Oral Biology
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Module - 03
Interaction of Immune System and Biomaterials
Lecture - 27
Protein mediated biomaterials

Hello everybody, today we will be seeing one of the lectures in module 3 of Oral Biology course. So, the title of the module is Interaction of Immune System and Biomaterials under the biomaterials 3rd module.

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So, what we will be learning in this? How biomaterials are interacting with the immune system in the host body? So, oral cavity is one of the place where almost all kinds of biomaterials are being used starting from metallic to ceramic to composites or polymers, and it has been used for replacement, therapeutic, regeneration for all kinds. So, a biomaterial by definition all of you must be by knowing this.

It is a material when it is placed inside the body to replace or augment a function of a particular tissue or a organ of the body or sometimes even to regenerate the part of the body. So, what is more important regarding biomaterials is, its property we are not going into that, that is not the scope of this lecture. Here we are going to see how the

body is responding towards any biomaterial? And if a biomaterial has to be successful without any rejection or if it has to perform its function optimally then what are the things that has to be considered from the host point of you.

And accordingly how we can design the biomaterial? Or how researchers are tuning the biomaterials? So, that is what we are going to see. So, if it to see the types of biomaterial, basically we can classify them as natural biomaterial which is from the origin of the biomaterial is going to be from natural sources or synthetic biomaterials.

So, natural biomaterials may be from plants such as cellulose or it can be from animals like collagen, gelatin right or it can be from insects like ketone. So, these are naturally originated biomaterial. So, mostly in case of dental application all kinds of biomaterial is used, but over for different application they use synthetic biomaterials.

So, commonly ceramic and metallic biomaterials are the one which is widely used in dentistry. So, we can classify the synthetic biomaterial as metallic, polymeric, ceramic, and composite; composite will be the combination of the above. So, here you can see the different application of these biomaterial in general, so, let us not go into this.

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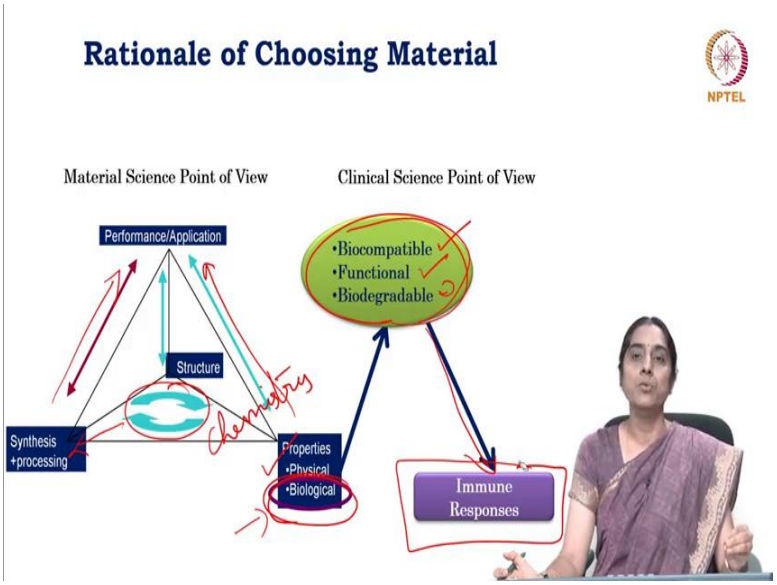

So, what is the rationale for choosing the material towards a particular application? So, when you talk to a material scientist; so, their perspective of choosing a material will be based on the composition its structure ok. So, the chemistry part is very important

wherein also the physical properties are important, how is the structure going to influence the property that they will be looking into.

And what is the way by which the synthesis is being carried out for that biomaterial and also the processing further processing to get the end product how it is being done. So, all these are going to you know influence the performance and application of this biomaterial; so, that is from the perspective of material scientist.

But when we are going to look from the perspective of a clinician from the clinical point of view what is most important is the biological property, the biological property does not mean that it is only biocompatible; so, it has been already written here. So, it should be biocompatible, it should be functional; so, for whatever purpose we are placing it should optimally perform that function, it should be biodegradable also.

After it has done its desired function say for example, regeneration of a particular tissue, it has to be either resolved in the body or it should be degraded and then removed from the body safely without causing any adverse reaction or at least with minimal adverse reaction. So, this is what is the will be the clinical point of view right. So, what is deciding this biocompatibility from the host side is the immune system?

So, immune system is any way going to cause a response even though it is termed as biocompatible, it is the foreign material that is being placed inside the body. So obviously, there is going to be a response or an action from the immune system.

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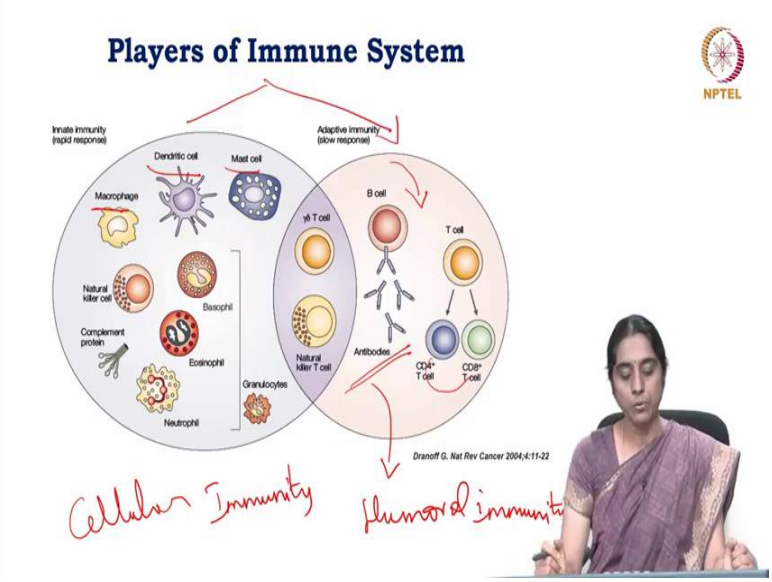

So, let us again recap something about the basics of immune system. So, immune system can be classified as innate immunity which comes from the birth. And it is mainly mediated by the cellular cells of the immune system such as macrophages, neutrophils right which are all granulocytes which belongs to the category of granulocytes and n k cells etcetera.

Then you have the other component which is the adaptive immunity which is a slow process, but is very specific. Whereas, your innate immune response is a rapid response, but it is not specific. So, this is mediated by the adaptive immunity is mediated by B cells T cells and CD 4 and CD 8 T cells. So, mainly mediated by the antibodies and the effective response of CD 4 and CD 8 cells.

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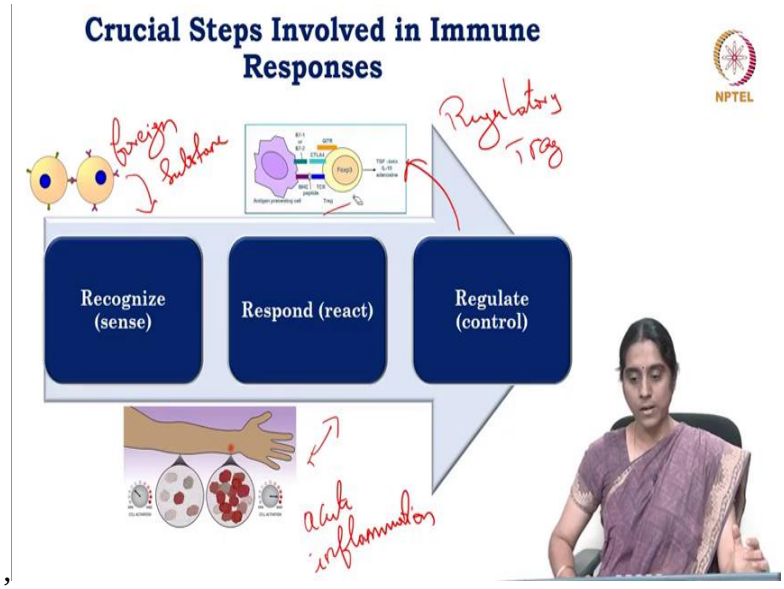

So, having known about it, the cells of the immune system and that is one way of classification. Another way is to say the cellular immunity and humoral immunity right. So, this is also another way cellular immunity is mainly mediated by the cells of the immune system and humoral immunity by the factors various factors in antibodies of the immune system ok. So, if you see the crucial steps, if the mechanism of how the immune system is function is functioning is very elaborate, but we will be seeing the key steps and the key process involved.

