Drug Delivery Principles and Engineering
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Lecture – 47
Blood Clotting and Hemocompatibility of Materials; Adaptive Immune Response

Hello everyone! Welcome to another lecture for Drug Delivery Engineering and Principles. My name is Rachit and we are discussing the Immune Response module at this point. So, let us do a quick recap what we learned in the last class.

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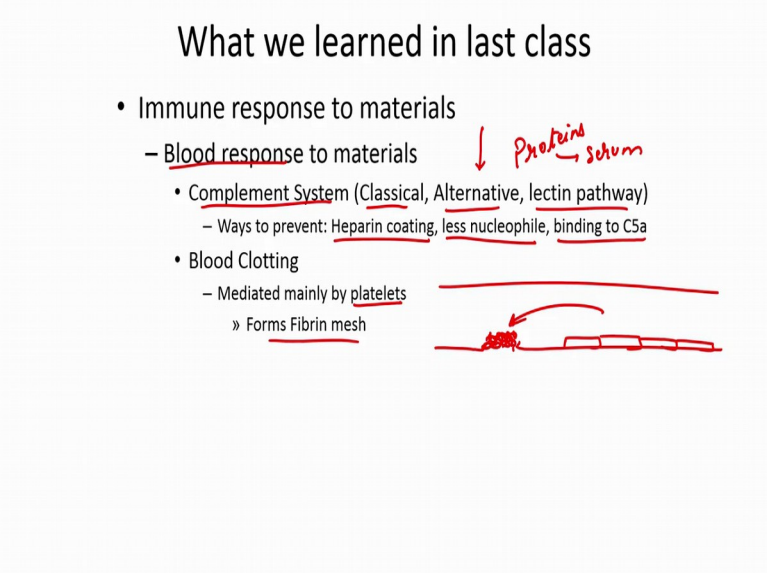

So, in the last class, as I just mentioned, we were looking at immune response to materials, and specifically, we were talking about looking at the blood response. So, we said that there are two ways in which the blood can respond, one is the complement system, which contains several proteins that initiates it. This is already circulating in serum and there are various ways in which this can be activated. The three major ways are named as classical, alternative, and lectin pathway. And, what we also discussed was that, alternative pathway is the major response that is causing the activation against the materials.

And there are few ways by which we can prevent that: one is to coat the heparin, which is going to inactivate the cascade, another is making your surface less nucleophilic. So,

making it such that they do not activate these complement proteins and then you can also bind it to C5a, so that will also result in prevention of activation. Then, next we talked about blood clotting. So, how does the blood clot? This is mainly mediated by platelets and they eventually lead to the formation of a fibrin mesh. So, if it is a surface or let us say this is a blood vessel and somehow the blood vessels are ruptured, these platelets just go in and form this aggregate of cells and then they reinforce it with a polymer mesh to plug this hole.

So, this prevents any kind of blood to leach out, but the problem is that, these platelets gets activated at any kind of surfaces which is not endothelial surface through various proteins that gets absorbed on these surfaces. So, once they are exposed to protein coated substrate, they will also do this and this can also lead to fouling of a surface. Maybe it is not going to perform the function that you wanted it to perform.

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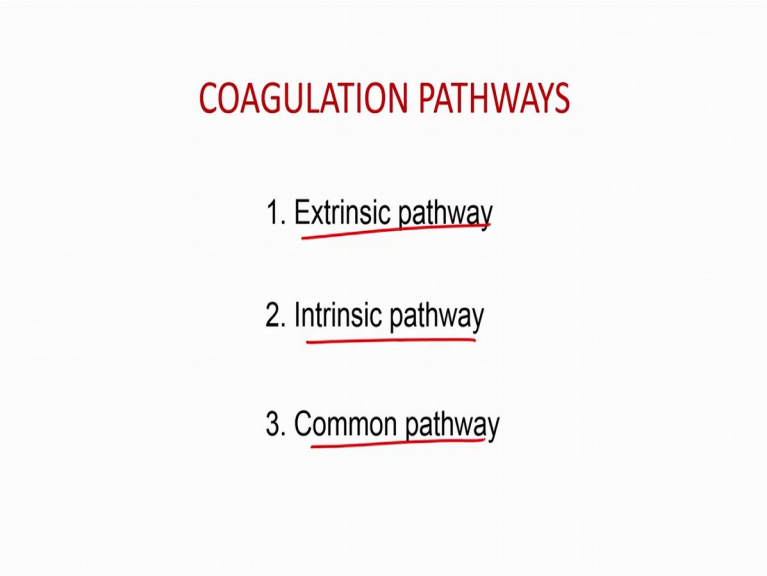

So, let us look at the blood coagulation pathway. So, just like the complement system and this is also divided into three common pathways, which is extrinsic pathway, intrinsic pathway, and common pathway.

