Drug Delivery Principles and Engineering
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Lecture – 46
Complement System and Blood Clotting

Hello everyone! Welcome to another lecture for Drug Delivery Principles and Engineering. My name is Rachit and we are going to continue our discussion that we were having on this topic.

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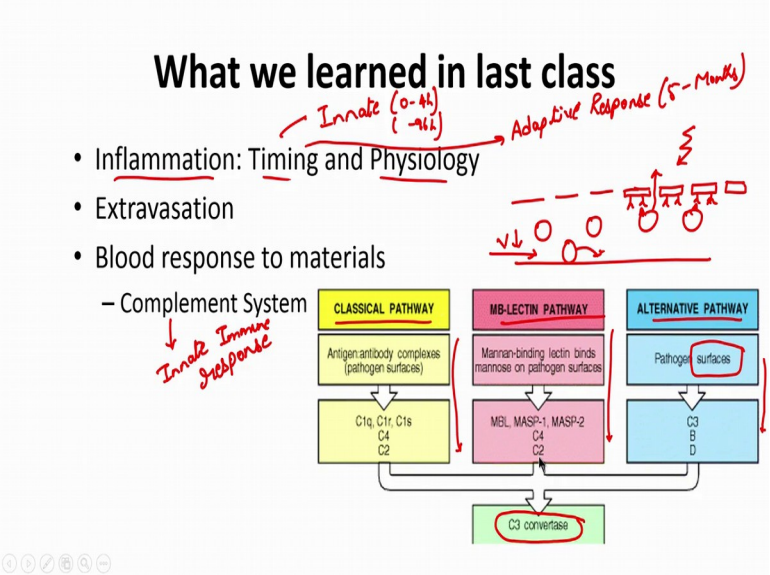

So, just a quick recap of what we learned in the last class, first thing we talked about was inflammation and the two major thing we looked at is the timing in the physiology. So, we discussed few things with the timing; we said that, initially innate immune system is the one that takes over and tries to eliminate any kind of pathogen and any kind of foreign substance. This happens fairly rapid, from the diamond production anywhere between 0 to 4 hours.

And then innate immunity can then amplify itself and continue this even all the way up to 96 hours. And even longer than that, but what happens after 96 hours is approximately after 4 days the adaptive immunity takes in and that is a much more amplified response.

So, it is a much more amplified response and that then kicks in from 5 days to all the way up to months if the infection persists and that is what then causes the clearance of anything that is pathogenic. And we discuss some of the players that are involved, so innate immunity is something that the body already has. So, it has some recognizing general patterns. So, maybe it recognizes a certain type of bacteria lipids like LPS or maybe it will recognize it something else, maybe single stranded DNA or something like that the or double stranded RNA.

In general, the adaptive immunity is more specific, so it will be specified actually to a specific sequence of a protein that is derived from the bacteria or from virus alright whatever it might be something that the body's never seen before. So, that is happens in the physiology part of it, we again discussed that, the blood vessels in a normal tissue are very well organized and they have a certain aspect to it where they are moving around with a certain pore size in the endothelial layers. Whereas, once the inflammation happens these vessels become leaky, these vessels then start to grow as well because there is lots of cells in the surrounding. So, the normal physiology also changes with ECM deposition and fibrosis can happen.

Then we talked about extravasation which is nothing, but how does these immune cells which are circulating in the blood vessel, come out at the site of injury. So, let us say the injury happened here and of course, because of the injury there is lots of cytokines that are being released from this surrounding tissue. These cytokines then activate these endothelial cells and do couple of things: one is that they make them leakier, so the vessel then changes, and becomes slightly leakier and then the other thing that they do as a consequence to of this the blood flow velocity also goes down. As the blood flow velocity goes down these now cells that are floating in the blood, have a lot more time to interact with the cells at these layers. And the second thing that this inflammation does is it causes these cells to then up regulate certain receptors.

Now, that these receptors are stimulated, these immune cells can then bind to these receptors. And then what we studied is, then there is something called a rolling adhesion where, these will roll over on the surface and continue to bind more and more receptors. And when there is another receptor bound to that it can actually stay at the site, it will then start to come out from these gaps that are created because of this leakiness. So, that is extravasation.

