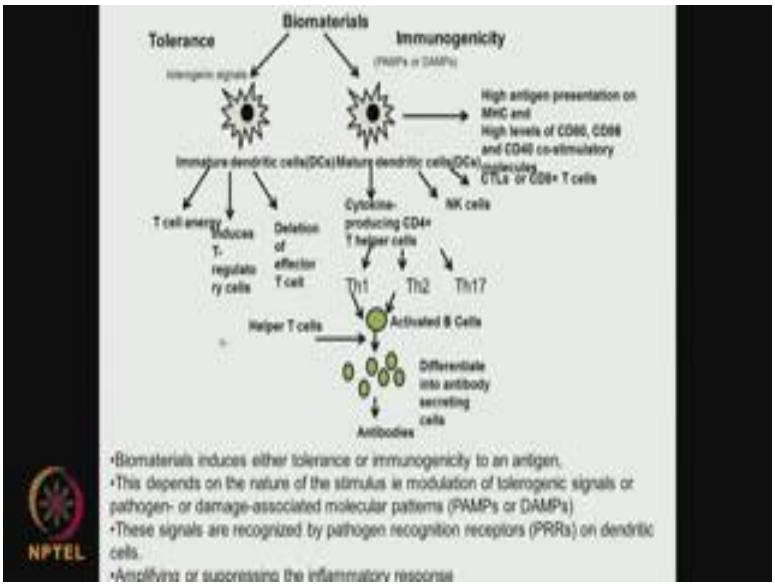

So, we have the immune response happening here, there is a long cascade. Biomaterial induces either tolerance or immunogenicity this is called the tolerance side, this is called the immunogenicity side. So, in the tolerogenic signals, we have T cells induced they are called T cells here where as in immunogenesis, we are going to have a polymorphous nucleosides, high antigen presentation, so many steps are happening. So, we get activated B cells then differentiated antibody secreting cells these are the real defense which tries to kill. The antibodies are produced which tries to destroy your biomaterial. So, the biomaterial induces either tolerance that is this side or it produces immunogenicity, so that you get antigen antibody.

So, this depends on the nature of the stimulus that is modulation of tolerogenic signals or pathogen or damage associated molecular patterns or damage associated molecular patterns. These signals are recognized by pathogen recognition receptors that are PRR as I mentioned here on dendritic cells. So, amplifying or suppressing the inflammatory response. So, biomaterial if it is either inert or bioactive can lead to tolerance or it can lead to immunogenicity. So, during the process of immunogenicity, we are going to have antigen antibody type of reaction taking place here through a series of steps. So, let us not worry about all these various factors that come into the picture, but that is what happens when the biomaterial is placed inside the body.

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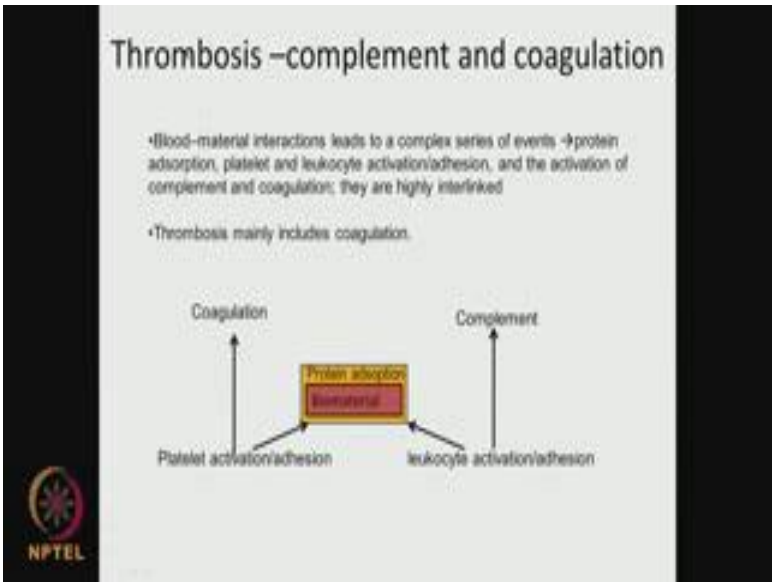

Now, let us go deeper. So, thrombosis complement and coagulation all these things are going to happen as soon as the material comes in contact with the blood, the blood material interaction. Especially if you are going to have a cardiovascular stent if you are going to have a diaphragm heart diaphragm or if you are going to have small diameter vascular graft or large diameter vascular graft, so all these are blood-contacting device. So, the material should not lead to the activation of the platelets or the leucocytes, all those which can lead to quite complications.