So, first it is the recognition of the foreign substance right, by means of some receptors right; so, that is the first step. The second step is how the immune cell is going to react towards this sensing; I have recognized something foreign how should I react. Whether I have to eliminate it immediately by means of engulfing it or should I recruit some other specialized cell. So, that is the thing that happens next the decision making.

But, most of the cases it is acute inflammation which tries to eliminate in case of it is pathogenic infection it will try to eliminate that infection; even in case of biomaterial this is the process acute inflammation happens. So, then comes the process called as regulation. So, once the job is done, the foreign substance or the antigen has been recognized, it has been appropriate response has been created during the response overreaction has been you know has been has happened say kind of inflammation.

But, if it is not controlled beyond a point that process itself will lead to damage or tissue injuries which is called this immunopathogenesis. So, the immune system has a regulatory mechanism mainly through the cells called as regulatory three cells in short called as T rags. So, these cells have certain molecules which are expressed and they will suppress the activated macrophages or other T cells effect our T cells.

So, this is a way by which you know the general mechanism by which the immune system functions; so, recognize, respond and regulate. There is also another set in case of infectious disease called the memory response ok; so, that also happens. So, again when secondary infection happens, these memory cells they come immediately into pay and at. So, that I am not discussing with regard to biomaterials.

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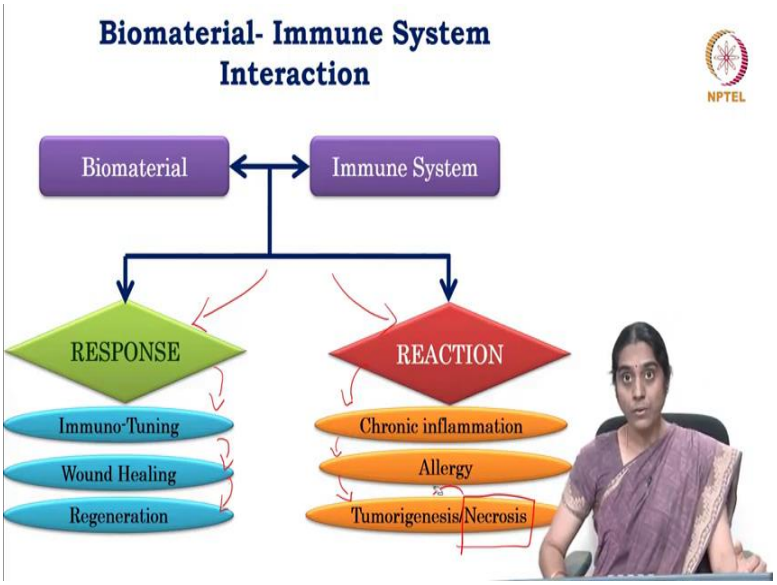

So, with biomaterials how was the interaction? So, you can see here in this slide that the interaction can either lead to a response or it can lead to a reaction. So, what is the difference between the response and the reaction? In case of response what we ideally require is a response; you need to have immune tuning which will elicit a type of inflammation. But that has to be converted that it should be a switching of the inflammation towards own healing and this own healing will lead to regeneration.

So, this is what is the ideal requirement for preserving a biomaterial for longer time in the body or for optimal functioning of the biomaterial and especially for regenerative biomaterials. So, what is the opposite which is happening leading to the failure of the

biomaterial? The inflammation is not controlled and it is continuously happening even after months or even years called as the chronic inflammation.

And in some extreme cases also other cells of the immune system most of the cells of the immune system are activated leading to allergic kind of reaction. And in extreme cases cell death at the place of implant placement happens due to necrosis of the cells surrounding the implant causing implant failure and in extreme cases even tumorigenesis happens.

Tumor formation initiation of tumor formation happens because of adverse immune reaction towards the biomaterial. So, now, you understand why the interaction of biomaterial between the immune cells another host cell is very important.

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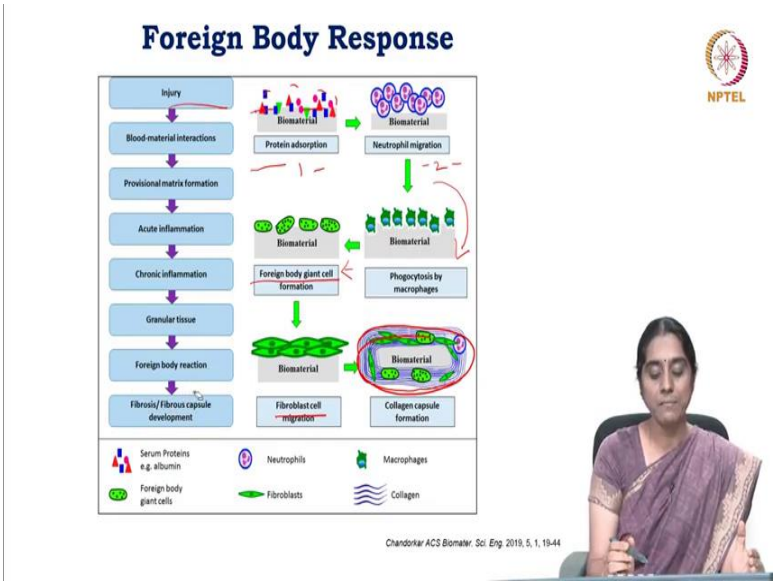

So, immune interface with biomaterial has to be elaborately studied before it is translated to the bench side ok. So, how is the foreign body response in towards the biomaterial? It usually initiates with injury right. So, how is the biomaterial placed inside the body? If it is going to be the oral cavity also definitely there is going to be an injury where the implant is placed and that causes exposure of various fluids including blood and either surrounding lymphatic fluids or in case of oral cavity GCF Gingival Crevicular Fluids.

So, all the material saliva etcetera; so, the proteins present in these fluids are going to get absorbed towards your biomaterial; so, that is the first step, the protein absorption is the

first step. The second step is the protein absorbed on the biomaterial is going to recruit polymorph nuclear neutrophils.

This is again going to recruit further other immune cells by means of secreting various chemo attractants. So, this is going to attract macrophages, these macrophages will try to phagocytose this biomaterial. Because, most of the biomaterial is not a small particle there are going to be large particles and engulfing these particles during that process the fusion of macrophages happen; so, that leads to the formation of the foreign body gain cell ok.

Then this foreign body gain cell will settle there and recruits various other cells such as importantly fibroblast migration. So, fibroblast you know are the cells which are going to secrete one of the extracellular matrix protein called as collagen. So, this collagen will form a capsule around the biomaterial; this capsule is going to encapsulate macrophages, fibro blast, and polymorph nuclear cells; so, you it will have the immune cells as well as the fibroblast. So, this is the general process of the host towards any biomaterial, this is how it is.

So, generally if you see here in the flow chart that the injury will cause blood biomaterial interaction, causing provisional matrix formation, leading to acute inflammation for few days. And if it is persisting for weeks it leads to chronic inflammation leading to the granular tissue formation, and then the foreign body reaction happens thus ultimately leading to the fibrous capsule development ok.