We then started looking into blood response to materials and we said that they are majorly two types of it; one was complement system which is what we were studying when we left it at the last class. And this has three different major pathways through which the complement system activates itself. Complement system is a part of innate immune response and it comprises of several types of proteins that are present in a body and get activated and then, leads to a cascade of events and these are the three major pathways through which these complement system activate.

One is a classical, another is lectin, and another is alternative pathway; alternative pathway is what is typically responsible for immunity against foreign surfaces and so in our case, biomaterials will also act as so on surfaces whereas, the other pathways are more specific to certain type of pathogens and all. And again this is a very complex route and we do not have to remember all the route for all these pathways, but eventually what happens is through all this process a major enzyme called C3 convertase is generated and that is where all the three pathways intersect and from there on it can then do multiple things that we are going to study as we go along.

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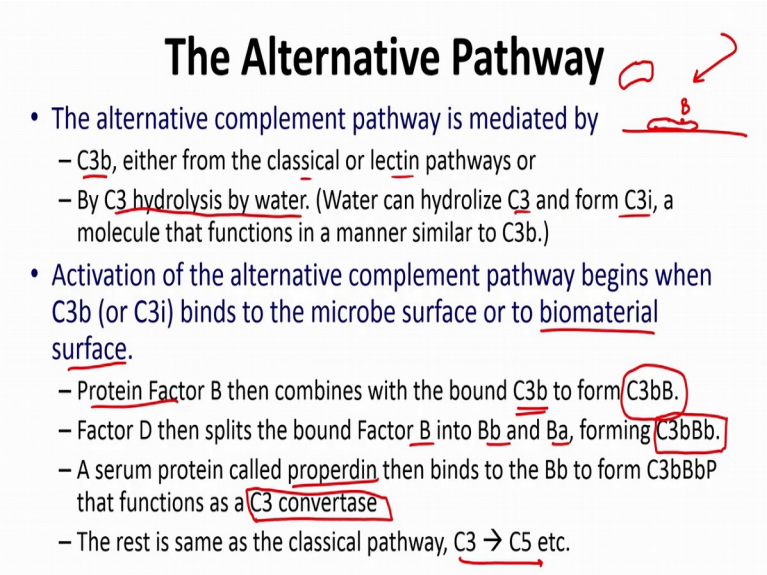

So, let us talk about alternative pathways, since as I said alternative pathway is the pathway that is majorly responsible for immune responses against some of the foreign materials that we are going to implant. So, let us look at how this pathway gets activated

so, that we can have some fair idea of what to do with it. So, alternative pathway is mediated by one of these, C3b either through a classical or a lectin pathway.

So, by C3 hydrolysis by water this can also be activated. So, basically what we are saying is both the classical and lection pathway can activate, this alternative complement pathway or the C3 hydrolysis can also do that. So, water can hydrolyze C3 and form a C3 intermediate; and this molecule then functions in a very similar way as C3b. So, if I go back what we find here is, this C3 is the major activating protein, C3b particularly that is responsible for this alternative pathway to get activated.

Then the activation of the alternative complement pathway begins when C3b binds to a microbe surface or in our concern a biomaterial surface. So, C3b is a protein that is fairly inactive when it is just floating in a body and does not find anything foreign. So, our own tissue, our own cells do not activate it, but once it finds a surface which is microbial in origin or which is something foreign in origin, this can then bind to that biomaterial surface.

Then there are several cascades of events that happens; a protein factor B then combines with this C3b because, once the proteins bind, they change their conformation. So, now, maybe the conformation site is available, then binds to form another intermediates the C3bB and then a factor D comes in and then splits this bound factor B into Bb and Ba, again these are lots of terms here that I am introducing. But you do not need to remember all of these, but it will be good to know at least the pathway.

So, this then results in the formation of another intermediate, which is written as this. And basically what you are saying is, once the C3b has come in and let us say the C3b conformation is something like this, but once it come in and let us say it has bound and changed its conformation, maybe it is an active site that is getting activated to which a factor B comes in.

Once the factor B comes in, then it can attract more factors from the serum so this is a cascade of event where a change in conformation on one protein leads to change of conformation of several other proteins. And this, then continues, now we are going to all the way to serum proteins called properdin which then binds and forms the C3 convertase which is the common junction for all the complement pathway.

