Medical Biomaterials
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Lecture – 19
Biological responses

Welcome to the course on Medical Biomaterials. We will talk about biological responses. In the previous class also I talked about biological responses. We will spend some more time on the biological responses, because as soon as a foreign body; whether it is a metal or whether it is a polymer or a ceramic placed inside the system short duration - long duration, immediately the defense mechanism starts acting and this defense mechanism may involve antibodies, coagulation factors, inflammatory factors, the formation of encapsulation of the biomaterial. So, many things can start happening actually.

Some of them are felt only when the material is placed for very long period and some of them are felt immediately as soon as the material is placed within a short duration. So, we have going to spend some more time on this because this information is very important. So, that to be very sure that the material does not cause cytotoxicity or any other problems to the host and before going into human; these materials are always tested in animal and they perform all these test to identify all these issues. So, what happens? So, what are the various responses that happen inside coagulation? So, many factors related to the blood coagulating factors come into picture here.

Then the complement; there is a set of proteins which help in trying to attack the material, this is the host defense system. So, there are some complement proteins which tries to identify this foreign object and there are some complement proteins which tries to attack them. So, all those things will happen.

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Then inflammation starts happening, the inflammation mostly around the site of implant inflammation is good because it helps the whole systems to adjust as well as attack the foreign body and then you also end up having capsule formation. So, the formation of giant cells multinucleated cells all those things start happening actually.

So, these are the various responses that happened as I said some of them start happening very fast within a few hours and some of them start happening within a few days and so on. So, ultimately the goal is to achieve biocompatibility of the material and try to address the cytotoxicity or genotoxicity of the material. So, the material has to be both biocompatible and it should not cause adverse reaction so; that means, it should not be cytotoxic. So, in order to understand this I thought we need to spend some time on the various biological responses of the biomaterial to the system. So, we will spend some of them and I did introduce some of these topics in the previous lecture as well.

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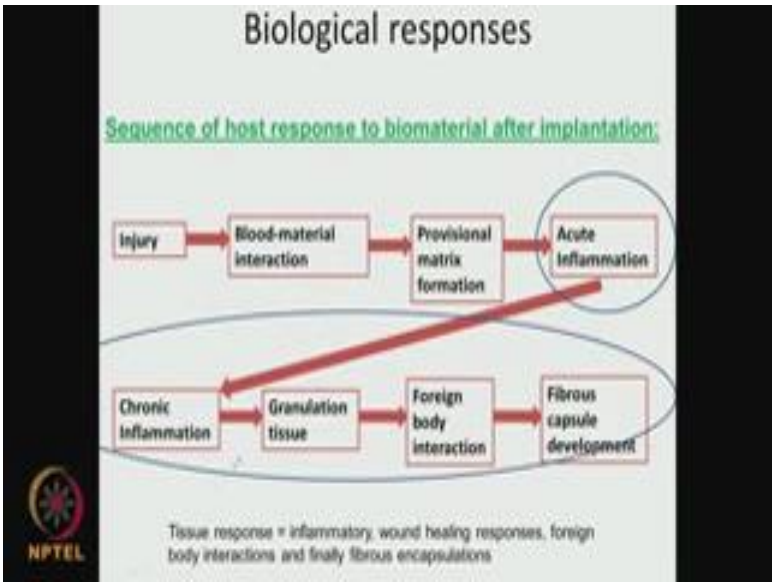

So, there is sequence of events that happen as soon as the biomaterial is implanted in to the system. So, from the place where it is implanted is considered as a site of injury. So, there could be a blood material interaction if it is a you will if it is a cardio vascular stent or if it is a diaphragm valve there is going to be a blood material interaction whereas, if it is in urethral region of course, this is not going to be happening then there is a provisional matrix formation. So, if there is a blood material interaction there could be coagulation things start happening there could be other things like complement activation could be happening then you have the acute inflammation that is at the side then it leads to chronic inflammation then formation of granulation tissue foreign body interaction and finally, the material could be completely encapsulated that is fibrous capsule development could happen.