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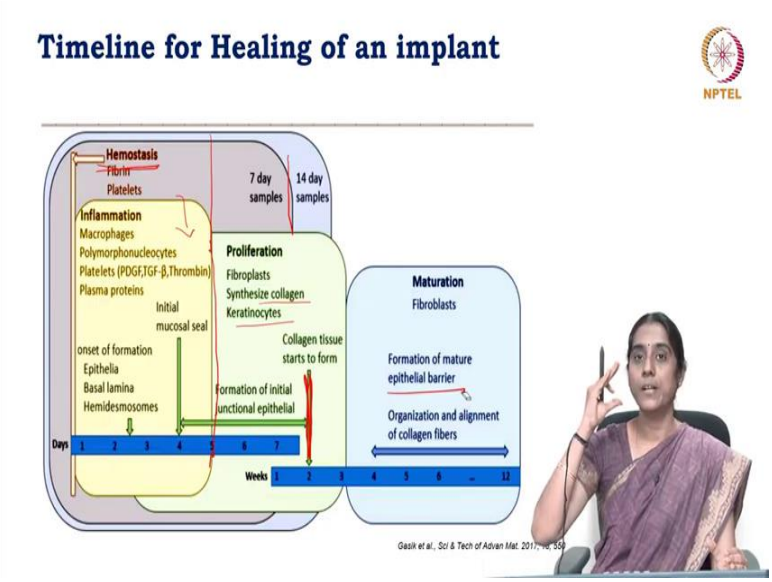

So, the if you see the timeline of all this; so, initially there is inflammation the homeostasis hemostasis happens right. This all happens in the first 5 days of implant placement that is your inflammation acute inflammation ensues within that time. The protein absorption later on neutrophil recruitment, macrophage recruitment, and you know all these in inflammatory cells clogging towards the biomaterial happens within the first 5 days; this is not exact, but most of the cases this is how it happens.

And later after 7 days the fibroblast cells are going to be recruited there which will synthesize collagen and there is also certain cells in certain areas where Keratinocyte will be recruited this will produce keratin. So, that will also participate in the capsule formation. So, this helps in the formation of initial junction are the biomaterial interface.

So, after the 7th day towards the until 14th day the whole process of capsule formation will happen in most of the biomaterial. And later on as the third week later it progresses to the maturation of the epithelial barrier and then there will be a proper organization and alignment of collagen fiber around the biomaterial making it thicker. So, this is how the healing of an implant happens starting from the tissue injury ok.

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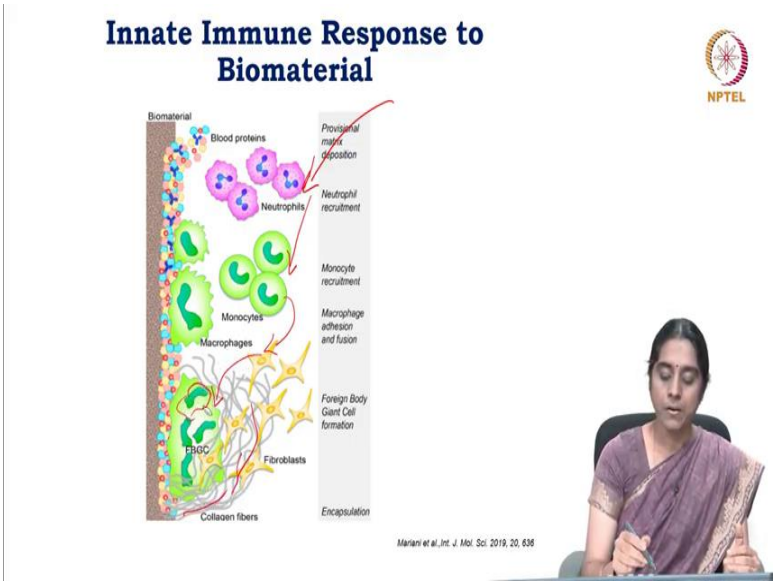

If you see here is what it actually is all this process. So, there is a innate immune response which acts rapidly towards the foreignly introduced bio material right. So, where you have you can see that neutrophils are coming and the neutrophils or I am sorry. So, neutrophils are recruited this neutrophil is further recruiting monocytes from the blood and the monocytes are maturing here into macrophages.

So, these macrophages further attract chemo attract fibroblast cells write and form the collagen fibers here. Here again these macrophages they become Foreign gained Foreign body gain cells.

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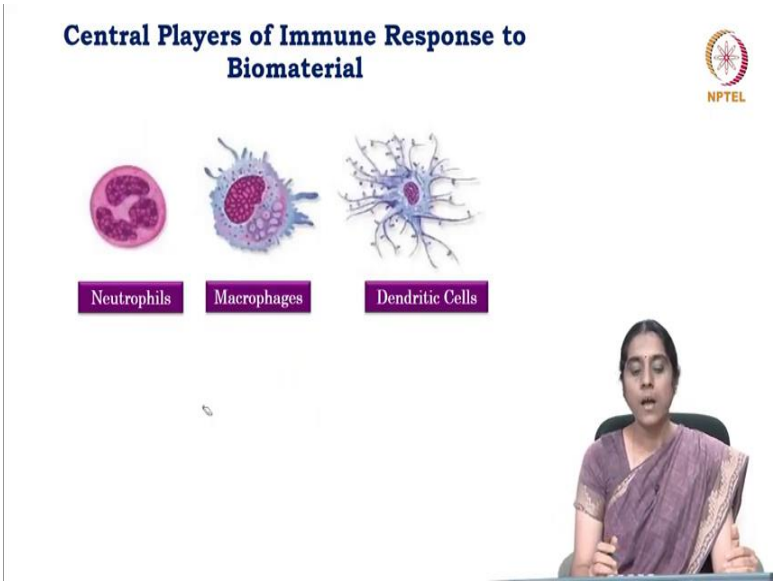

So, if you see here the central players are neutrophils, macrophages, and dendritic cells, even though we did not see dendritic cells here. But dendritic cells are in are cells of the innate immune system which plays a role here in switching towards wound healing as well as in the inflammatory process. So, let us see how it is being done.

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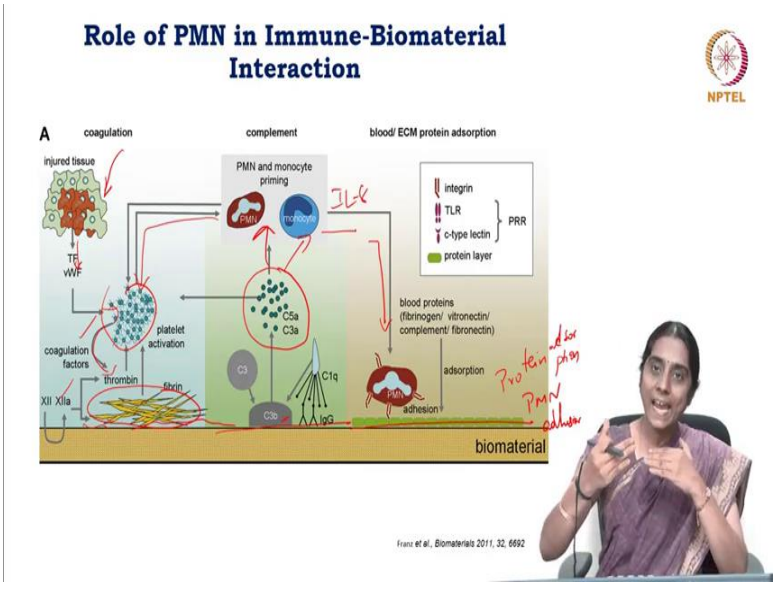

So, if you see the how the polymorphonuclear cell such as neutrophils are interacting and playing a role in the interaction. So, you see here once the tissue at the implant site is injured, tissue factor and other coagulation factors are released. So, thrombin platelet is

activated platelets are activated which is releasing thrombin and that causes the fibrin formation; so, this fibrin is going to get absorbed on the biomaterial.