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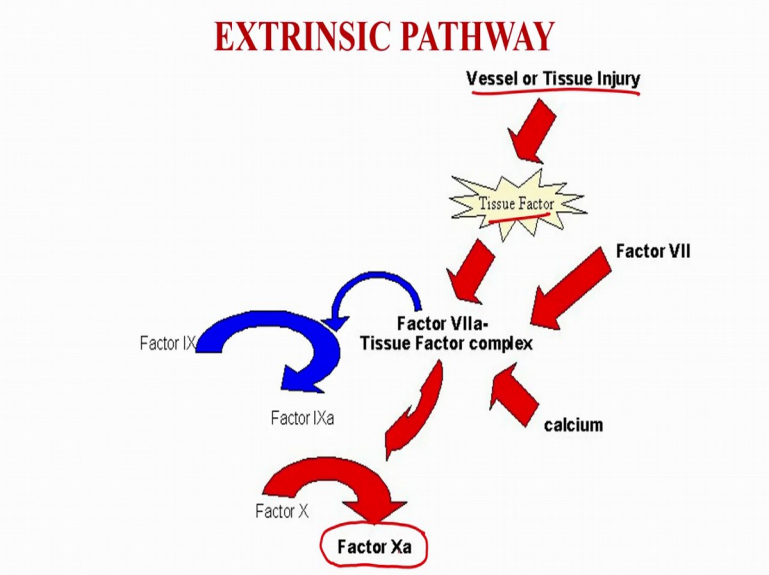

So, let us look at the extrinsic pathway. So, this is again a very simplified form that I am showing you. Just listing out the major proteins and major components that are involved. It is actually having huge lots of proteins are involved with this, so let us see how this happens. So, you have some vessel or tissue injury that has happened: this is because maybe you put in a needle, maybe you put in an implant or maybe there is some accident that the patient suffered.

So, that causes lots of tissue factors to get activated. This will then combine with several proteins that are present in the serum. And, eventually for the purpose of this talk what you have to remember is eventually that leads to formation of factor Xa, so that is a protein that gets generated.

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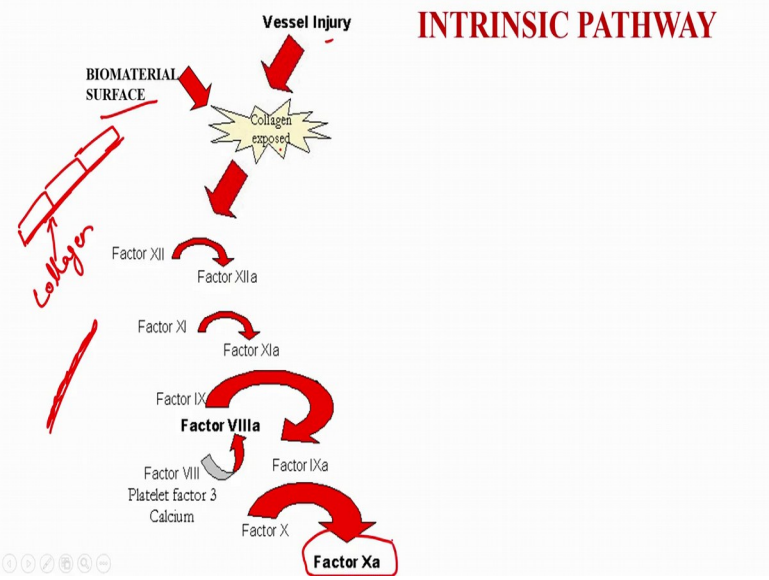

Intrinsic pathway is again same, so you have some vessel injury: this is the one that is also responsible for biomaterial surface, so this may be some biomaterial surface. So, because of this vessel injury to the basement membrane that is below the endothelial cells. This membrane is made up of primarily collagen. And so, again the platelets never really see collagen in a healthy vessel, but when there is an injury or there is a biomaterial surface, this biomaterial can also code collagen on to it is surface.

So, now these platelets get exposed to collagen. Once that happens, then you have again cascade of event where factor XII activates factor XIIa, this gets further cascading into factor XIa. And further and further it goes on eventually leading to the same molecule factor Xa to be generated on the surface.

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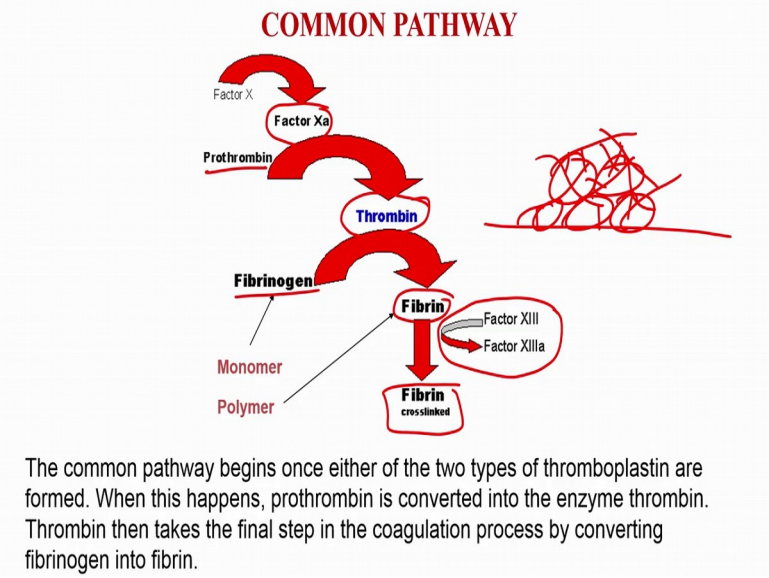

And then there is a common pathway which goes and picks up from the factor Xa and factor Xa then activates prothrombin. So, prothrombin is a protein that is circulating at very high amounts in a blood and results in formation of thrombin, and then this thrombin is another enzyme that cleaves fibrinogen or polymerized fibrinogen into fibrin. Fibrinogen is a monomer and thrombin itself is an enzyme which is causing essentially polymerization of fibrinogen causing long fibers of fibrin to form. This will then use some more serum proteins to get cross linked as well.