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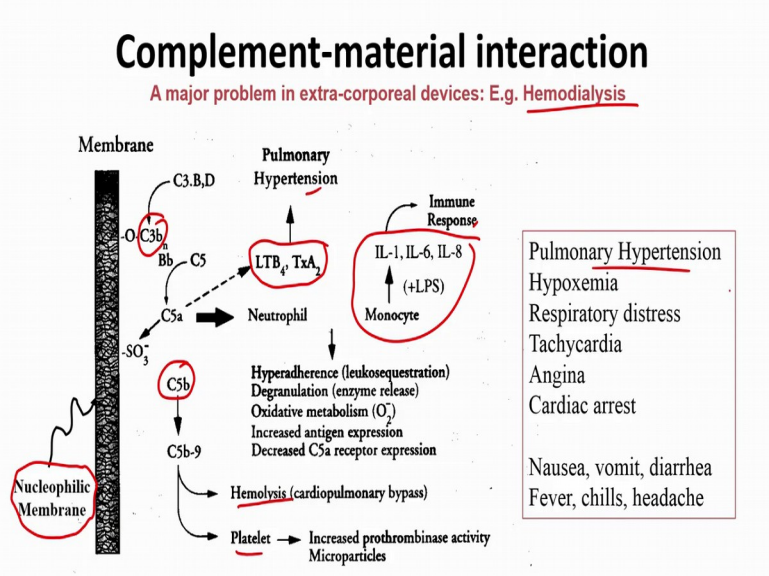

So, the rest then remains the same as other pathways the C3 goes to C5 or the C5 and that causes the further activation of the complement system. So, this is what it is written here, so complement system. So, you have an external surface, you can have first the C3b coming in and then all kinds of protein come in, leading to the formational of C5.

Once the C5 is formed, this can then cause lots of other things to happen. So, up regulation of several cytokines, up regulation of several other lipid-based molecules happens and that can then really result in pulmonary hypertension or increased immune response. Eventually, this may also cause haemolysis and platelets coming in and we are going to talk about that in few slides from now. But, as you can see let us say if this is a device that you are using for haemodialysis, which is basically if the kidneys are not working well and you want to filter the blood from any toxic region you can use some membranes like this. But then the problem is these membranes could be: let us say their nucleophilic. then they cause attachment of C3b and the activation of the alternative pathway.

So, these are some of the few things that need to be kept in mind, when you are designing things like that. So, as I said it can lead to pulmonary hypertension, further leading to hypoxemia: it can lead to respiratory distress several other things can happen.

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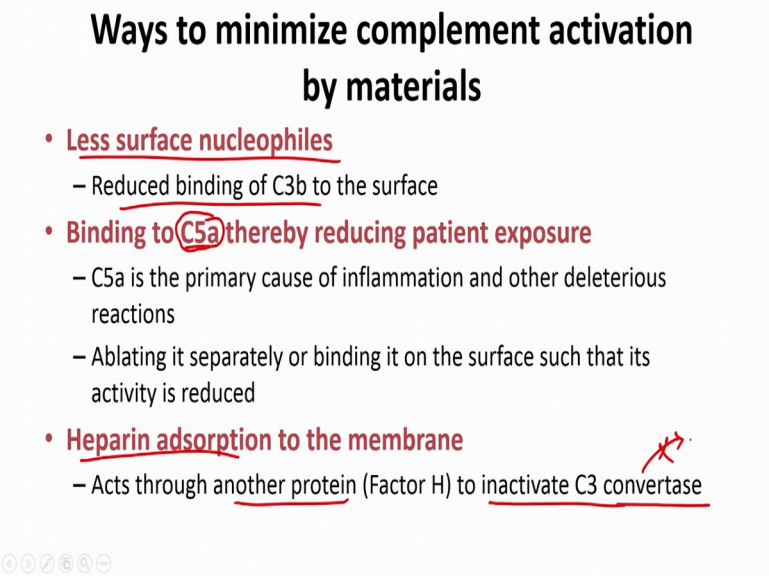

So, how can you minimize the complement activation by your material? So, it is important right? I mean if you are designing all these drug delivery vehicles or tissue engineering vehicles and you are implanting it, you want to make sure that those do not really activate any kind of complement system.