The next thing that is happening is from the complement cascade system; the complement pathway of the immune system is also a pathway of the innate immune response. So, they are set of proteins which play a role in eliminating bacteria's as well as foreign antigen. So, these complement proteins are also activated specifically C5 a and C3 a; they in turn activate nuclear neutrophils and monocytes ok; not only that these neutrophils activated neutrophils further activate the platelets. So, this is like amplification of the fibrin formation.

And here if you see the activated monocyte further releases certain factors like IL 8 which is going to cause recruitment of more neutrophils ok. So, here you see that there are also other proteins like C3 b complement protein and the complement protein C1 q the complex formed is being adhered on the biomaterials. So, once the addition of the neutrophil happens various other proteins like, fibrinogen, vitronectin, fibronectin also adhered to the surface of the biomaterial.

So, the first step is protein absorption we saw right in the previous slide along with PMN adhesion. So, what I mean to say here in the slide is PMN is the first cell to come when a biomaterial is placed. They are the neutrophils or the one which immediately recognizes that it is a foreign material and it plays a role in the absorption of proteins on the biomaterial.

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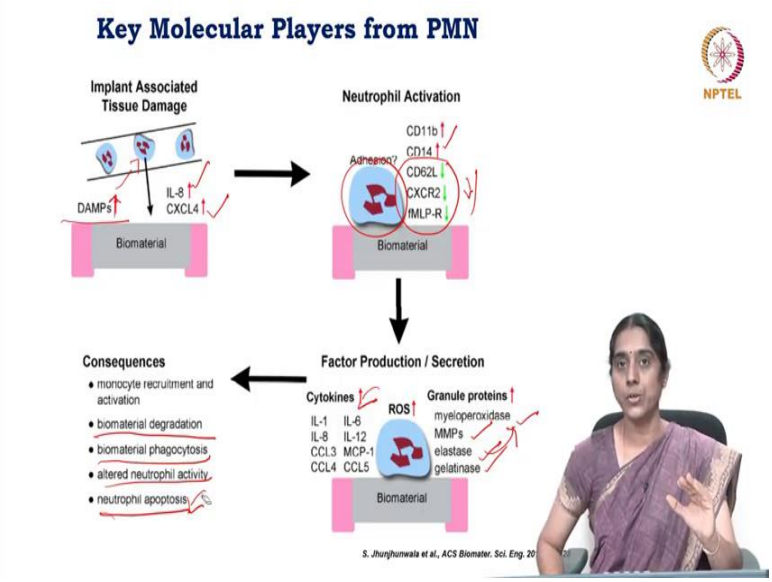

So, what are the key molecular players from neutrophils? So, you can say that when neutrophil is activated these molecules, CD11b CD14, CD62L, CD11b and CD14 are increased whereas, these three molecules are a it is not express well their expression is reduced ok. So, if it is going to be implant associated tissue damage. So, whenever there is a tissue injury these damps are expressed which will activate the PMNs and they will cause the secretion of chemokines such as IL8 and CXCL4 which is a chemokine ok; that will lead to activation of other neutrophils and this is the process.

So, once the activation of neutrophil happens the next step is the production of ROS, Reactive Oxygen Species and also you know the increased expression of granola proteins. Especially; myeloperoxidase, these are the enzymes which are more secreted from the neutrophil granules then matrix metalloproteases, elastases and gelatinases.

So, these are all enzymes which are going to cause it is a proteolytic enzyme, they are going to cause destruction of proteins in and around them; so, that is mainly for eliminating bacteria and bacterial proteins. But, here in spite of it does not recognize something is biomaterial or a bacteria; but, even for the biomaterial the same process ensues. And on the other hand cytokines such as IL6, IL12 these are all pro inflammatory MCP1, CCL5 chimokine legend and IL1, IL8 these are also increased once the neutrophil is activated.

So, what are the consequences of this activation of neutrophil? There will be biomaterial degradation, there will be damage to the biomaterial immediately. And the biomaterial could be phagocytosis, because even neutrophils are phagocytic cells they can phagocytose and engulf the biomaterial not the whole of this at least part of it; so, then alter neutrophil activity.

If they are going to be activated continuously sometimes there will be exhaustion in the ROS production due to which when there is real infection happening there at the sight of the biomaterial implant placement, the neutrophil may not be able to kill the pathogen. Because, there will be exhaustion of the neutrophils, due to which there will be even biophil film formation.

And also the neutrophil apoptosis finally, they will be because too much of activation leads to the apoptosis of neutrophils. As you know neutrophils are short lived, they maximum their lifetime is from 18 hours to 24 hours; so, when they are activated, they undergo apoptosis immediately. So, this is how your neutrophils are going to alter the site of biomaterials.

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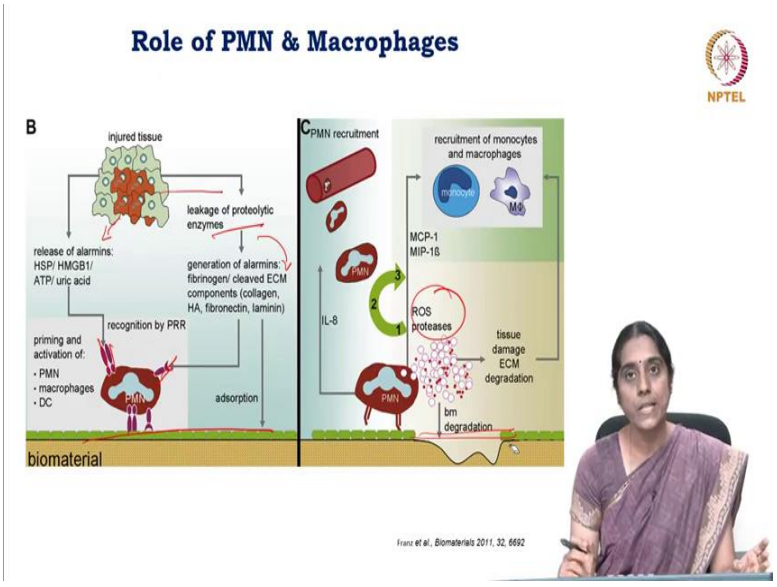

So, what is the role of macrophage and PMN together? So, when your tissue is going to release alarmins heat shock proteins and uric acid during its injury, it will cause leakage of proteolytic enzyme. Now, that is going to cause the generation of alarmins, alarmins are nothing but proteins which are released during tissue injury which will help the body

to recognize that there is there has been injury happened at a particular site. So, these cleaved easium components will be absorbed on the biomaterial priming the neutrophil, this is what we saw in the saw in the previous slide.

But these PMNs will be having patent recognition receptors called a stole like receptors. These receptors are going to again further recognize some of these uh proteins and more and more release of cytokines and chemokines will happen; so, attracting lot of immune cells from the blood towards that site right. Now, the next thing which happens is this PMN will recruit more of monocytes from the blood and will differentiate them into the macrophage at the biomaterial site, these macrophage can have two phenotypes that we will see here.

But here you see this recruitment of monocytes may lead to cause generation of ROS and further protease secretion which will cause tissue damage and extracellular matrix degradation which will be on the top which were absorbed. And further even the biomaterial will be damaged by the immune cells together with neutrophils and macrophages.