So, the blood material interaction leads to a complex series of events, protein absorption, protein gets absorbed in the biomaterial, platelet and leukocyte activation, activation of complement and coagulation. So, as soon as this forms, you are going to have blood coagulating and settling down on the biomaterial, which may lead to thrombosis. So, the blood material interaction this leads to protein absorption first, blood plasma protein, and fibrinogen and all so on. Then we are going to have platelet and leukocyte activation, adhesion activation of complement and coagulation leading to thrombosis also.

So, we have the biomaterial, we have the protein absorption here. So, we can have one side platelet activation leading to coagulation, we can have leukocyte activation leading to complement. So, both these things can happen for blood-contacting devices. So, you need to understand little bit of this as well as little bit of this and that is what we are going to show in the next slide.

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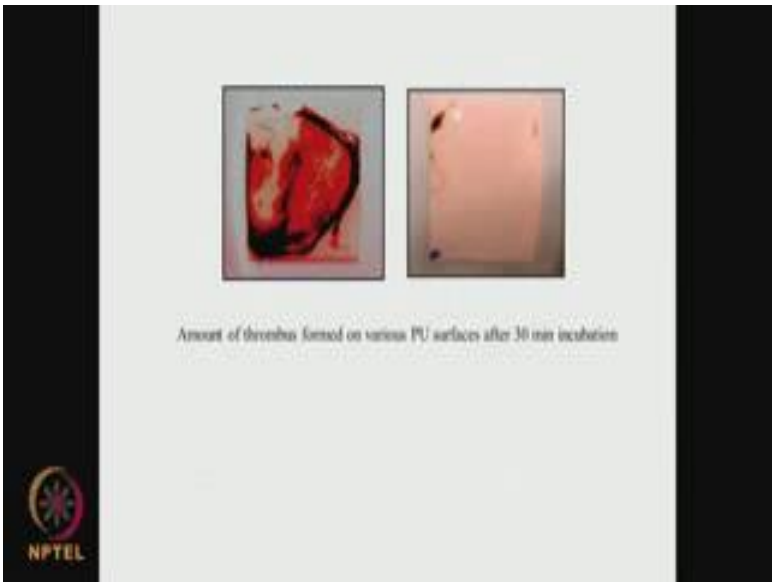

For example, look at this we used to do some experiments on polymers which are in the area of blood-contacting devices. So, when we incubated with the blood, after 30 minutes of incubation, we can see thrombus formation. Here we modify the polymer, so that it does not lead to these activation and hence the thrombus formation. So, this is modified polymer, we can see there is no thrombus formation. So, there is lot of difference. So, blood contacting device ideally you should prevent the thrombus formation, whereas here you can see it is thrombus formed polyurethane finds application quite a lot in biomaterials, because it is very flexible almost like a rubber and it is very pliable. So, we can do lot of things on that that is the advantage of using polyurethane. So, polyurethane is used in diaphragms and so on actually.

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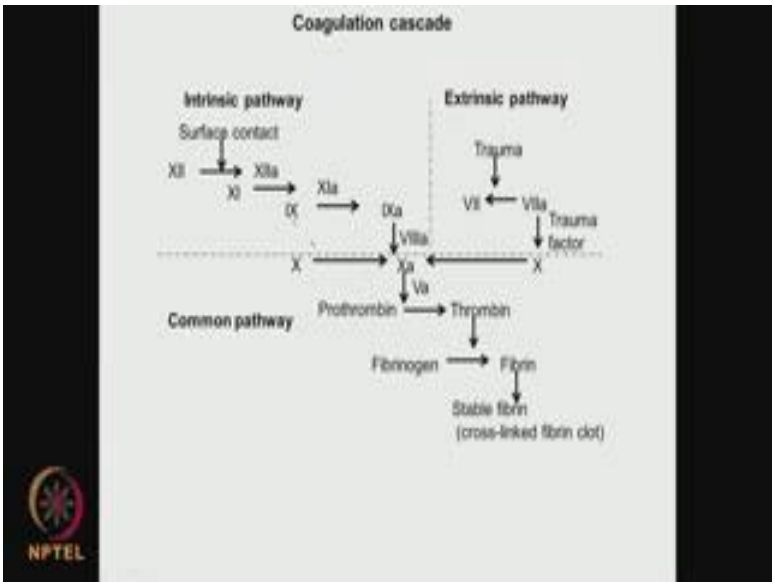