So, there could be inflammatory activation and there could be immunological activation. So, you could have immunological things happening immediately then inflammatory reactions happening and then again immunological activities happening later actually and that is how the system the human system or the whole system starts responding to this biomaterial. So, we need to understand this and whether this leads to complications to this host needs to be also recorded and. So, these are related to the tissues. So, inflammatory wound healing responses. So, it is like an external injury a wound. So, there could be several steps of responses which are called wound healing responses and

there is a foreign body interaction because you have you have placed of material which is a foreign body and then finally, fibers encapsulation.

So, when a biomaterial is placed after a few months or few years, it will be completely encapsulated by the tissues and it may be impossible to remove the material from the host at all because it is completely encapsulated. So, if somebody wants to remove especially if there are certain join failures and then they want to replace it and place a new joint the physicians, the surgeons find it very difficult because the material is completely encapsulated even if we take cardiovascular stents it may get encapsulated by tissues. So, it may be very difficult to remove that old stents and try to place another new stent that is why there is now lot of interest and looking at biodegradable stents. So, that stent will completely go way. So, with if the cardio cardiac surgeon wants to place another stent in the vicenary; he or she will not have any problem.

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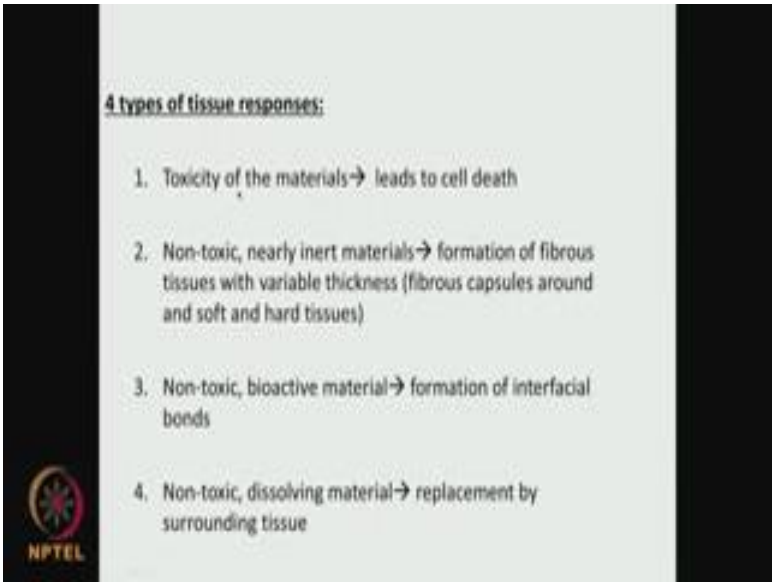

Now, let us look at each of them little bit more in detail. So, there could be 4 types of tissue responses here because there could be different types of material that is coming into the human system toxicity of the material the material is very toxic; that means, the material itself may be toxic or there could be some lichens that could be toxic there could be some acids getting lich lactic acids or acrylic acids or some other may be silver nano particles all this could be very toxic because the local concentration gradients. So, there could be death; cell death.

So, this we can even look at it in vitro for example, I talked as couple of classes back an assay called MTT assay where we can look at the viability of cells; that means, how many percentage of cells that are viable with respect to control and. So, we can incubate the biomaterial with the cells like LSECs or 3T3 which are mostly muscle related cells and then after 20 hours, we can count and see; what is the percentage of cells that are live? So that is called MTT where we are using a Araich MTT or we can look at cytotoxicity and so on actually there are so many ways to determine whether the cells are dying now whether the cells are dying naturally when the cells die naturally we call it apoptosis when the cells die because of a foreign material and then it is called necrosis.

So, we can monitor whether the death is apoptosis are necrosis if the cells die in because of necrosis the cell in membrane is damaged. So, the materials inside the enzymes inside are leaked out. So, we can find out what is the enzyme that is coming out for example, LDH type of enzymes which gets leaked out when there is a in a crosses. So, this can be easily studied through in vitro. We can look at different cell which are related to the human system and then find out the toxicity of the material if you have non-toxic nearly inert materials for example, PTFV originally if you look at a biomaterial history the second generation it was felt and can we have inert material as biomaterial PTFV, PVC, they are extremely inert.