At the end of this, you will have a fibrin that has been cross linked. If again this is a material surface, first you will have aggregation of these cells and then you have fibrin. So, first you have fibrin polymers and then eventually cross linking of them as well, so a very tight network is formed. So, for all practical purposes this is nothing but a hydrogel carrying platelets and blood cells encapsulated and plugging the hole, where the blood was leaking out of, so that is the major pathway.

So, this is of course, required for functioning of a healthy human because if you have any defects anywhere this will cause problems. This is because the moment the person gets injured there is a heavy risk of them bleeding out. So, this is of course, a very essential pathway, but the problem is that if we are trying to do any kind of biomaterial-based therapies and these things will occur on the biomaterial, and will not let the therapy to succeed. So, what is the way around?

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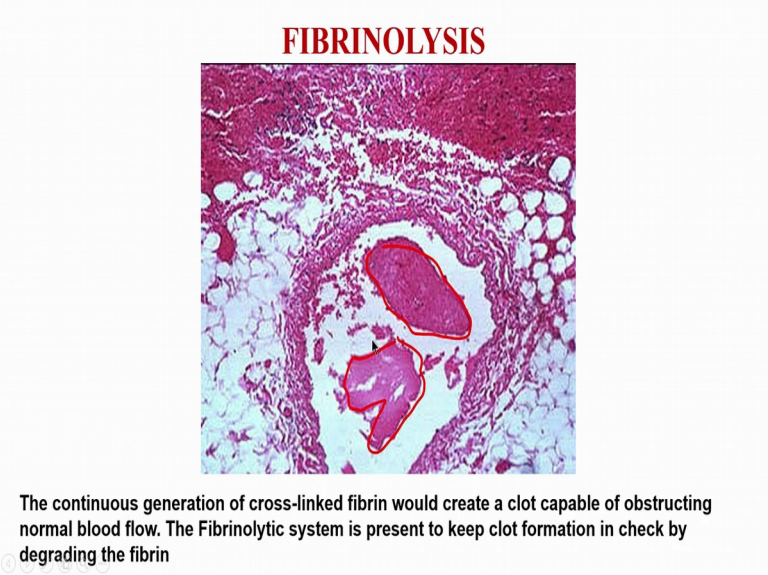

Once you are activating this continuously even the serum proteins and the levels of the serum of all these enzymes that are causing clotting to happen are increasing. Here, you are seeing a blood vessel and you can see these fibrin clots, that are actually floating in the blood vessels. And, this could be a big problem because once you have all these, they can actually clog the smaller blood vessels. This takes us back to previous discussion wherein, it causes stroke or heart attack or anything can happen causing loss of function of a particular tissue.

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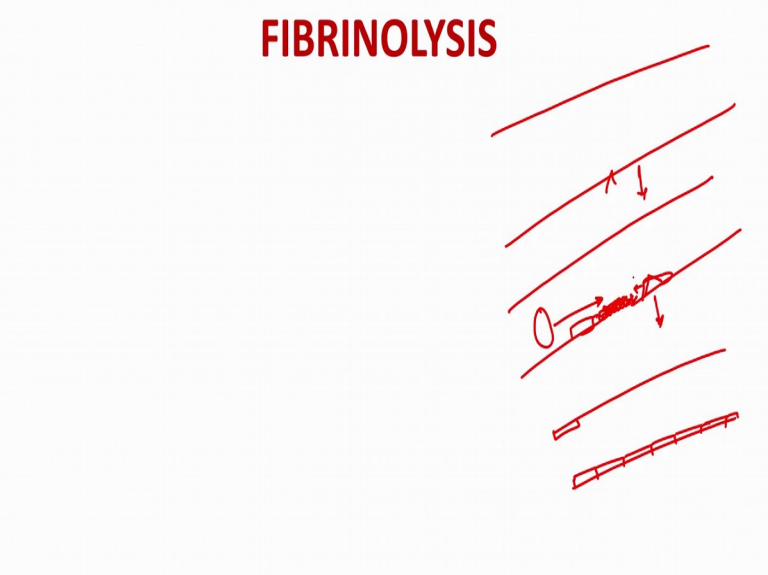

So now the clot forms. What is the normal way through which the body then removes the clot? Let us say this is the healthy blood vessel; this got injured and now you have a blood vessel which has a hole in it. Now, you have this clot that is formed with the process that I just described: aggregates of cell with a polymer. And now, eventually the body wants to return back to this original blood vessel, because this is still obstruction from cells to the cells that are flowing through them.