So, let us look at this coagulation that is this coagulation here. There are many factors in coagulation, one is called the intrinsic pathway other is called the extrinsic pathway. So, we have the surface. So, there are many factors factor 12, factor 11, and factor 9, 10 and so on. In the intrinsic pathway, intrinsic as the name implies the material is in touch with the surface the blood is in touch with the surface. This is extrinsic because of trauma some factors are getting activated.

So, there are lots of factors here as we can see many factors due to intrinsic or due to extrinsic. So, we have prothrombin, it gets activated by this factor five A leading to thrombin, which activates fibrinogen to fibrin. And this fibrin is stable cross-linked which forms the blood clot; it is useful because when there is an injury we need the blood to clot. So, these factors are very, very important so that the blood clots.

But then when we are using a biomaterial the biomaterial is elucing this particular response for the blood to clot. In that case, we it is not desired where as in real life we need the blood to clot if there is a injury whether it is open or inside injury. So, all these factors are very, very important for the clotting of the blood especially the fibrin which comes on fibrinogen activated by thrombin. The prothrombin gets activated to thrombin which converts fibrinogen to fibrin this stable fibrin is the clot. But in the presence of a biomaterial, these two pathways could get activated. So, there are many factors which get activated. So, there could be formation of blood clot like as I have shown in this

picture. Whereas in this picture as you can see another modified polymer, there is no blood clot formation. So, one side of it is coagulation blood clotting, the other side is the complement which comes from leukocyte activation.

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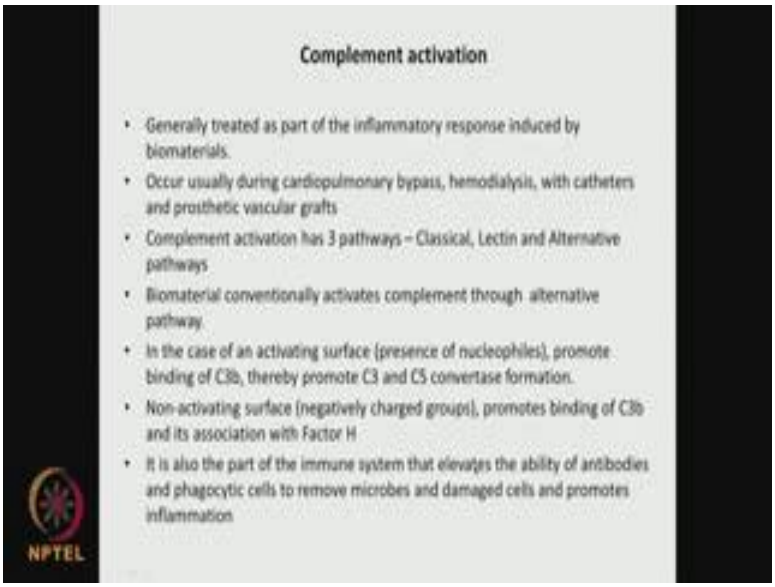

What is this complement activation? This generally is a part of the inflammatory response. This occurs usually during cardiopulmonary bypass, haemodialysis with catheters and prosthetic vascular grafts. So, we are talking about materials like polysters, polytetrafluoroethylene and so on. This complement activation has three pathways classical, lectin and alternate. And biomaterial generally works on this alternate pathway. In the case of an activating surface, presence of nucleophils, this promotes binding of something called C 3 b which promotes C 3 and C b convertase formation where as non-activating surface promotes binding of C 3 b and its association with factor H.

Now this complement activation is also part of the immune system that elevates the ability of antibodies and phagocytic cells to remove microbes and damaged cells and promotes inflammation. So, the complement activation is also important in normal human system, because it helps to repair damage cells, it also helps to remove microbes through the phagocytic cells. So, in a normal human being, this process is very important, but when you have a biomaterial placed and this process is not desired.