So, what are the different ways to minimize it? So, first is I briefly told you, it can make the surface less nucleophilic. So, as I said the nucleophilic surfaces tend to attract the binding of C3b. So, if you have it such that they are less nucleophilic, they will have a reduced binding of C3b. The other is you can by in basically blocking the C5 ways, so C5 ways is the primary cause of inflammation and the deleterious reaction so if you ablate it, you can decrease it.

So, let us say if I know that I am going to put an implant in a patient, why do not I first knock the C5a out from the patient either using antibody or using some other method? so, that there is no more C5a present in the serum or its present in a much lower quantity; and because I have ablated it, it cannot really go and bind to the implants. The other method used is to adsorb heparin before implantation so, what this does is, it actually inactivates the C3 convertase.

So, even if the C3 comes and binds and starts the cascading, this heparin along with another protein called factor H, which it will acquire from the serum itself it can then

prevent this pathway from getting activated, so this cannot go forward. So, these are three of the ways that you can do this.

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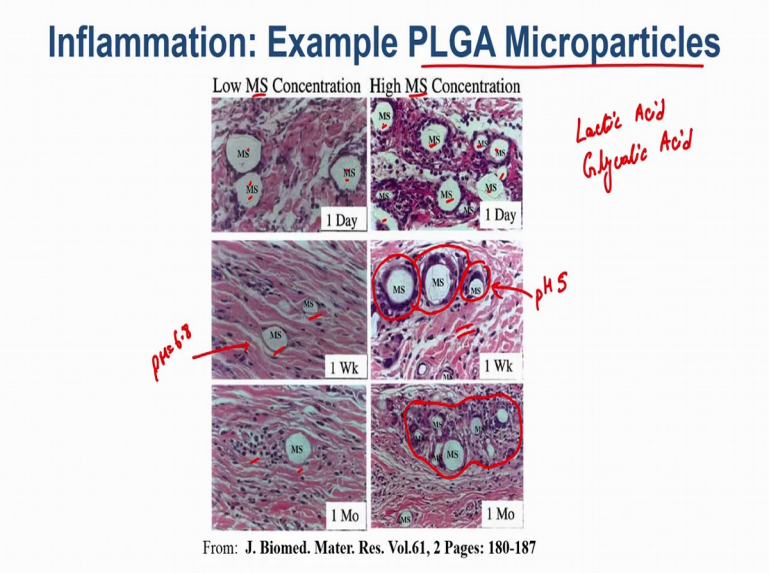

So, here is some examples not necessarily for complement, but in general inflammation. So, and this is just showing how quantity can also matter. So, in this case you are using PLGA micro particles. You can see all these white regions written with MS or microspheres. And what you are seeing is you have two sets of patients: two sets of animals, in which one you have put in a low concentration of PLGA micro particles and another you put in a high concentration of PLGA micro particles and even just that can really result in quite a dramatic effects.

So, what you see here is in case when you put low concentration, so you have some concentration of micro particle at day one; these are PLGA micro particles so, they will degrade both hydrolytically enzymatically as well as (Refer Time: 13:58) by hydrolysis. So, then they are degrading as you can see quite a little of them are left in the tissue at the vicinity and it does not really look too much inflamed; You do see some cells coming in, but it does not really look anything out of the usual, the tissue has a good architecture as it should be. And in the other case you have high concentration of PLGA microsphere.

So, you can see quite a lot of microspheres in that same amount of area and what you can see is, even after week 1, you are now starting to see you all this region around the microspheres looking very different than the rest of the tissue. So, what this suggests is

the body is not liking these microspheres and eventually, what you are seeing is more and more of this expansion of this abnormal region has happened along the microspheres. And the tissue does not look anywhere close to how it should look in the healthy being.

So, this is just showing how just having different concentrations of PLGA micro particles can have very different outcomes when you are studying between the two. So, how can this go? We know that PLGA degrades into lactic acid and glycolic acid. So, maybe because of the high concentration when they degraded, they created the pH which is much lower than 7. So, maybe the pH here in these regions is, let us say 5 and maybe since there is less PLGA load here, maybe the pH here is only, let us say 6.8 or something.

So, the body will respond very differently because pH is also a trigger for something that is not supposed to be there. So, that can also result in activation plus, not to mention there is more particles, with more chances that immune cells are coming in and binding to it and maybe they are getting activated too. So, there are some possible reasons of why you might see this.