So, in a steady state you want it back to a vessel like this, which is fairly well formed and has no hole in it and no obstruction either. Then the body also has mechanism by which it can break down fibrin. Once the healing has happened, these endothelial cells which are at the surrounding have proliferated and migrated. This will eventually lead to a very nice covering of these endothelial cells and no hole that is remaining. But for that to happen there is again a cascade of events that causes the lysis of this cross-linked fibrin. Let us quickly look at that.

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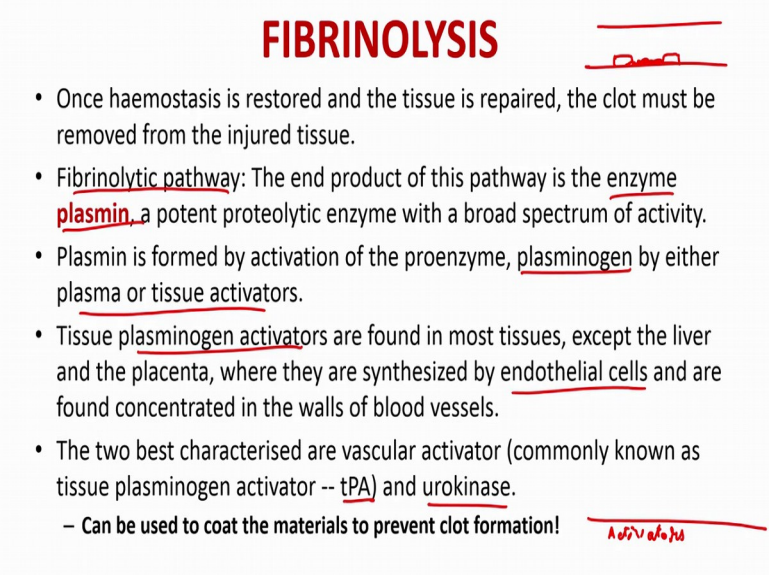

So, as I said, once hemostasis is restored and the tissue is repaired; the clot must be removed from the injured tissue. So, there are fibrinolytic pathway, as the name suggests to break down fibrin, and the end product of this pathway and just like those all these previous pathways there are few major proteins that are involved. In this case there is an enzyme called plasmin, and this is extremely important in terms of cleaving this fibrin clot and it has fairly broad-spectrum activity.

Plasmin is formed by the activation of a pro-enzyme which is called plasminogen. First this plasminogen which is already present in the serum gets activated either by the plasma or the tissue activators and then these tissue plasminogen activators are also found in most tissues except liver and the placenta. And these are typically synthesized by endothelial cells and that is why they are concentrated in the walls of the blood vessels. So, it helps because now these endothelial cells, so let us say this is the obstruction and these endothelial cells are now migrating towards this obstruction, they will themselves secrete this fibrinogen activators or plasminogen activators to cause the degradation of this clot here.

Some of the well characterized activators out there are urokinase and some tissue plasminogen activator called tPA. So, these things are actually very well characterized and actually used in humans too, and these can be used to coat materials to prevent clot formation. So, now, what you can do, is you can take your material you can pre-coat with these activators. So, if the activators are present on the surface then the first time the fibrin comes in it is going to get cleaved off.

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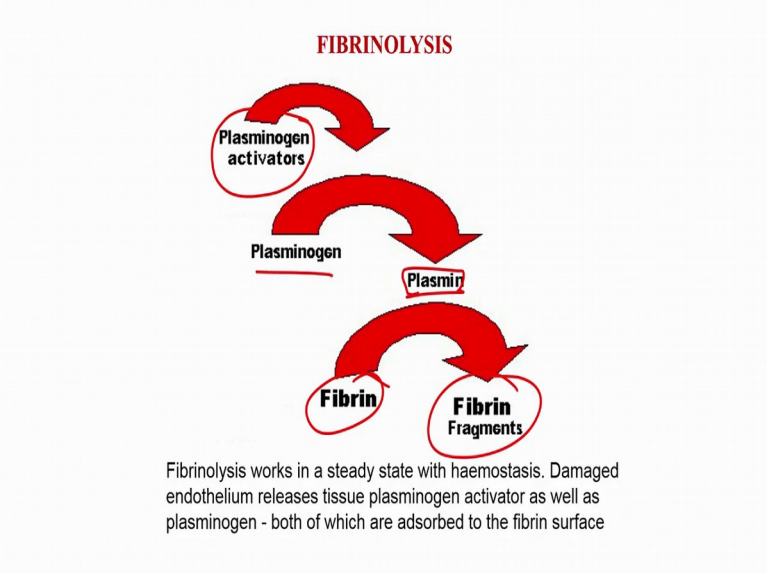

Thus, you would not let the clot to form. Here is an example: you have plasminogen activators that is going to change plasminogen to plasmin which is the major enzyme and then this plasmin is going to take this fibrin and degrade into fibrin fragments. So, there

are always some steady state levels any damaged endothelium releases these activators as well as plasminogen.