So, what formation of fibrous tissues with variable thickness? So, you will going to have the entire material encapsulated by fibrous tissue tissues this called fibrous capsules around this could be soft and hard tissues that was in the second generation biomaterials it was always felt and the biomaterial should be completely inert PTFE is supposed to be very good titanium is supposed to be every good. So, small diameter vascular grabs completely inert. So, such materials are non-toxic. So, there is formation of fibrous tissues as you can see here it could be a thin fibrous soft tissues or hard tissues or thick and it completely encapsulates your material.

Now, non-toxic bioactive material; that means, the material is active it is not inert. So, there could be is formation of interfacial bonds between the tissues and the biomaterial. So, it is not completely engulfing or encapsulating your biomaterial, but its form a interfacial bonds it is very difficult later on to remove if there is a fibrous encapsulation or even when it is form an interfacial bonds because the material is completely become part of the host system 4th type non-toxic dissolving for example, it could be a

biodegrading material or bio absorbing material. So, what happens after sometime maybe a year 2 years the material is completely disappearing dissolving like your p l a or some PLGA and so on. So, what happens is that area is getting replaced by the surrounding tissues. So, the tissues start growing as if the material is not there material has completely gone and ideally it could be used in tissue engineering.

So, I am growing tissues with some polymers which are bioresorbable then you are placing it inside and the tissue grows material disappears maybe one day we will have industry the cardiovascular stents which is completely bioresorbed. So, the cardiovascular stent may completely disappear unlike the current stents which are made of nickel titanium. So, 4 types of situations toxic biomaterial or leaching leachents from the material that will kill lead to cell death non-toxic nearly inwards. So, there could be fibrous encapsulation for not toxic bioactive there could be formation of interfacial bond, non-toxic dissolving material and so it is replaced by surrounding material, but here we may say non-toxic dissolve way there could be some toxic dissolving material also for example, polylactic acid and it is dissolving or it is bioresorbing with the lactic acid that is produce is little bit toxic.

So, there could be some cells dying and in the vicinity there could be little bit local toxicity because of the local acidity. So, there could be some variations here toxic dissolving material. So, we have different situations and the tissues responds in different ways that is a beauty of our defense system which takes which looks at coagulation which looks at complement activation mainly to attack the foreign material a leads to inflammation the chronic and the acute inflammation then it leads to fibrous formation encapsulation giant fib cell formation all those things happened and you need to know that such things can happen that is very very important for us to understand.

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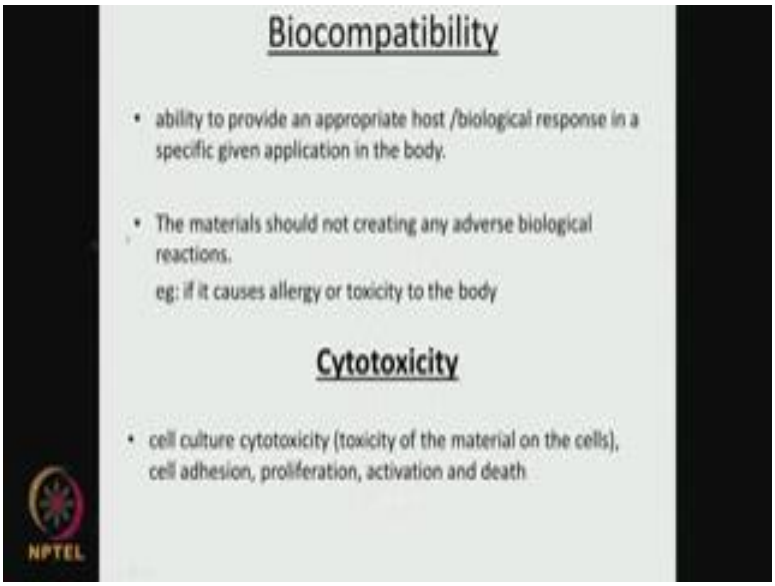

That is look at to each one of them little bit in detail. So, biocompatibilities as I mentioned the ability to provide an appropriate host biological response in a specific given application in the body. So, specific application, so we have different applications like we have been talking about it could be short duration long duration permanent stay it could be in blood contacting non-blood contacting urinary region, it could be a inert biomaterial, it could be inactive biomaterial, it could be bioresorbable material, it could be a metallic which does not re so given application so, it should have appropriate host response the material should not create any adverse biological reaction we do not want the material to lead to systemic toxicity or acute toxicity genotoxicity or metal leaching which could be problems to the host creating a certain side effects.