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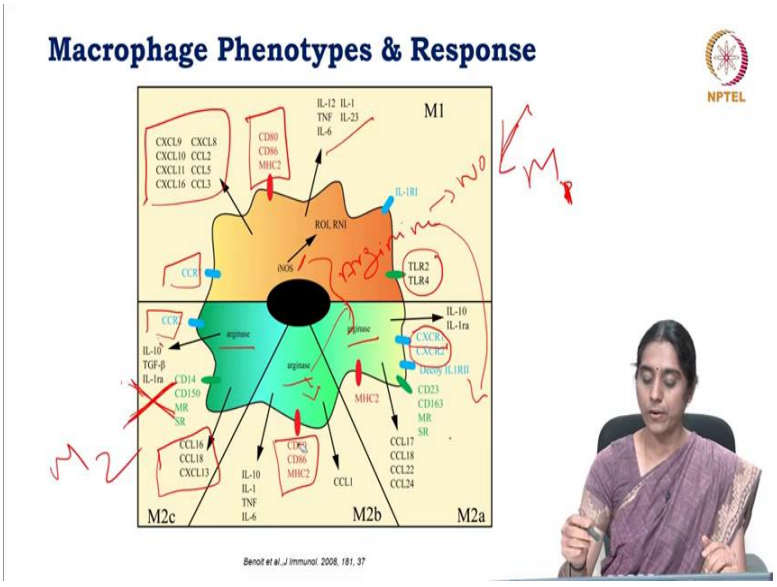

So, if we see the next uh role of how the macrophages are going to be, before we go further we need to know the different phenotypes of macrophages. Macrophages can be of you know M they usually polarize at a particular site termed as M1 polarization and

M2 polarization M2 and M1 ok. So, in case of M1 we do not have subsets, but in M2 we have subsets like such as M2a, M2b and M2c.

So, what is this M1 and M2? So, based on its phenotypic marker expression based on the enzyme type of enzyme it secretes and its function and the cytokine profile it expresses they are classified as M1 and M2. So, you can see here the set of chemokine here and the set of chemokine by M2 is different. Similarly, the set of phenotypic markers by M1 is CD80, 86 and MHC2, here also it is CD80, 86 and MHC2. However, you can see that there is expression difference in the expression of the chemokine receptor.

You will have high CXCR 7 expression in M1 while in case of M2 it will be CCR2 and CXCR1 and CXCR2; so, these are the main differences. So, this is going to be the same; however, the expression rate may be slightly different and also the expression of TLR2 and 4 is more seen with M1 phenotype while here it is not.

And specifically the important differences with the cytokine pattern. Here you can see that the cytokine IL10, TGF beta, IL1 ra which are mostly anti inflammatory is secreted by the M tif M2 phenotype. While here it is proinflammatory cytokines that are secreted in higher quantities like, IL12, TNF alpha, IL6 etcetera. Another important difference is by with the arginase.

So, you can see here arginase is secreted by all the M2 phenotype and whereas, your M1 phenotype is secreting iNOS; so, both are enzymes. Arginase will act on both iNOS, iNOS is inducible dielectric oxide synthase and arginase again both are enzymes which will act on the substrate called as arginine ok. So, while iNOS will produce nitric oxide ok, inducible nitric oxide also it can lead to its a reactive nitrogen intermediates or reactive oxygen intermediates.

While this arginase will act on arginine and that will lead to the formation of importantly a molecule called a ornithine. It leads to the formation of ornithine arginine, leads to a formation of ornithine and ornithine leads to it leads to the formation of arnethine; so, this is the difference.

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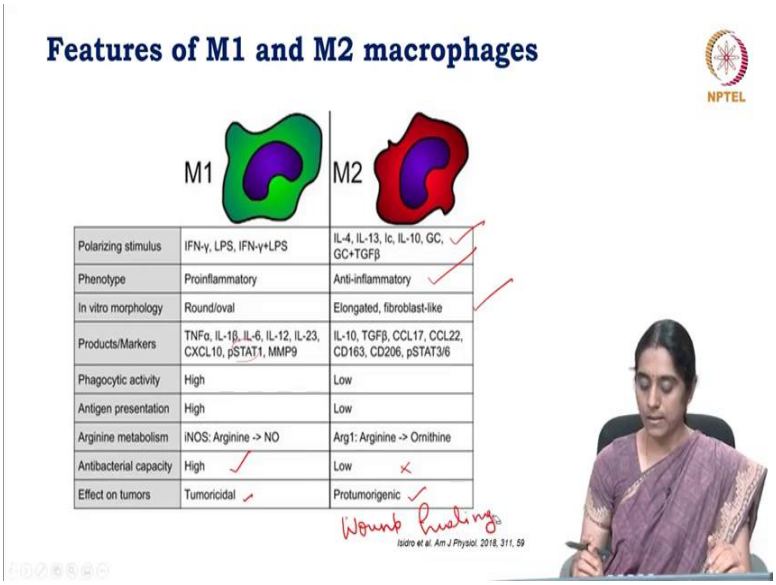

So, here you can see the features in has been given in a tabular form. So, the polarizing stimulus here is interferon gamma LPS etcetera, while for M2 it is IL4, IL13. The phenotype for M1 is pro inflammatory for M2 it is anti inflammatory and for in the in vitro morphology is round or oval for M1, while for M2 it is like fibroblast products which means elongated.

The markers for M1 is if you are particular about the cytokine it is TNF alpha, IL1 beta, IL6, IL12, 23 and it also secretes chemokines CXCL10, then MMP9 and it also has activated pSTAT1. While in case of M2 phenotype it is IL10, TGF beta, CCL17, CCL22, CDs163, CD206 etcetera. Phagocytic activity is high with M1; whereas, with M2 it is low, antigen presentation is again high; whereas, with M2 it is low, arginine metabolism leads to nitric oxide production while in case of m two it leads to ornithine production.

So; obviously, M1 has high anti bacterial capacity; whereas, M2 has high low antibacterial capacity; so effect on tumor is it is tumorsidal in nature, it is pro tumorigenic. So, in what way then M2 is important? They have an important role in wound healing; so, this is the this is why biomaterials they prefer M2 polarization.

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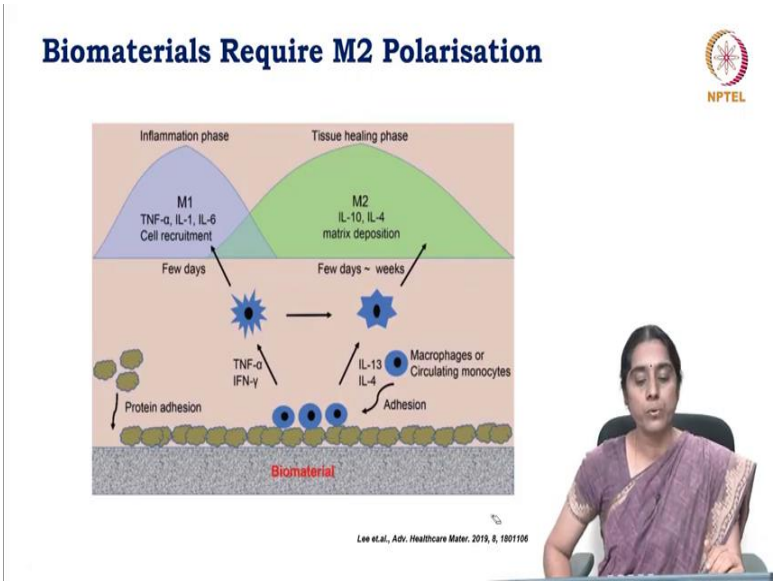

So, they require M2 polarization, the macrophage should polarize towards M2 polarization M2 phenotype once the acute inflammation happens. So, you can say that after the initial inflammatory phase here after few days the tissue healing phase happens. So, when we check the macrophage phenotype then you can see that it will be in M2 type ok.