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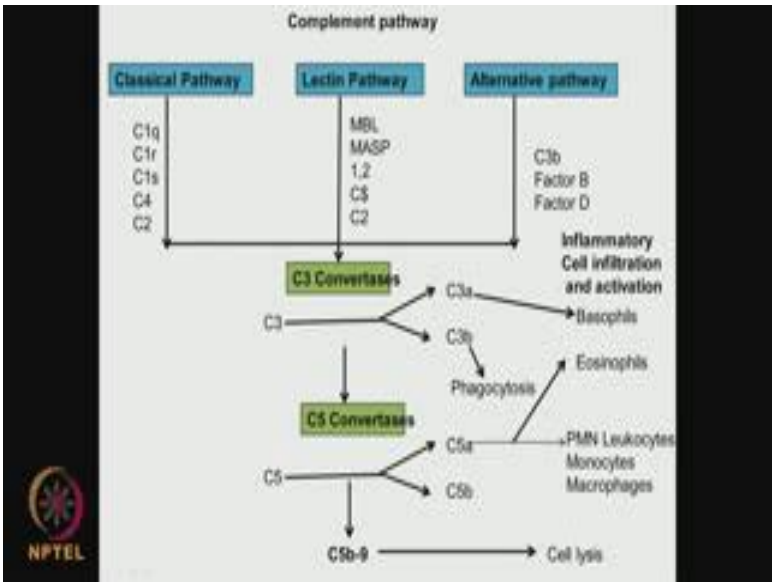

So, this complement pathway just like we looked at the coagulation cascade, we have lot of factors. Here also we have lot of complement coming into. So, classical pathway, lectin pathway, alternate pathway as I said generally biomaterials or foreign bodies elicit this pathway. So, we have inflammatory cell infiltration, activation then it leads to basophil, eosinophils, monocytes, macrophages and you can have cell death happening here. So, because of this pathway lots of complements are activated, this can lead to cell lysis. So, again going back protein absorption biomaterial, so we can have two things happening, the platelet activation, blood clotting coagulation or we can have leukocyte activation leading to complement cell lysis that is cell death.

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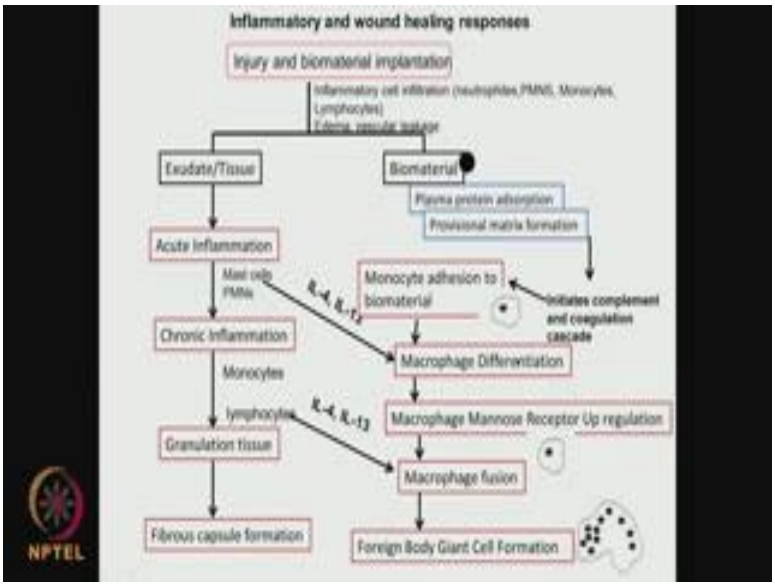

Then comes the inflammation and wound healing. So, we are going down if you go back and look at again our old picture, here we have the inflammation we finished the blood. So, we have the inflammation happening here. Or again if you go back here inflammation that comes slightly later, but we cannot really say later so things happen, so fast. So, it is very difficult to tell. So, there is inflammation and wound healing because the surgeon has opened the human part to place a biomaterial. So, wound is created and there is a presence of a biomaterial which also leads to certain inflammatory response and wound healing responses. So, injury and biomaterial implantation inflammatory cell infiltration like neutrophils, PMNS, monocytes, lymphocytes, oedema, vascular leakage all these things happen. So, two things can happen one is fibrous capsule formation or foreign body giant cell formation which just encompasses your biomaterial.