So, it could cost allergy metal leachents can be allergic to the provision or lactic acid leaching out could be allergy acrylic acid leaching out may be from dental implants to biology cytotoxicity cell culture cytotoxicity. That means cytotoxicity of the material to the cells and especially the addition of the cells to the surface proliferation activation and death. So, you want the cells to adhere proliferate get activated and finally, should go through the process not through in a crosses. So, the biomaterial should be compatible as well as not create cytotoxicity.

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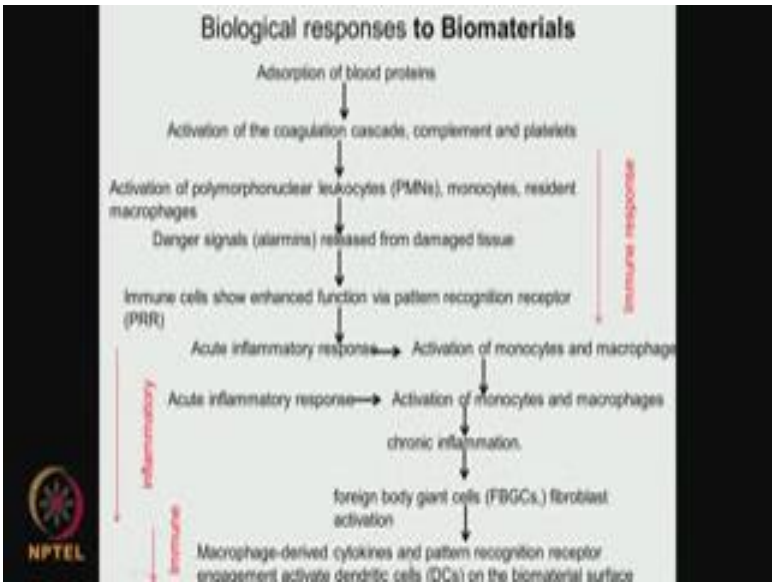

So, look at the biological responses, let us go little bit in more detail. So, absorption of blood proteins is happening here if it is a blood contacting device then we are activation of the coagulation cascade, this is a fantastic cascade. So, there are many coagulation factors one after another after another keep on happening and finally, you are going to have the coagulation then you are activating the compliment these are set of proteins which get activated. So, that they are the host defense mechanism activation of the platelets. So, all these things happen actually then we are having activation of polymorpho nuclear leucocytes monocytes and then macrophages these are macrophages are certain cells which tries to form the encapsulating of the biomaterial.

Then danger signals that is called alarmins released from the damaged tissue that is the next step immune cells show enhanced function by a pattern recognition receptors. So, they are able to identify that the by the material has certain pattern. So, there are some factors getting released all these are called immune response activation of coagulation activation of complement term activation of platelet then its activating the polymorphonuclearly co sides macrophages which is involved in the engulfing or the encapsulation of the biomaterial then signals are produced and then all these immune cells show enhanced activity.

Then comes the inflammatory things you are going to have acute inflammatory response activation of monocytes and macrophages activation of monocytes in macrophages then

chronic inflammation and happens, after that you are cured and then you are getting foreign body giant cells fibroblast activation. And finally, macrophage derived cytokines and pattern recognition receptors engagement activate dendritic cells all these start accumulating on the biomaterial. So, again immune response takes place. So, we have the immune response to start with then we have the inflammation which is acute and chronic happening and then we have again immune responds foreign body and giant cells farming fibroblast activation macro felt derive side against.