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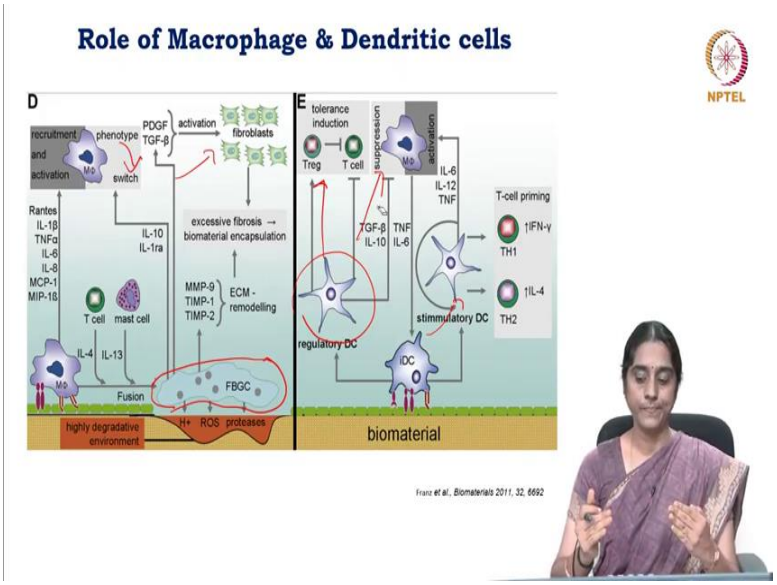

That was about how macrophage plays a role in shifting the inflammation towards wound healing, in when a biomaterial is placed. So, the next one is how macrophage and

dendritic cells leads to resolution of the inflammation. So, here you see here that the switch happens right; the phenotypic switch from M1 to M2 happens, leading to the activation of fibroblast. So, wound healing is mediated by secretion of collagen right that is done by fibroblast. So, excessive fibrosis will happen and biomaterial encapsulation will happen.

So, this is how it is and not only that the fibro foreign body gained cells are formed and that will lead to slight damage at the implant place site. So, this is a reason why there is loosening of the implant are the placement implanted site leading to tissue loosening as well as the implant failure; so, this is highly degradative environment.

But, if you have to have a ideal environment for the successful placement as well as for performance of the biomaterial. Then it should be that the disease which are going to be activated by the macrophage and neutrophils should gain a regulatory phase regulatory. So in already you must have been well exposed to immunology.

So, dendritic cells are professional antigen presenting cell, they will be in immature state. Once they are exposed to antigen they become activated and a process called maturation happens to the dendritic cells and they are stimulated either they can be stimulated or they will enter into a regulatory mode.

So, in regulatory mode what happens is they further activate the regulatory T cells; so, tolerance is induced towards the foreign body ok. If it is a stimulatory DC, then it dendritic cell it will lead to activation of the macrophage while regulatory dendritic cell will lead to the suppression of the dendritic cells ok; so, this process we need to remember.

So, if the biomaterial has to be successful and has to perform active optimally there should be a initial inflammation. We cannot say that I have to totally avoid inflammation, I need immune cells at the site of the implant which is going to initiate the wound healing and then it should resolute over a period of time; so, that the failure is avoided right.

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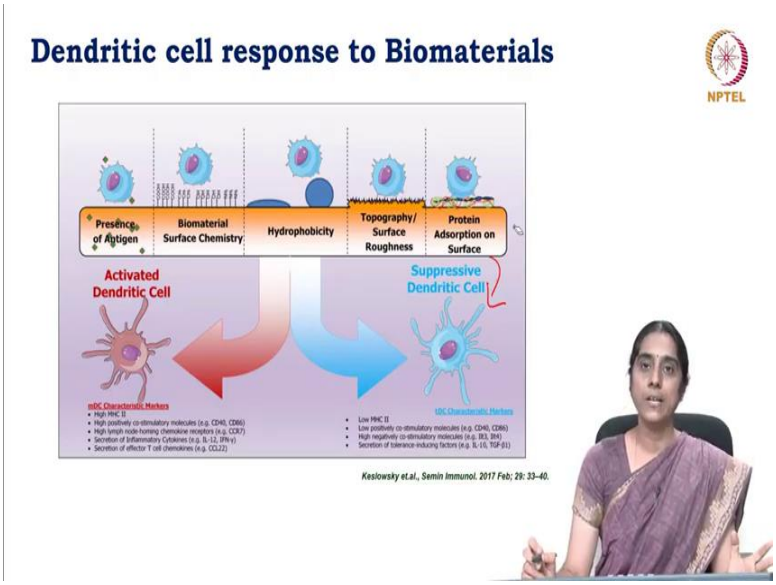

So, how can the dendritic cell response to biomaterial can be tuned then? So, that can be done by various tuning the properties of biomaterial. Starting from surface chemistry and then whether the surface is going to be hydrophobic or hydrophilic or the topography and the surface roughness. All these parameters can be tuned; so, that we can achieve a idle state of dendritic cell.

If we want to activate certain properties or tuning of properties has to be done or if we need an suppressive dendritic cell that can also be done. So, specifically if you see when there is protein absorption on a surface that leads to suppressive dendritic cells ok.

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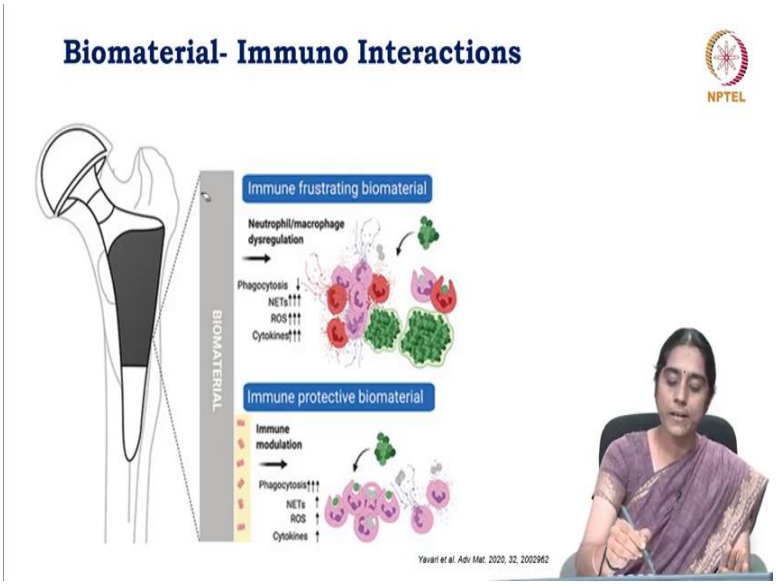

So, altogether what we saw is a biomaterial has the ability to cause frustration of the immune cells or it can interact with the immune system in such a way that it is protective to the biomaterial from the surrounding other tissues. So, either the immune system will itself can destroy the biomaterial. So, summary from all these slide what we understood is that, the immune system can itself degrade and damage the biomaterial by if because of the frustration created by the biomaterial.

Or it can be protected by the immune system itself that all depends on various factors host factors as well as the various you know physicochemical properties of the biomaterials. So, when a immune system is frustrated what happens is the phagocytic capacity becomes reduced while the reactive oxygen species. All the inflammatory type of responses increased like, necrosis increases the inflammatory cytokines are expressed in high quantity; so, all these stuff happens.

Whereas, in case of protective response phagocytosis process is increased, the netosis happens even the ROS that is reactive oxygen species release happens, but it is not so high.

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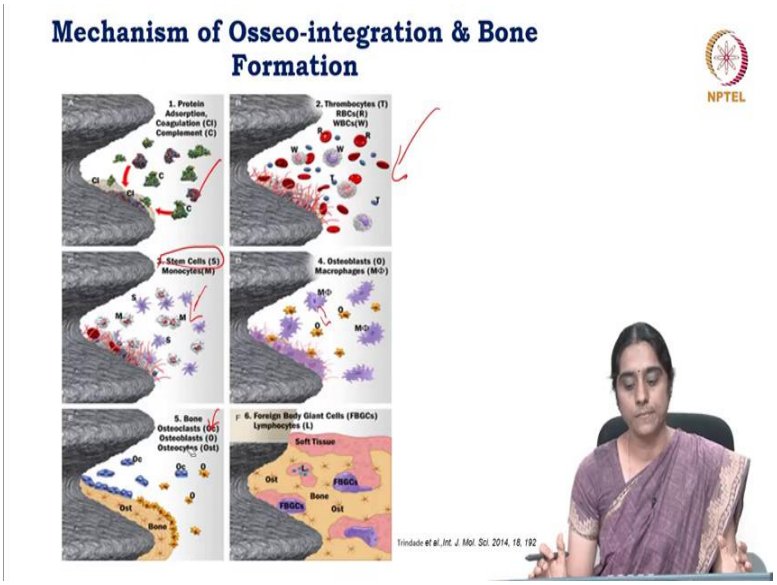

So, in case of dentistry, they are mostly even interested in bone formation right. So, most of the bones are either replaced kept as a replacement for bone tissue or they are used as a regenerative material graph material for bone formation or they are used to preserve the alveolar bone, bone present and at the site right.