So, we have let us look at this pathway biomaterial. So, plasma protein absorption, provisional matrix formation this like I talked about complement and coagulation, monocyte adhesion to biomaterial, macrophage, differentiation, macrophage fusion foreign body giant cell formation. So, all these are happening in this route. Whereas we have exudate tissue, acute inflammation, these are called mast cells PM chronic inflammation then we have granulation tissues fibrous capsule formation. So, this is another route which tries to repair your injury and which tries to lead to inflammation.

So, we can have this route which leads to foreign body giant cells formation, we can have this route which leads to fibrous capsule formation. So, when you have a biomaterial and you are testing it out say for in preclinical trials in animals after a month may be you can see whether it leads to foreign body formation, it leads to fibrous capsules and granulation tissues. What is the extent of these tissue formations with respect to control all this tells you the response this biomaterial is creating in the host system.

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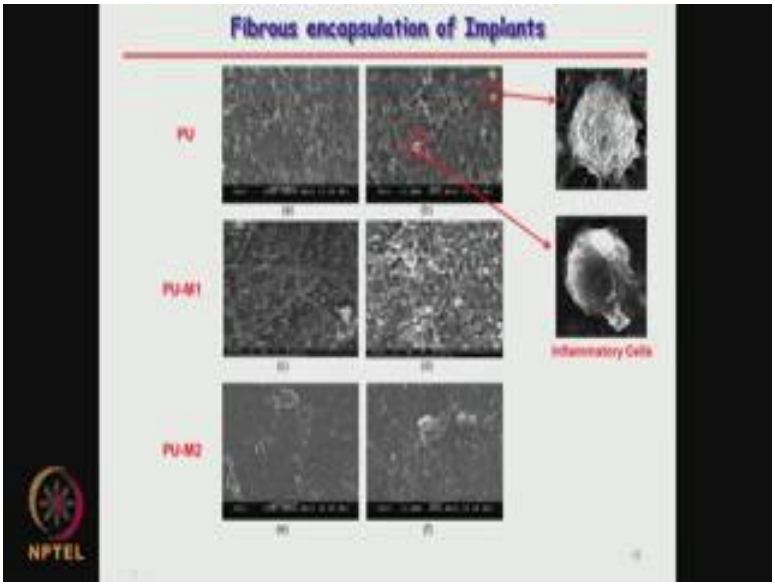

I just want to show some pictures scanning electron pictures of ours, where we are looking at fibrous encapsulation of implants. You can see this is a polymer, polyurethane, this is another polyurethane modified system, and this is another polyurethane modified system. We can see here this shows you how the fibrous encapsulation of implant is going to take place. So, we can see here, all these are called inflammatory cells.

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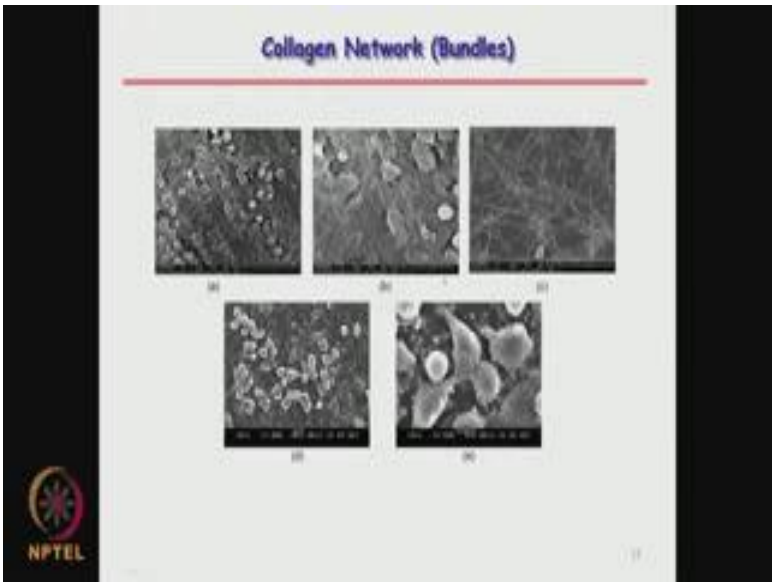

Similarly, collagen network bundles are also getting formed on the biomaterial. So, trying to encapsulate as you can see collagen network here collagen, as you can see here that is happening there.