All these things start happening as the material is placed inside the system this is where the time of events could be several hours here.

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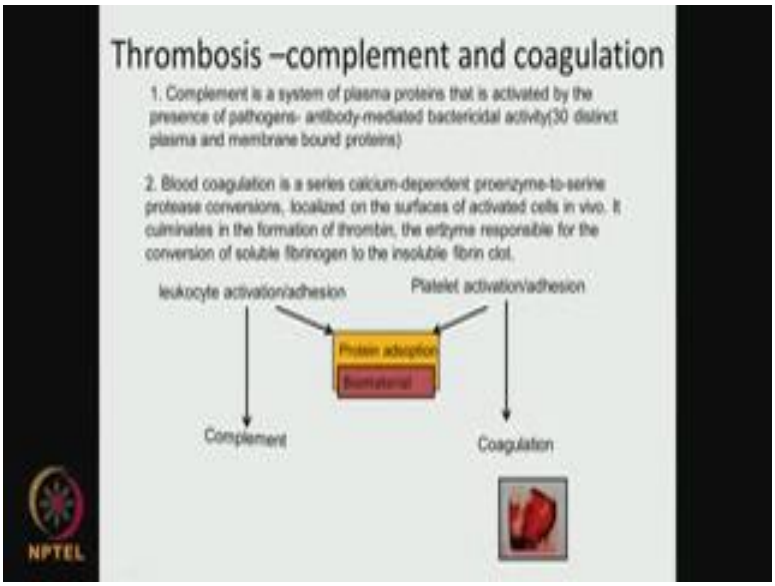

So, let us look little bit in more detail each of these items. So, we are going to have 2 things happening when the material is in contact with the blood the compliment and the coagulation. So, what is this complement this is a system of plasma proteins that is activated with the presence of pathogens antibody mediated bactericidal activity. So, these are formed when there are any pathogens these are formed when there are any foreign bodies they get activated and there are thirty distinct plasma and membrane bound proteins these proteins are found in plasma these proteins are found in membrane.

So, what is there act job and they try to identify these pathogens they try to identify this bacteria they try to identify this foreign body so that they can be attacked by the host immune system. So, there are 30 proteins here that is what is this compliment means then

next one is the coagulation blood this is a series of calcium dependent proenzyme to serine protease conversions look likes on the surface of the activated cells this is envoi it happen what happens finally, there is a formation of thrombin thrombin is an enzyme which converts fibrinogen to fibrin. Fibrin is a insoluble blood clot. So, and when there is a wound up blood comes out after sometime and it starts clotting right that is called fibrin.

How does it form it forms from fibrinogen into fibrin this conversion happens because of this enzyme called thrombin it is an enzyme thrombin which converts fibrinogen into fibrin and this is what is blood clot. So, if this thrombin is not formed blood will start oozing out it will not clot ultimately you need to form this fibrin for example, you can see this blood clot on a polymer or a biomaterial surface. So, both are very important. So, as soon as the material comes in contact with blood complement system gets activated; that means, about 30 proteins get activated which are involved in identifying pathogens or bacteria and which tells the at the defense system of the host that there are some unwanted foreign body present and this is the compliment than the blood starts coagulating how does it happen there are the series of actions taking place and finally, end up in the formation of an enzyme called thrombin which converts the fibrinogen present in the blood to fibrin, fibrin is insoluble.

So, it is the blood clot. So, two very important act actions take place. So, you need to understand it as soon as the material comes in contact with the blood now this com complement there are thirty proteins involved in the compliment each protein has different type of activity.