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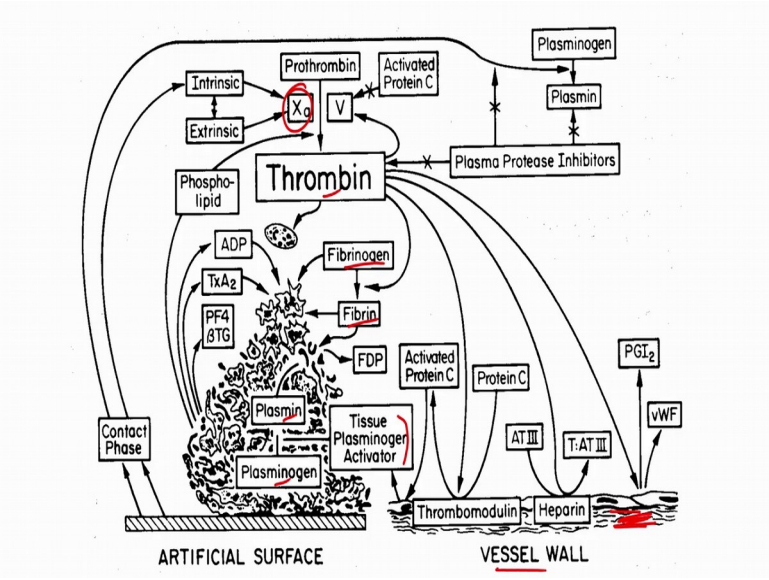

So, these can be then used to degrade the clot. This is again a complex figure I do not really want you to remember all this, but just want to show you how it is typically done. So, you can have an artificial surface or you can have a vessel wall. In this vessel wall these endothelial cells are secreting all kinds of enzymes and making sure that this is not happening.

So, you have these endothelial cells coated over basement membrane which could be collagen and thrombin of course, plays a major role. Plasminogen, plasmin we have all talked about. The tissue plasminogen activator again is a major role and this is essentially what caused what is the pathway that happens. Again, as I said you do not really need to remember this, but there are some key players such as factor Xa, plasmin, plasminogen, fibrin, fibrinogen these are some things that you should remember.

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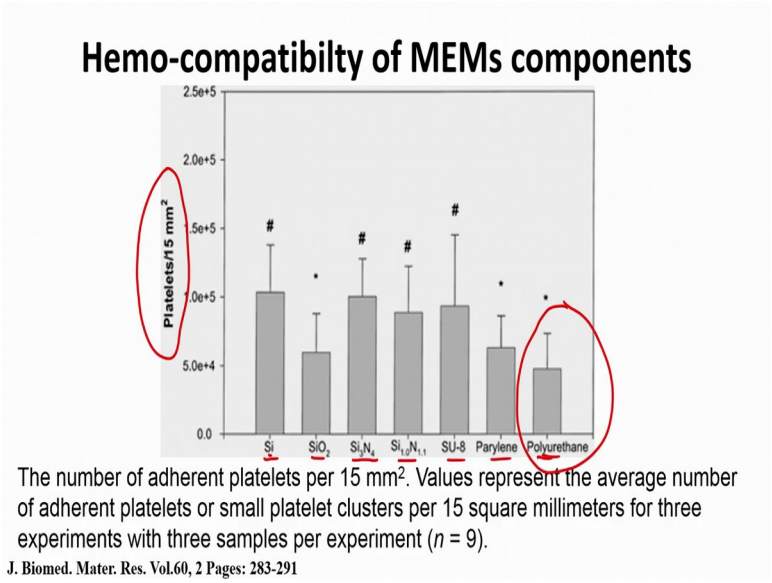

So, here is some example say we are talking about hemo-compatibility or MEMs component. So, what a MEMs? These are devices which are used as electronic devices which are used for measurement and mostly for diagnostic purposes in human body and what you are seeing here is you have different choice of materials. So, mostly silicon based, but some other materials as well and what you are seeing is, if you incubate them with platelets how many platelets are seen per centimeter square or per millimeter square in this case.

And so, what you find is different substrates will have different affinity for platelets and that may have to do with that proteins that are getting absorbed, the type of proteins and the amount of protein, and as you can appreciate that the different materials can have different effects. So, maybe if your application it does not matter whether you use polyurethane or silicon, then polyurethane may be the better choice just because it is going to cause less adherence of platelets and thereby less clot formation over its surface.