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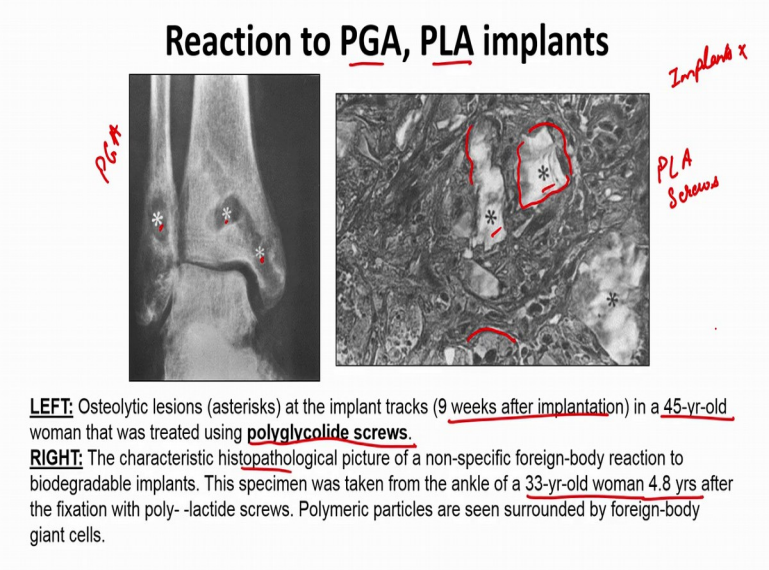

Something more on this on the same line, so here what you see is PGA and PLA implant very similar to PLGA, basically instead of copolymer now you have poly glycolic acid or poly lactic acid and here you have some bone implants. So, what has been done is a poly

glycolic acid screws have been made on the left side, so this is for the PGA. So, they have made PGA screws and what you find is these screws actually are causing lesions to develop right?

So, you can see all these lesions where the bone density is fairly low, where these screws were implanted. 9 weeks after implantation, the screws are probably gone at this time, but what you are seeing is the bone has really not grown and this is actually in a human patient; it is a 45-year-old woman that was treated with these screws. And what you are seeing is these lesions being developed this is not going to be any good, because instead of providing now stability to the bone, this bone is going to become weaker and weaker.

And similarly, on the right you are seeing some histopathological picture where you are seeing nonspecific foreign body reactions. So, you have again these implants and these are again supposed to be biodegradable implant, but they are creating foreign body reactions. So, you can see how disorganized they are, that if you look, nowhere close to how it should be, and this is also taken from a patient this is 4 and a half almost, 5 years after the implantation of these screws that were put in.

So, this is PLA and you can see that these screw residues are now surrounded by some giant cells. So, you can see these giant cells that are just surrounding these and lots and lots of them which have they are not able to degrade this and what they have done is they just world it off and lots of immune action is happening.

So, the conclusion from here is I mean, we know PLGA is an extremely biocompatible material. But then there is a limit to how much a body can tolerate a foreign material and that is true for any material, we saw that even PEG which is used in patients is developing now antibodies.

So, the whole idea here is to know how the material is acted upon by the body and maybe if the pH is an issue, the PLGA implants is not the way to go right. Because the body only has certain capacity to clear things; obviously, anything that degrades there is interstitial fluid that will take the degraded products out. But if let us say the degradation rate is much higher than the clearance rate then, what will happen is you will have accumulation of the assets and that is going to cause further inflammation to happen.

So, in this case it might be even better to not put a big implant of PLGA, but small particles upto a certain dose may be very tolerable. So, that is why the PLGA works very well the PLGA, PGA, PLA all of these works very well in terms of drug delivery. Where you have these small units of micro and nano particles floating around or maybe at a site which can degrade soon enough and the body can handle them in terms of clearing out the degraded products.

But these big implants when you put those, they have problems because now a lot of surface area for a given site. And these will degrade, these will create a local pH that is much lower than the physiological pH of 7 and it may cause inflammation and something like these outcomes might be achieved which is not what you want. Okay!