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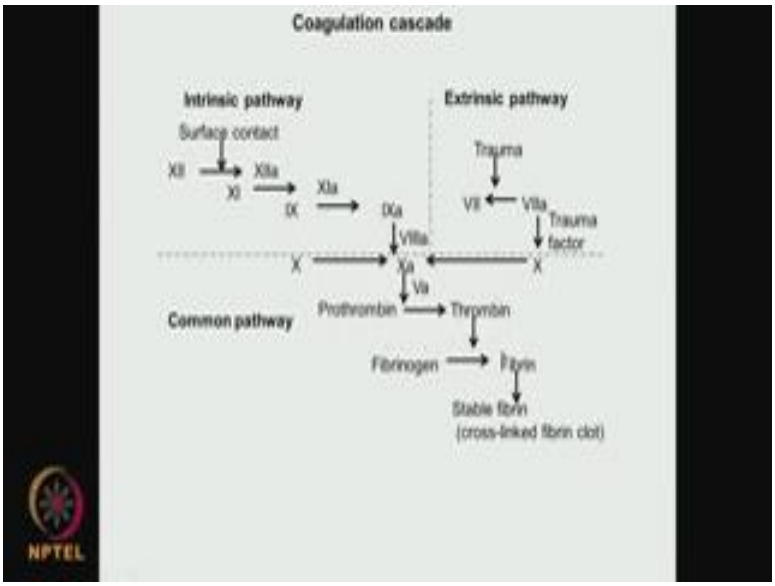

So, we will look at that later. So, coagulation, I said thrombin is here which converts fibrinogen to fibrin this is this fibrin is an insoluble. So, this is called the blood clot this is a blood clot. So, thrombin is a protein which is doing the job of converting fibrinogen to fibrin, but look at this before that there are. So, many factors which lead to the formation of thrombin which is formed from pro thrombin which is formed with the help of factor X a or ten a 10 9 9 and so on.

One is called the intrinsic pathway other is called the extrinsic pathway. So, 2 pathways through which you have this particular factor 5 a happening which catalyzes the conversion of pro thrombin to thrombin and thrombin is the main enzyme which converts your fibrinogen to fibrin and which leads to the formation of stable fibrin which is in our colloquial term call it blood clot. So, these are series of things happening and of course, formation of blood clot is very very important for the survival of the human being if the blood does not clot and there could be continue oozing of blood and the person can lose blood and the end up having a shock because of shortage of blood. So, especially if somebody has cardiovascular problem they are giving given drugs like Warfarin or even aspirin which helps in the thinning of blood.

So, those people who take Warfarin or even aspirin have the problem of the side effects; that means, if they have injuries there could be lot of blood loss. So, they have to be very careful they can even have internal bleeding because these factors are affected or these

factors are retarded or inhibited by those drugs especially those who are taking drugs related to cardiovascular for blood thereon. So, coagulation is very important for the survival of human being, but then when you keep this type of biomaterial they also lead to some of these clot formation. So, you do not want your biomaterial to initiate this cascade of events and ending up with formation of thrombin which catalyzes the conversion of fibrinogen to fibrin.

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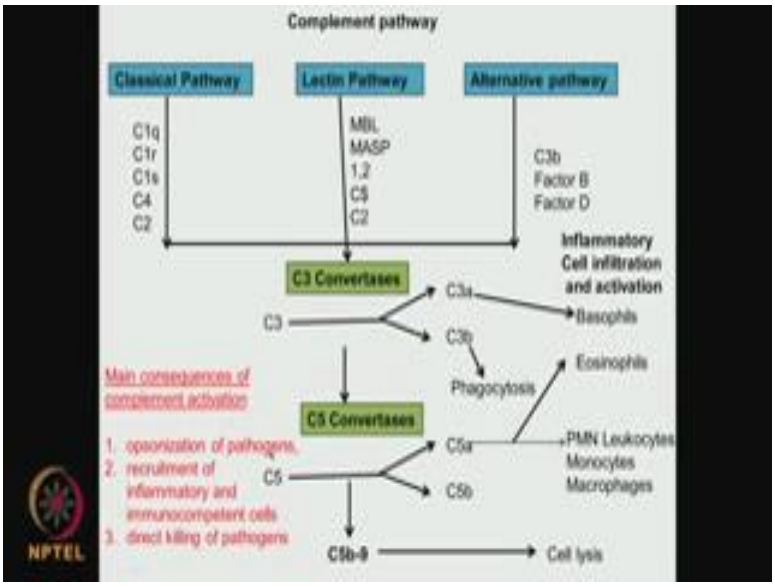

So, it is fantastic things set of things happening inside the human system I did talk about complement these are the system of plasma proteins. So, they also get activated which are involved in. So, many antibodies mediated bactericidal activity. So, we look at that complement pathway there are again 3 pathways here classical pathway lectin pathway alternate pathway and as I said there are almost thirty distinct plasma or membrane bound proteins. So, they all get activated at different sequence of events and they in turn activate snowfields basophiles monocytes macrophages. So, many things they can activate once they activate they get activated they can even kill the cells. So, this cell lyses also takes place.