So, how this is happening? If it in case it has to regenerate, the whole process see the absorption and happens on the biomaterial; then you know the recruitment of the immune cell is happening at the site of the biomaterial. Along with the recruitment of immune cells, the stem cells are also recruited towards the site of immune cell and biomaterial interface.

So, in case of oral cavity it is middle thymol stems which comes from its origin. So, they are differentiated into osteoblasts by means of factors secreted from macrophages. It might be certain factors or it might be some receptors secrete interaction with the stem cell that causes leading to the formation of osteoblasts osteoblasts or bone forming cells.

So, these the it is not just the osteoblast alone that will help in the formation of bone, but also the balance of osteoclast, osteoblast and osteocytes. So, this balance is mediated by the macrophages because osteoblast will go on forming the bone matrix, but the remodeling at the site will be mediated by osteoclast right ok.

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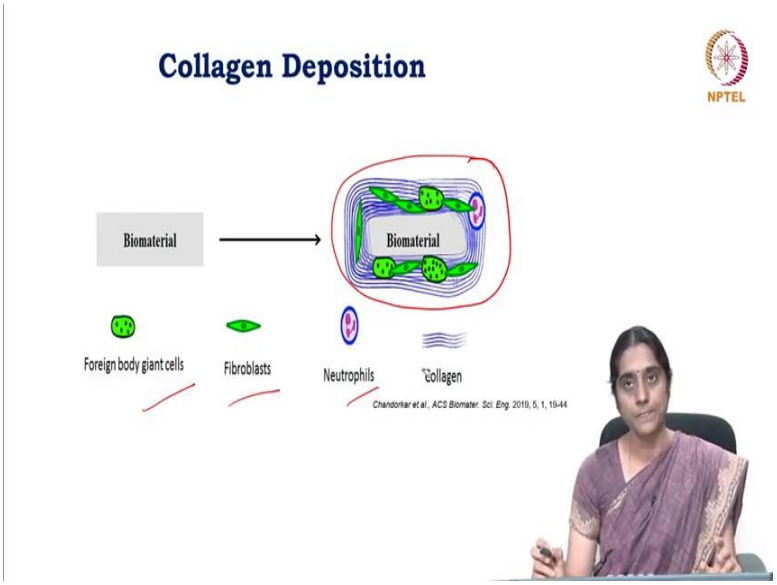

So, this is a schematic representation of how the collagen deposition happens or encapsulation happens on the biomaterial. So, you can see that mostly fibroblasts are there, foreign body gain cells nothing but the fused macrophages, and the neutrophils are there with a shell of collagen ok.

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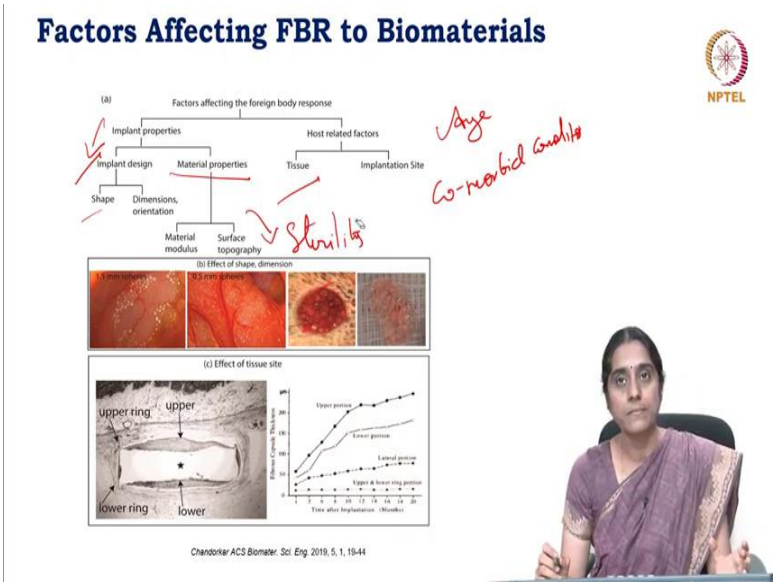

So, what are the factors which affect this encapsulation or formation? As already mentioned it is due to the properties of the it may be influenced by the properties of the implant. Such as implant design starting from shape, dimension, orientation and also the

material property which I will discuss in detail in the next slide; material modulus, surface topography also the host related factors.

Here we have mentioned about the tissue and implant side, apart from that age of the patient and then uh co morbid conditions. So, all these also play a role in uh how the foreign body reaction is going to be there and also on how the sterility is maintained in the (Refer Time: 45:2)

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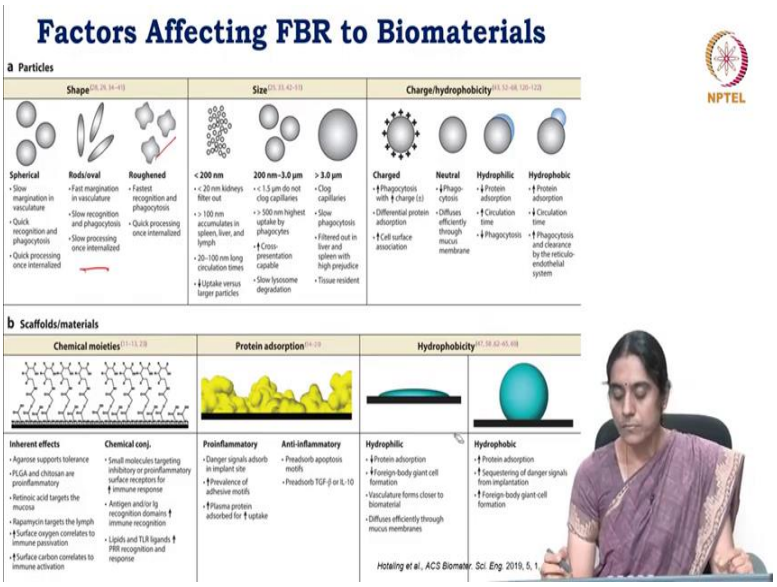

So, in detail you can see the how the physicochemical properties of the biomaterial is influencing the foreign body reaction. So, here you can see the shape; if it is spherical there is slow margination and vasculature and it is quickly internalized. Rod and oval it is slowly processed and once it is internalize, but it has fast margination in vasculature. Rough shape roughened you know it will cause fast recognition by the phagocytes and phagocytosis is initiated.

Likewise, size also has an influence; so, less than 200 nanometer, then it is easily filtered out and accumulates in the liver, spleen, lymph etcetera. Whereas, particle size between 200 to 3 microns are clogged on in the capillaries. Then it is cross presented they are can be cross presented to other immune cells causing further amplification of immune reaction and it is slowly degraded by the lysosomes.

And greater than three micron, again it will clog in the capillaries. It will be phagocytes very slowly they are filtered out in the liver itself, because they are bigger and spleen they will be resident tissue resident they cannot move much faster; so, we will they will be resident at the tissue.

So, whereas, if you have and also if you see the charge. So, the charge of the particle again has effect on how the biomaterial is going to respond towards the immune cells. So, all these things basically have a influence on the phagocytosis and then movement, circulation how they are going to be circulated in the body and where they are going to get accumulated.