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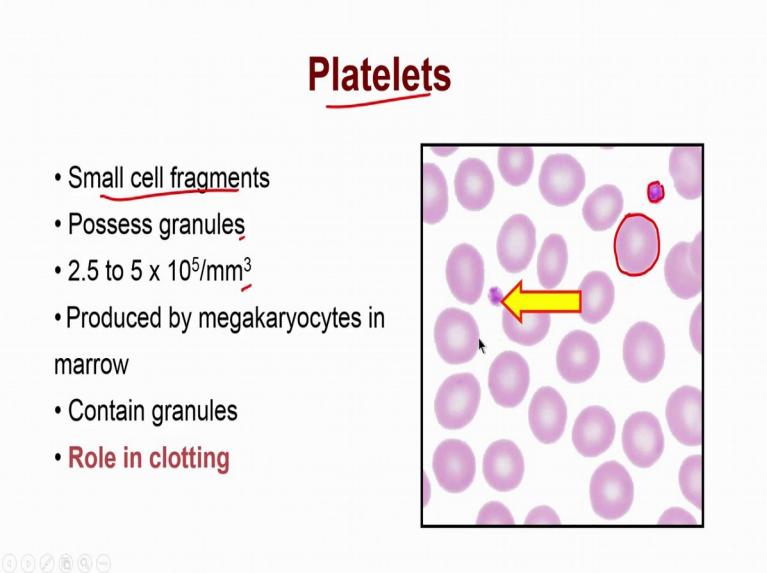

So, before I describe you the next sort of how the blood reacts to the foreign material I need to talk about platelets. And most of you may already know platelets are something that continue to float in our blood, these are small cell fragments and they possess lots of granules they are fairly small in size. So, here you can see here you have blood cells versus a platelet. So, you can see they are fairly small much smaller than the size of blood cells and their major role is to help with the blood clotting and how do they do that is what we are going to study next.

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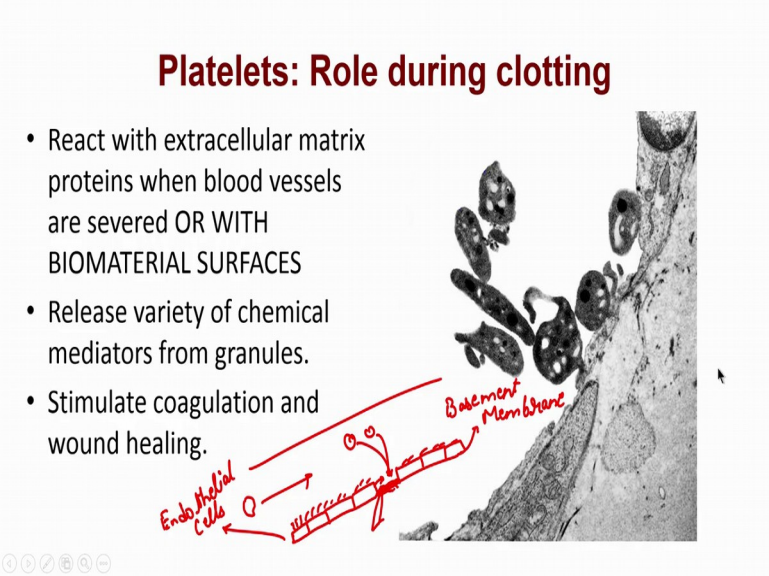

So, what platelets do is they react with extracellular matrix proteins when the blood vessels are severed or with biomaterial surface. So, typically in the body let us say if this is a vessel most vessels are lined up very nicely with the endothelial cells which; obviously, have glycoproteins on their surface. So, when these platelets are floating around and moving through the blood, the only surface they see is a very nicely covered surface with glycoprotein.

So, they do not really have any side to attach to it, but now let us say the blood vessel gets injured, let us say I put a needle or there is some accident that has happened. And it is blood injury that happened; what happens is now that these endothelial cells let me write this down. So, these are endothelial cells, this is basement membrane.

So, now that this basement membrane has been exposed to these platelets, these platelets are going to then bind to that new surface. So, what will happen is the platelet will come and bind it will get activated and it will then release variety of chemical mediators the granules that it is carrying and then it is going to cause more and more platelets to come in.

So, more and more will come in and they will start to do the same thing. So, what they will do is they also then start polymerization of some polymers that are present in the blood as well. So, it is let us say this was completely ruptured and the blood was

probably leaking out from here, because you have put a needle lot it is because of some accident.