So, many different types of compliments and they end up killing cell here inflammatory and cell infiltration. So, we do not want the complement activation also which may lead to shelter what are these. So, main consequences of complement activation opsonization of pathogens what is this opsonization these compliments are fantastic proteins they

identify what are the pathogens. So, that the host defense can go and kill them, is it not? So, they are like you are in the war Special Forces which identifies who are the terrorist. So, that is what it does opsonization of pathogen. So, some of these complement proteins or job is to just identify pathogens that is one some of the compliments recruitment of inflammatory and immunocompetent cell. So, they will start collecting inflammatory and immunocompetent cells towards the target size.

They all want to go towards the place where the biomaterial is placed and third is direct killing of pathogens they directly go and [FL] they will go and kill the pathogens it could be a bacteria because the biomaterial is always considered as a foreign object. So, for the immune system they are unwanted. So, 3 things these complement proteins do they identify the pathogens or the foreign body to the host defense. So, the host defense once they are identified the host defense system can go exactly and kill. So, it is like you know and the path the special force goes and identifies who are the terrorist in a village and the army comes and exactly targets those people and leaving the innocent people that is the job of complement they recruit inflammatory and immunocompetent cells.

So, they bring the cells towards closer to the foreign body they directly kill the pathogens. So, there could be pathogens killed. So, some good cells also get lysed and so on. So, all these are the main activities of this compliment. So, once they get activated they start doing these 3 different jobs. So, we do not want that to happen especially when you place a biomaterial that is why especially when a when a patient undergoes a implant they try to give drugs. So, that the immune system is slightly is suppressed we do not want the immune system to become active and creating blood clots and complement activation and then a systems becomes a inflammated and so on. So, they give lot of drugs where the immune system can get little suppressed.

So, the patients can have the risk of having diseases where because the immune system is compromised here. So, they can have bacterial infection they can even have a long term h I v type of infection because their immune system is suppressed. So, these are the job of the complement proteins. So, one needs to keep in mind when the biomaterial is placed that the biomaterial does not activate the complement proteins at the same time we do not want the biomaterial to activate the coagulation pathway because there could be lot of blood clotting taking place as I have been explaining formation of stable fibrin which are insoluble around the biomaterial formation of clots.

So, we do not want both these things happening when the biomaterial is placed inside the blood clot attacked area.

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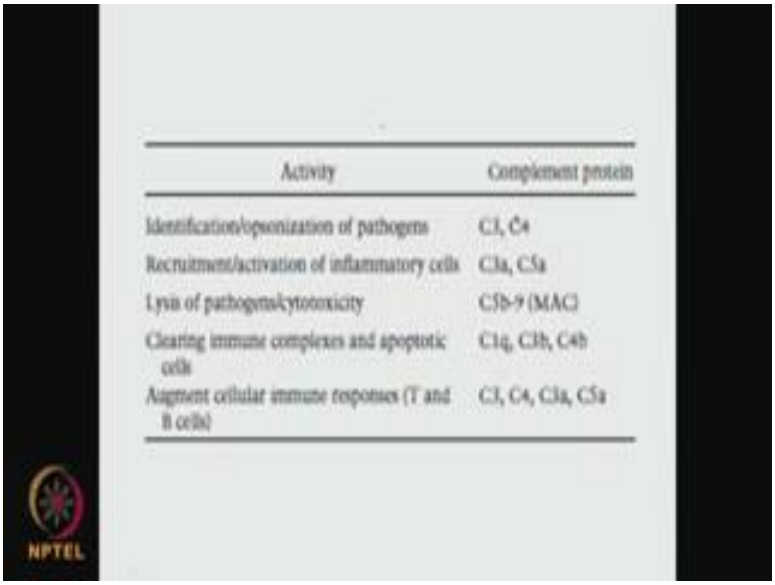