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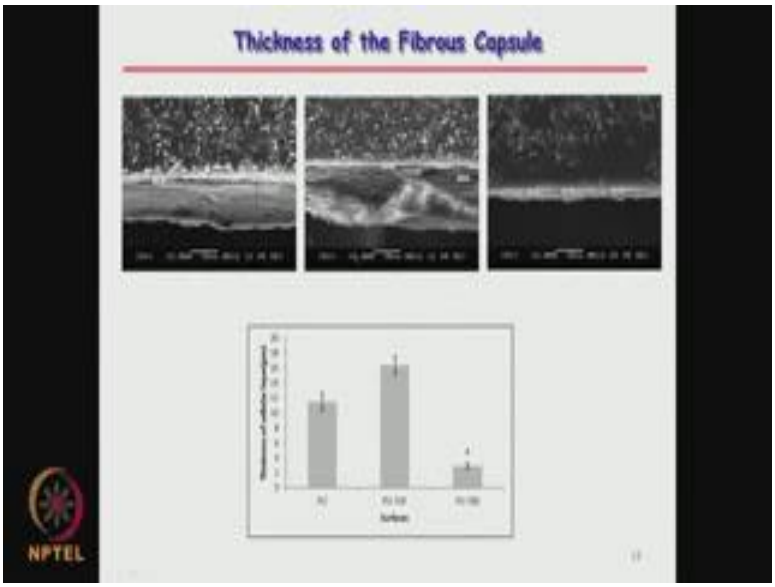

So, the fibrous capsule is trying to encompass the biomaterial. So, you can see in some cases thick bundles are formed, in some cases thin bundles are formed. So, the thickness some cases very large almost 18 microns in near about 30 days, in some cases we get only 4 microns as you can see here, so the fibrous capsule.

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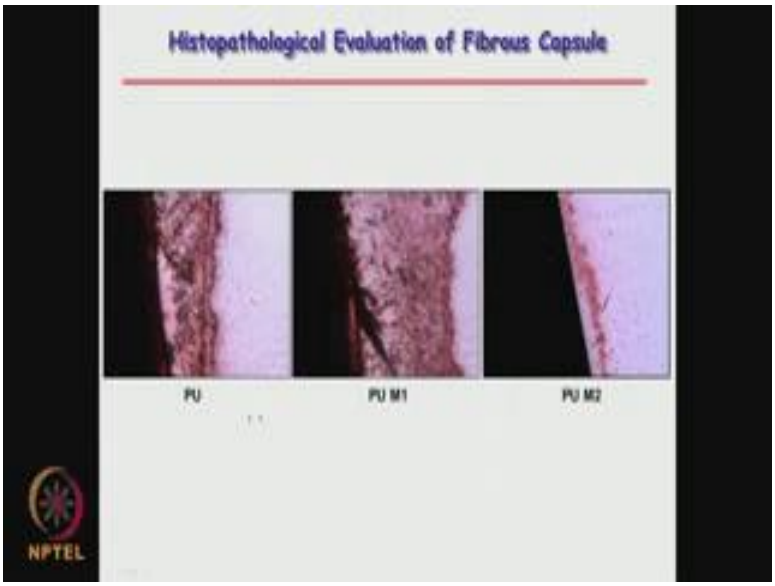

So, when you look at the histopathological, we can see the same thing fibrous capsules. So, in one case thickness of the fibrous capsule is very minimal, in some cases it is very large.