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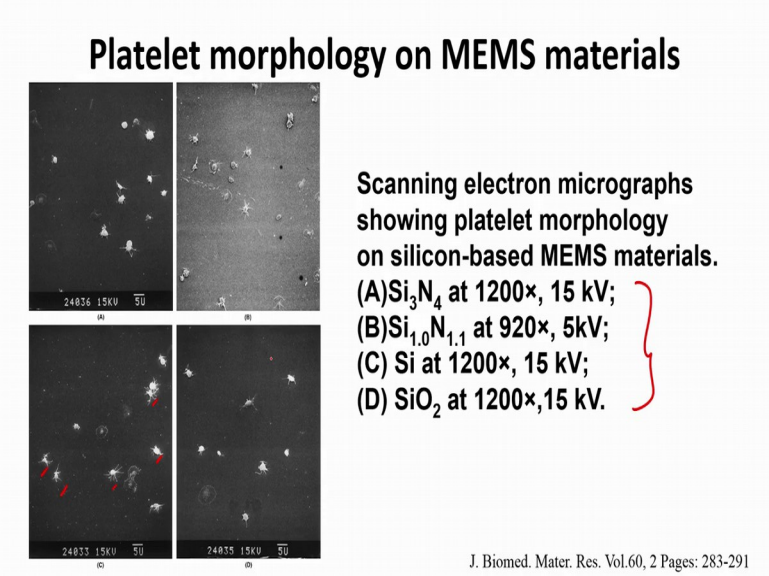

So, just one more SEM image; I told you that these platelets when they get attached to a surface, they lose their round morphology and actually become spiny and start to a spread around. So, that is what you are seeing here in this SEM image, and in this image, you are looking at three, four different types of surfaces at a higher magnification. And again, you can appreciate that all of these are causing attachment of platelets some at a higher or some at a lower amount which was seen in the previous slide. But this is what if you then give the whole serum do it these things can start forming and following the surface with blood clot.

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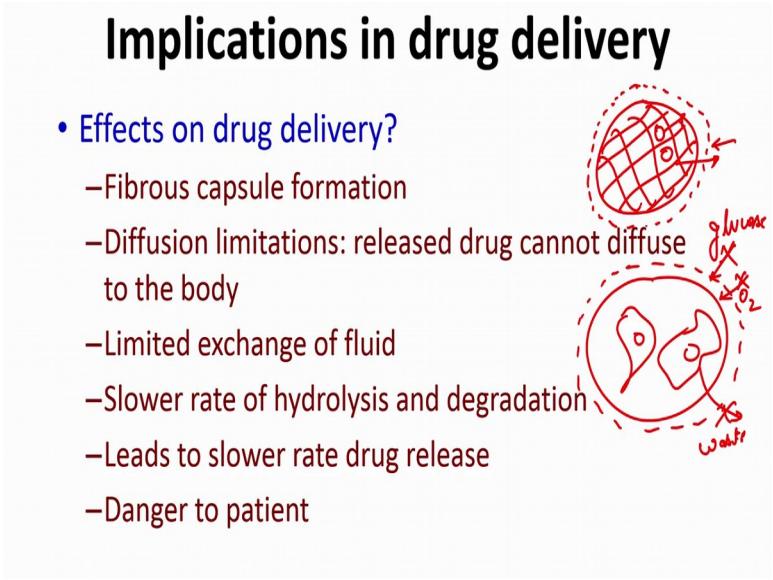

So, what are the implications in drug delivery, what are the effects of this in the drug delivery? First is there is a fibrous capsule that is formed consisting of fibrin clot. Again not very good: your molecule release it will get altered. So, it is causing diffusion limitation because earlier what you had done is you had modeled let us say this was PLGA particles and you thought that is going to degrade at a certain rate or the drugs are going to diffuse out from these pores at a certain rate. And, come into the system immediately, but that is not going to happen anymore, because now this is coated with a thick layer of the blood clot which is going to change the diffusion.

So, that is a parameter that can cause no diffusion to happen. So, that is a parameter that you had not accounted for and that is going to cause the failure in the therapy you have limited exchange of fluid. So, let us say if this was carrying cells and was used for tissue engineering. So, let us say I have this material that is encapsulating cells within it.

Now, these cells cannot get external glucose, oxygen because there is a same thing: there is a blood clot that is preventing the diffusion from happening and neither they can get the base products out. So, all of these have been blocked off and that will eventually result in the death of these cells. So, again not ideal or it may be an osmotic pump.

So, that will not work, not only that, the things that are coming in is going to decrease, but the things that are going out as well. So, since the water is not coming in as much

amount as you had hoped, the degradation may change, the hydrolysis may change. So, these are some of the things that will start to change the whole kinetics of your device.

So, all of this leads to slower rate of drug release, when we are talking about drug delivery applications. And it is a danger to patient, maybe because that that amount of drug is required for the beating of the heart or for the functioning of the brain and those things are fairly sensitive for even small minor changes. So, any of this can cause serious complications to patient.