So, these has many complement proteins the C 3, C 4 as I said there are thirty different plasma bound or membrane bound proteins which are com complement these proteins C 3 and C 4 identification that is opsonization of pathogens C 3, C 5 a, they are recruitment activation of inflammatory cells C 5 b 9 lysis of pathogens cytotoxicity C 5 b nine. So, you are looking itself lysis C 3 C 4 are involved in identifying the pathogens C 3 a C 5 a, are involving in the recruitment of inflammatory cells sorry C 3 a, C 3 b recruitment of inflammatory cells clearing immune complexes and apoptotic cells.

So, once the cells have died you need to remove there that is C 1 q and so on. C 1 q and then argument cellular immune responses again bring back the cellular response with the help of T and B cells; so C 3 C 4. So, these are the jobs of these complement proteins very interesting such fascinating things are happening inside the host system. So, I think it is very fascinating the complement system it identifies the pathogens it brings the inflammatory and immune competent cells it directly kills the pathogens. So, all these 3 things are done by the complement system. So, complement activation, how does it happen?

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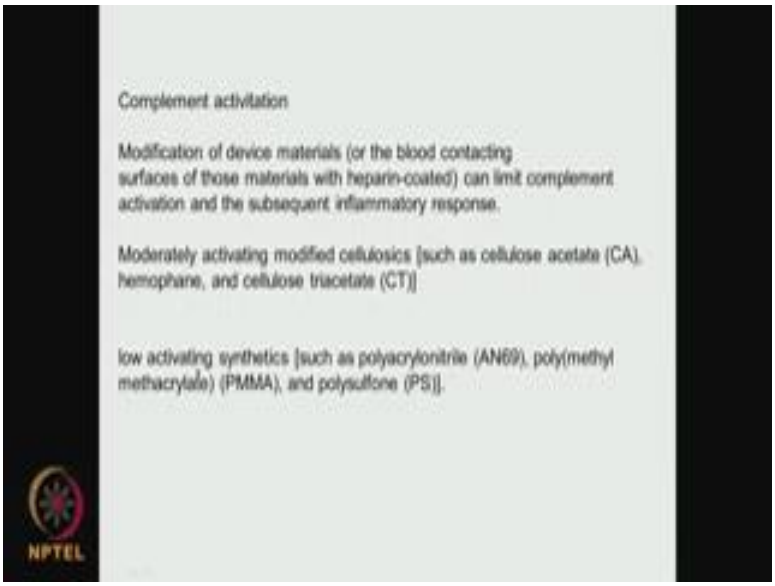

So, we can or how would; we can prevent this complement activation? So, we can have modification of divide device materials or the blood contacting materials people have looked at heparin coating heparin by coating this heparin it will prevent a both complement activation as well as it can reduce the coagulation of blood. So, this can limit complement activation and subsequent inflammatory response. So, that is modification of the devices heparin coating.

So, there is lot of interest now a days on heparin coating there are some polymeric material which are moderately activating the complement based on cellulose like cellulose acetate homophone cellulose triacetate all these biomaterials moderately activate the complement system that some biomaterials which very low activating polymethylmethacrylate poly cell phones polyacrylonitrile that is why polymethylmethacrylate it is widely used in the oral or dental poly cell phones are also used in some of the guide wires guide tubes because polyacrylonitrile also are very useful because none of them activate the complement system they are all synthetic material.

So, one needs to keep their in mind and if one is designing biometrical where blood clot contacting biomaterial and we do not want the complement activation. So, one is modifying the surface and heparin is widely nowadays used which can prevent both the coagulation as well as compliment or we can think of using this type of natural material

which moderately activate or this type of synthetic material which does not activate the complement system. So, we will continue more on these biological responses in the next class also.

Thank you very much for your time.