So, this is going to form a polymeric coating over this severed area to which then endothelial in grow on to; so, this is their normal major rule that if they find that the blood is oozing out from somewhere or the blood is exposed to an environment where it should not be. I mean technically the blood cells and the platelet should never see anything which is not this endothelial surface. But if it is seeing it then these platelets come in and make sure that we do not drain out of the blood, because what will happen if these platelets do not clot you will continue to lose blood and eventually the person will die, so that is their major function.

But the problem is now that we are putting biomaterial surfaces, those biomaterial surfaces are again something which are not endothelial surface. So, these platelets will also get activated on these biomaterial surfaces and cause a blood clot to happen on these surfaces which is not ideal. Again, first of all it can result in these polymeric coating to happen on your material thereby changing the release rates, they were changing the tissue dynamics. And then the secondly, what can happen is you these if the material is huge and you have so many of these clots can then come off and start floating in the blood and which is again not something that you want.

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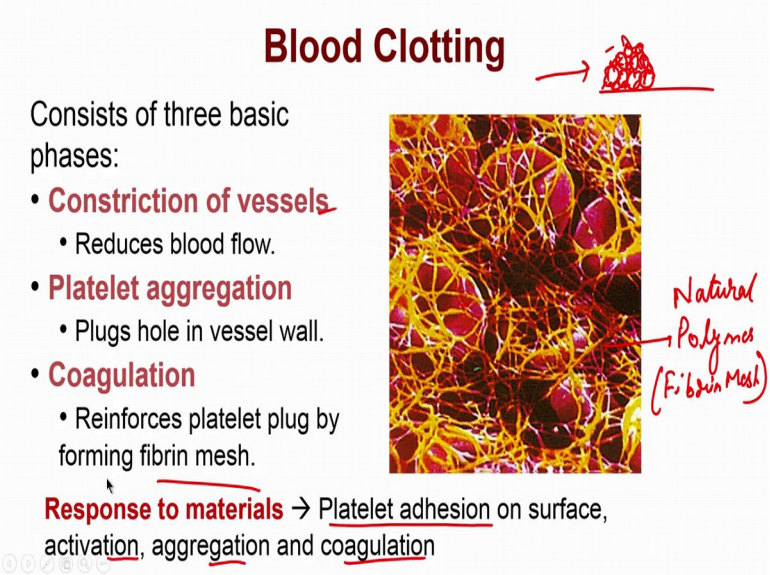

So, here is the blood clot example, so you can see these RBCs and they are being entrapped by this polymer and this is of course, a natural polymer. And these RBCs have got entrapped and you can see a nice mesh network which can then prevent any sort of more blood from leaking out from this system. So, the blood clotting then essentially consists of three basic phases: one is a constriction of vessels, so that reduces the blood flow in that area.

The next is the platelet adhesion by finding a new surface and then gets aggregated on that area, so that plugs the hole in the vessel wall fairly rapidly. And then the coagulation happens where in these platelets now are just a mass of cell piled on let us say a hole that was there, but this is still fairly loose.

So, these platelets, with the more and more flow coming in and getting washed away, but what these platelets do is then they form this fibrin mesh. So, thus is now I have defined as fibrin mesh and that will cause these cells to then, get entrapped along with some RBCs as seen here to then form a very tight network which, will then stay until the healing can happen. So, you are not going to bleed out at that point.

And response to the material, there is platelet adhesion on the surface, so the platelet will adhere to any foreign material because of course, that is not the environment that they are used to and we talked about that the platelets will adhere to any new surface. They will get activated, they will aggregate, thinking that this might be something, that where the blood vessel is ruptured they will start aggregating on that surface and the major goal there is to prevent any bleeding out from happening and then it will cause coagulation using this fibrin mesh. So, that is the major blood clotting mechanism.