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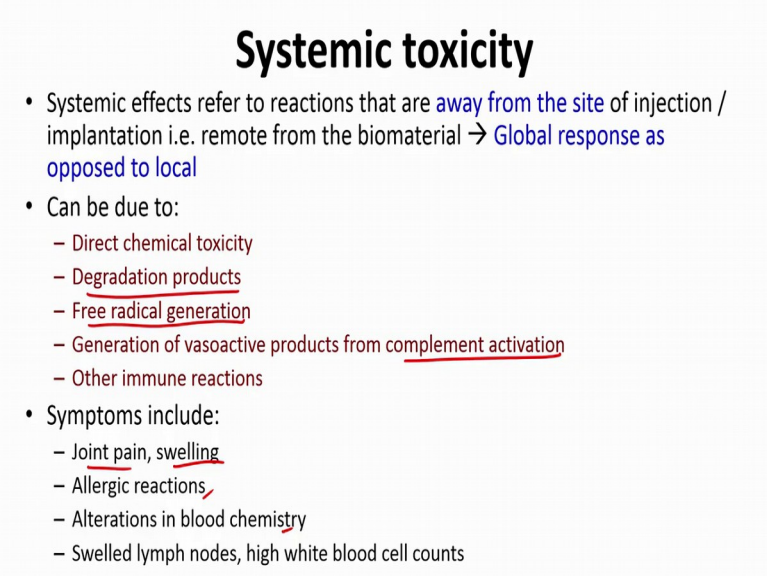

Now, let us talk about systemic toxicity. So, so far what we have talked about is when it comes to the surface both the complement and blood system are involved. So, the complement proteins are coming in binding to the surface and then on the platelets where they are coming in binding the surface and causing falling of the surface. So, what about systemic effects? Systemic effects refer to reactions that are far away from the site of injection or implantation.

So, if I let us say put something in my arm what happens to the rest of the body? So, arm maybe gets inflamed, but does the rest of the body also suffers from this? And so, this is more looking at a global response rather than just locally at the arm or whatever the site of injection or implantation may be. So, lots of systemic toxicity is seen and this can be due to several reasons, first is maybe the chemical is small and can diffuse and it is toxic.

So, there is a direct chemical toxicity that is causing this systemic toxicity to happen, or maybe whatever you put in the degradation products are toxic. So, we only talked about the PLGA degrading into PLA and PGA or lactic acid and glycolic acid: although those are not toxic. But they are causing drop in pH, but then products themselves can be toxic maybe it is some metal that is releasing out leaching out metal ions that is causing toxicity. So, could be anything maybe it is causing free radical to generate. These are oxide species that are highly reactive and will react with lots of surfaces on lots of proteins.

So, maybe these are being generated and then they are circulating out into the system, because these are small species and you have generation of vasoactive compounds from complement activation. So, again the complement has binding to the surface, then it is going to start activating and releasing lots of molecules that can cause the immune system to build up.

And, then immune system is going to travel everywhere in the body and your body will become fairly immunogenic at that point and then you have other immune reaction that can also happen. And, some of the symptom that you will see once this has happened is you can start seeing joint pain, you can see swelling in different regions, you can see allergic reactions. So, maybe it has happened a lot of the time, because if your body is allergic it may suddenly start to react with a piece of cloth that earlier you had no problems wearing with. This changes the blood chemistry whole together your lymph node gets swelled, so I am sure you might have heard about your lymph nodes getting swelled up.

So, sometime you go to the hospitals and doctors of your choice and they will try to touch your back a lower neck to see if you have any lymph nodes getting swelled up. Resulting quite a lot of immune cells now floating around in the blood that may cause several other side effects. So, this is a part of systemic toxicity of how this is a global response rather than just localizing at this point.

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So, we are going to now talk about adaptive immune response, so far, we were looking at innate immunity and some blood response. This was something if you remember and this is something that is nonspecific for the most part where it does not really distinguish between let us say a bacterium or a virus. It just recognizes some patterns and on the basis of that it causes amplification of immune response to try to kill it off immediately.

But what happens if it does not happen what happens? if the material remains in the body that the body does not like, or what happens if the bacteria is able to colonize the surface and maybe form bio-film or maybe persist due to various other evolutionary advantages that it has. And then how does the body tackle it? that is where the adaptive immune response comes in.

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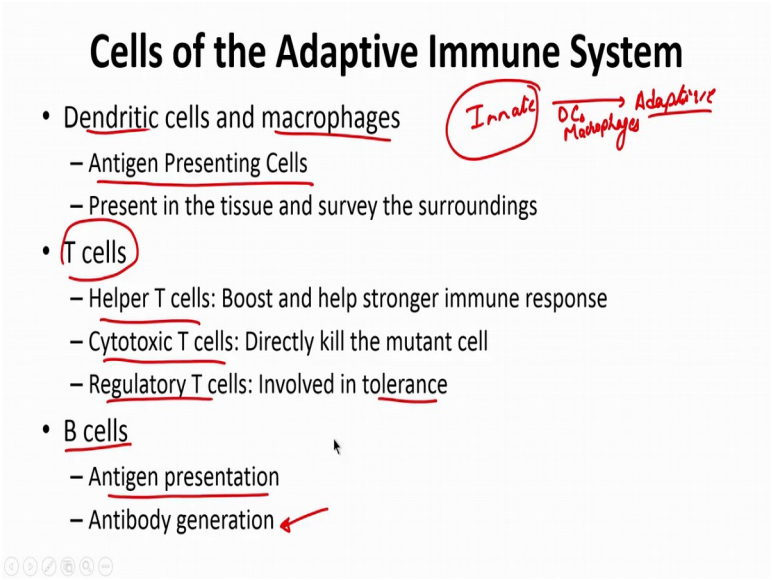

So, before we go to the adaptive immune response let us talk about what are the different cell types that are present in the adaptive immune response. So, by far the most you will hear here about are these dendritic cells and macrophages, you have already heard about them; these act as sort of a link between the innate and adaptive immunity through these DCs and macrophages.