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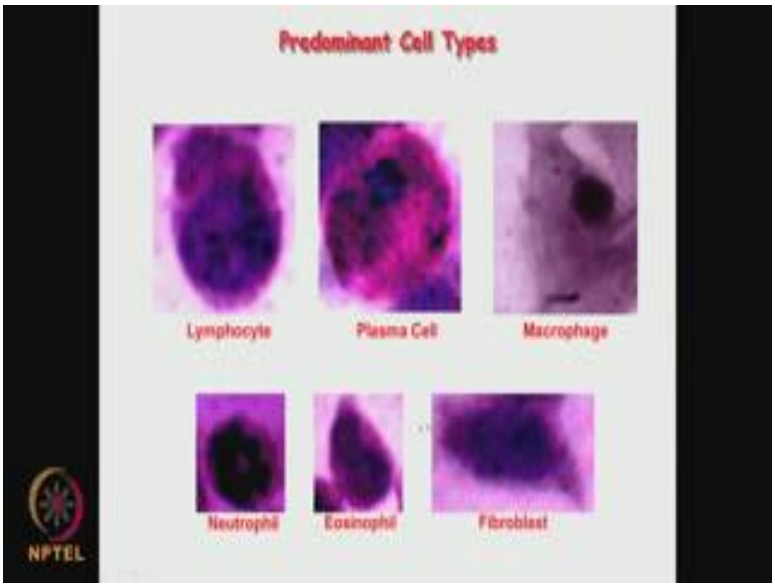

We can see different types of cells that are present whatever like lymphocytes, plasma cell, macrophages, neutrophil, eosinophil, fibroblast all these are various cells that are formed due to the inflammatory response due to the presence of the biomaterial as well as due to the surgery that was performed on the host.

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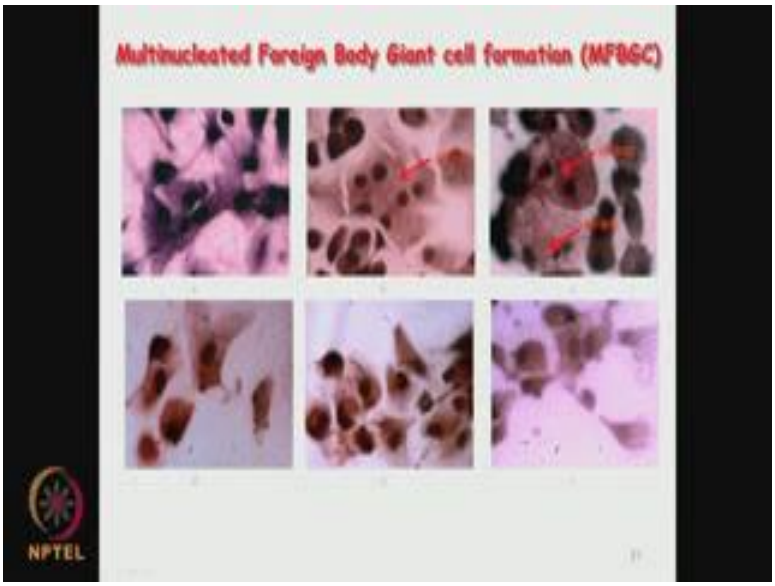

Again you can see multinucleated foreign body giant cells; you can see multinucleated foreign body giant cells. All these are pictures from our research when we place a biomaterial in a animal model, after 30 days we can see what these biomaterial, what type of response these biomaterials create in the host system. So, as you can see these are all multinucleated these are multinucleated right multinucleated foreign body giant cells formation after 30 days. So, we see lot of things happening, and all of them are towards the defense host defense system, especially if there is a blood-contacting device, you immediately have the blood plasma adhesion sorry absorption we would say, blood plasma absorption then the platelet activation as well as complement activation.

Then we also have the immune system response, and then we have the inflammatory response. So, the inflammatory response also is useful for the wound healing process and during that process we are going to have different types of cells form like the foreign body giant cells. And we are able to see fibroblast macrophages, plasma cells, eosinophil, neutrophil, lymphocytes and so on actually. So, the biological response is extremely complicated and little bit of this knowledge is very useful for a biomaterial researcher, so that how the system responds, and how does one modify the biomaterial to prevent this type of system responses.

And also depending upon the time duration of the biomaterial; for example, some of these pictures which I showed you now, these are all of almost 30 days of implantation.

So, if the implantation is much very, very less, you might not be really seeing this type of inflammatory response. And then again if the device is not contacting with the blood then you are not going to have platelet activation, thrombin and thrombosis and blood clotting, those aspects could be completely ignored.

So, we will continue more on these biological responses to biomaterials by the host in the next class as well.

Thank you very much for your time.