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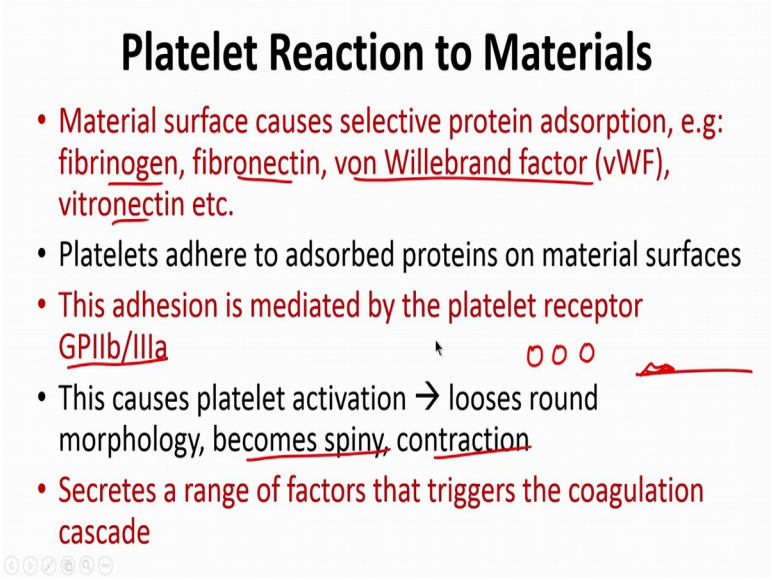

So, let us talk about how to measure this platelet reaction to materials. So material surfaces can then cause selective protein adsorption. So, as I said that any new material will cause protein adsorption and there could be proteins like fibrinogen, fibronectin, vWF, vitronectin etc. These are again some ECM proteins which can then also adsorb onto this new material surface that gets exposed to the serum.

Platelets adhere to these adsorbed proteins. So, as I said the major mechanism through which the platelet adhere, is to adhere to ECM. So, this is what they have receptors against and once they find that there is vWF or some other protein that they are aware of they can then bind to it which, they do not typically see in a normal blood vessel. So, then platelets will adhere to these adsorbed protein on the material surfaces using one of these or some other proteins.

So, platelets have adhesion molecules so, one of them is defined here, which is widely present on platelets. And because they bind through this particular receptor, this receptor then further causes activation to happen in the platelet, further signalling to happen. And so, as we have seen before, platelets are typically round, but when they go on the surface they then lose their morphology and they start to spread out.

So, they become spinier and they start to contract and their morphology changes by quite a bit. And then they start secreting a range of factors lots of enzymes that then starts the

cascade of the polymerization of the fibrin mesh that I was referring to earlier. So, that is going to cause this mesh to form and the blood to start clotting on that surface.

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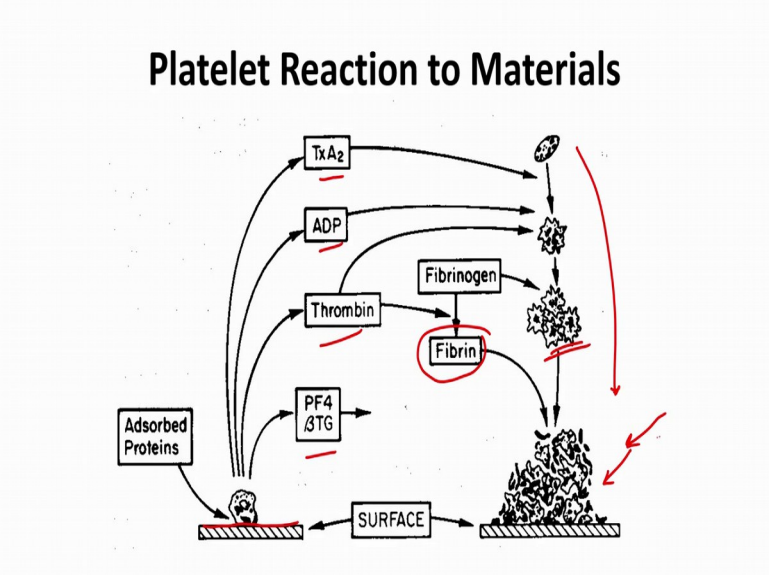

So, here is just a quick example, so you have adsorbed proteins on the surface to which the platelets as come, inbound this is now becoming activated. So, because now this is becoming activated you have several secretions of enzymes which will then cause first of all more platelets to come in and get aggregated. And secondly, it will cause the formation of fibrin polymer through a cascade of events and we will talk about the cascade of events in the next few slides.

But that will lead to formation of a whole clot to happen and this will plug the whole or at least that is what platelets are thinking that they are plugging; because they are now being able to see a new surface apart from the traditional endothelial surface with they have been used to. But in this case, they are not really plugging a hole; all they are doing is your material is getting completely fouled with some fibrin mesh with platelets and blood cells. So, this is the last thing that you want to happen on your surface. We will stop here and we will continue rest in the next class.

Thank you.