And why I am saying is that because; obviously, we know that DCs and macrophages can engulf anything foreign that they find. So, and that is a part of an innate response maybe it is not really a very specific there is a just engulfing happening or this could be an adaptive response that will come in just a bit. So, these for adaptive immune response they act as an antigen presenting cell. What does that mean? Anything foreign is an antigen which an immune system can recognize.

So, these cells DCs and macrophages they can process this and then they can present it to the rest of the immune system. See here take a look at this: This is the foreign material that is there and the cells are present in very large number in the tissue and they survey the surrounding especially macrophages that most tissues have their own distinct macrophages. If we have already learned about that lungs having quite a bit of them when we were talking about inhalation base route delivery; we had learned that liver cells have Kupffer cells which are nothing but liver macrophages. So, all of that is present in very high numbers.

Then the next start T cells; so, these are leukocytes. they are purely adaptive immune response and there could be several types of them. There is a helper T cell which helps in boosting the adaptive immune response for a particular antigen. There are cytotoxic T cells which will go and directly kill a mutant cell or a cell that the body thinks is not normal, and then there are regulatory T cells. And these are involved in tolerance which basically means that you do not really want what happens, if let us say immune cell like a T cell for some reason for due to some abnormal signaling starts to recognize your eyes as foreign.

So, the last thing you want is your own body cells to kill off your eye tissues. So, then we also have the body as also mechanism for these regulatory T cells that involved in tolerance. So, maybe these will go to the eyes site and we will make sure that they are not letting these cytotoxic or helper t cells to kill off the eye tissue. And, then the other major leukocytes are B cells and like DCs and macrophages they are also antigen presenting cell. So, they can also present foreign antigens to the immune system, but the major function that they are known for is the antibody generation.

So, all the antibodies that are generated these are generated through B cells they are mainly involved for external pathogens these antibodies. And that is how once the antibody binds to a pathogen that is a sort of a flagging a pathogen saying that this is the position here is an antibody and all of these immune cells or most of these immune cells have receptors for that antibody it is called Fc receptors. And we will we will talk about that in a moment, but that is what it causes them to recognition by the immune system and clearance.

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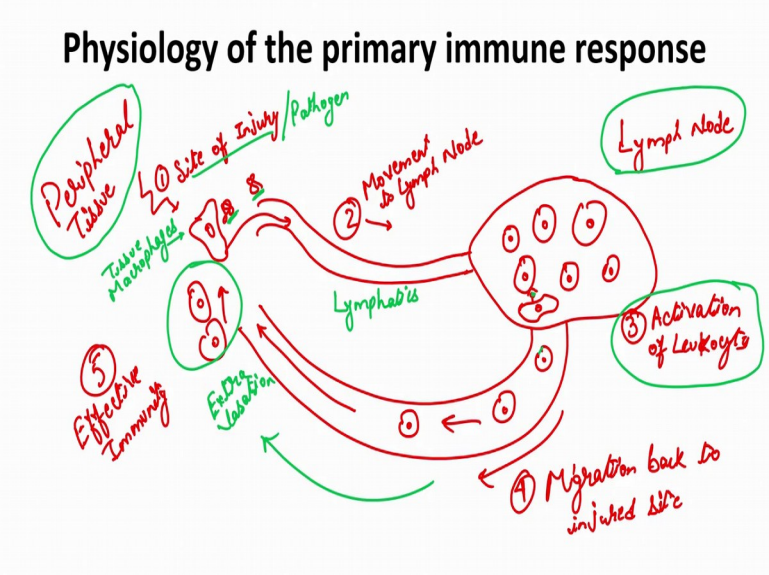

So, let us look at the physiology of the primary immune response. So, let us say you have a peripheral tissue, I will change color, so let us say you have a peripheral tissue and there is some injury or there is some pathogen that has come in. So, because of this pathogen and the injury what will happen to you may have pathogen or external proteins, antigens in that case they represent the site then you have these tissue macrophages that are going to take up these antigens.

So, these macrophages will then go to a secondary lymphoid organ, which are the secondary immune response organs; one of them is called lymph node. So we have lymphatics here. So, these things will go through the lymphatics they will go and then present whatever antigen that they have acquired to these leukocytes the T cells and the B cells that will cause activation of the leukocytes at those lymph nodes.

Once these cells get activated, they will start moving around in the blood and again get to the site where the injury has happened because these things are leaky, so extravasation will happen. And now, you have more and more leukocytes coming in which are actually trained on how to handle pathogens that are this particular antigen.

So, then they will come in and start to clear away whatever pathogen or whatever foreign material is present. That is how this adaptive immune response kicks in and this is the physiology of it. We will stop here and we will continue our discussion with the adaptive

immune response in the next class, it is fairly complex and quite exciting also. We will look at that to let us to how this builds up, how the body then differentiates between self and non self and what do we do with our materials, so that we can prevent the immune system from activating ok.

Thank you.