Likewise, if we take the chemical moieties on this surface functionalization that also is going to influence these processes. Again protein absorptions like, if the there is more of danger signals absorbed on the implant site; then that will cause high inflammation leading to adverse foreign body reaction and rejection of the implant. Whereas, if there is going to be absorption of anti inflammatory motifs on the you know in biomaterial that will lead to less reaction foreign body reaction.

Likewise, hydrophobicity also has a influence high contact angle and low contact angle. So, hydrophilicity causes very less protein adsorption and it will also decrease foreign body gain cell formation. So, hydrophilicity should be preferred if the foreign body reaction needs to be decreased; so, vice versa happens with hydrophobic surfaces.

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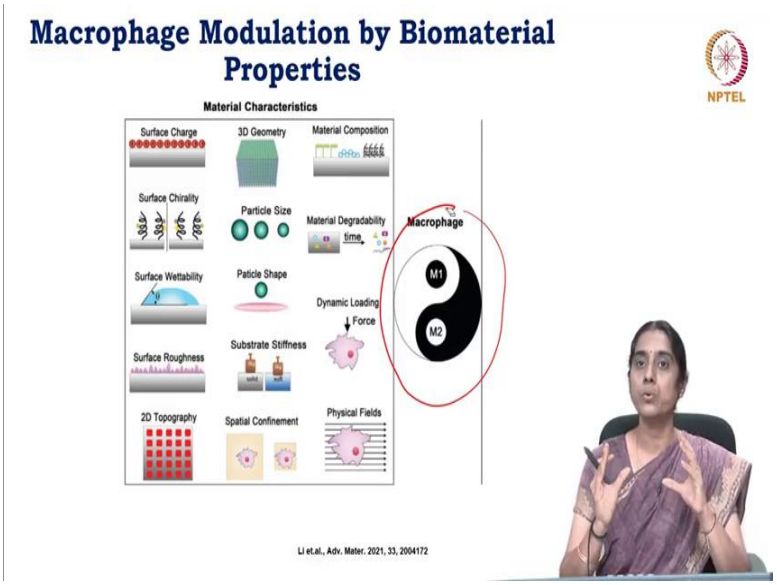

So, how the macrophage modulate modulation can be mediated by biomaterial. Now, we saw on a general how foreign body reaction is being affected by various factors. Likewise, these factors such as surface charge, geometry, material composition can influence this M1, M2 phenotypes. Lot of research study has reported that the difference in particle size difference in surface charge can tune the or can cause switching of M1 to M2 or M2 to M1.

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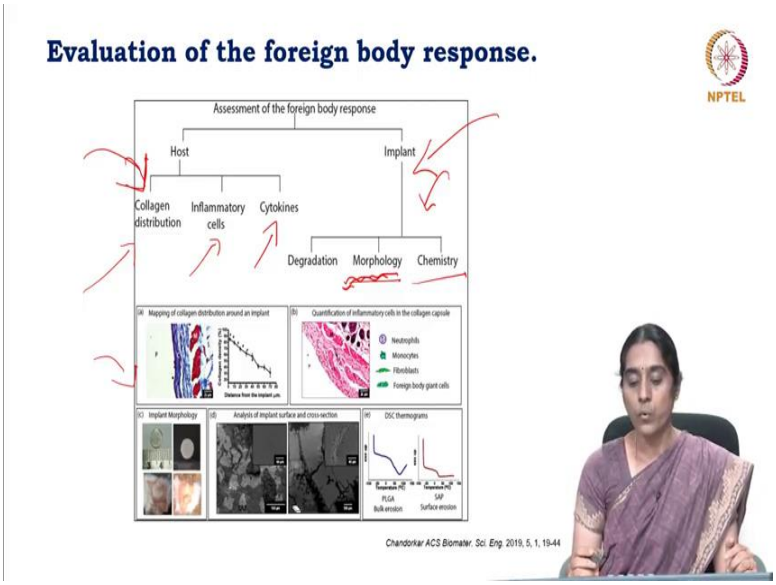

So, how do we evaluate this foreign body response? So, if a biomaterial has to be translator. So, if somebody is developing a new biomaterial and they see that it is very efficient and regenerating certain things, certain cells and tissues and invitro condition. But, if it has to be translator, then the important aspect that has to be studied is how far is the foreign body response.

So, we can assist the foreign body response from host point as well as from the properties of implants. So, from host when the implant is placed, we should see how the collagen distribution is the density of the collagen around the biomaterial; so, collagen estimation needs to be done. Then the inflammatory cell accumulation at the site of biomaterial can be studied either by immune histochemistry or by you know flow cytometric methodology or we can study the profile cytokine profile from the site of implant placement.

So, all these parameters will tell us the degree of foreign body response towards the biomaterial ok. From the implant point of view how can we study? We should see the degree of degradation. Whether a implant is degraded immediately when placed along with immune cells, even in invitro condition we can see that. And we already saw in the previous slides that the physicochemical properties can influence the immune reaction or immune response.

So, morphology we usually you know rod shape is going to cause more activation. So, we can tune in the morphology, we can study the morphology and say that whether it is going to cause high foreign body response or low body foreign body response. Similarly, chemistry of the implant material will also give us an idea about how far is going to be the foreign body response.

So, here this is an example of the mapping of collagen around an implant. So, this implant they have seen how the collagen is been deposited around the implant. So, the density of collagen has been estimated with regard to the distance from the implant. So, away from the how far it is depositing around the biomaterial it is being estimated.

Then quantification of inflammatory cell there is just h and d staining, histological staining; through that itself basically we can say how far is the degree of accumulation of various innate immune cells around the biomaterial. So, these are the morphology that may cause or influence the foreign body reaction ok.

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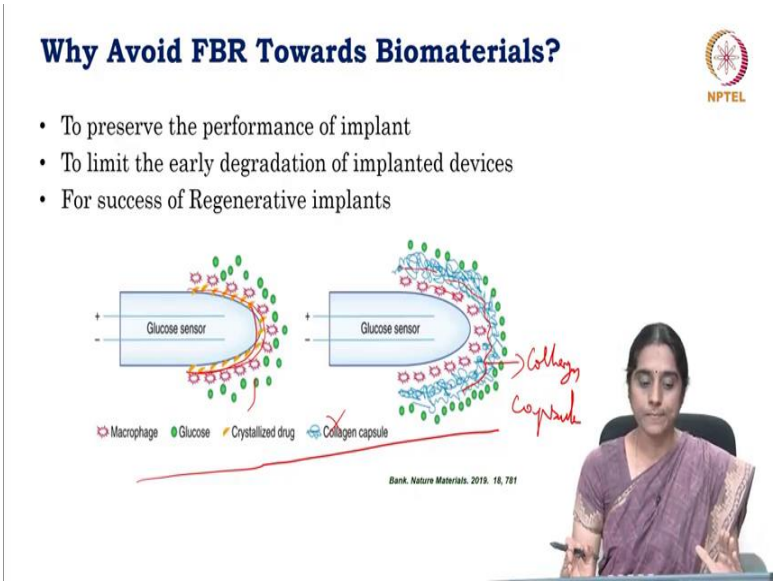

Foreign body reaction as we saw that is inevitable and unavoidable and it actually is required to certain degree or certain extent. Then why it should be avoided? See to a certain extent it is required for some biomaterial, but in some cases like where we want to place a biosensor in vivo biosensor implanted. Then if there is going to be foreign body reaction causing collagen deposition around the sensor and what will happen?

The sensitivity of the sensor will not be optimal, we cannot rely on that and over a period of time its functionality will be gone. So, we may have to replace it often? For example, in this schematic illustration you can see that this is a glucose sensor which they try to place inside the body for getting continuous monitoring of change of glucose level in a person. But, after three days itself they see that the glucose levels are not as sensitive because the formation of for the capsule around the sensor.

So, as a strategy what people do is they have coated it with a crystallized drug which is you know causing the inhibition of collagen capsule formation. You see here without the drug there is collagen capsule formation right, but so, the there are various strategies by which we can avoid it.

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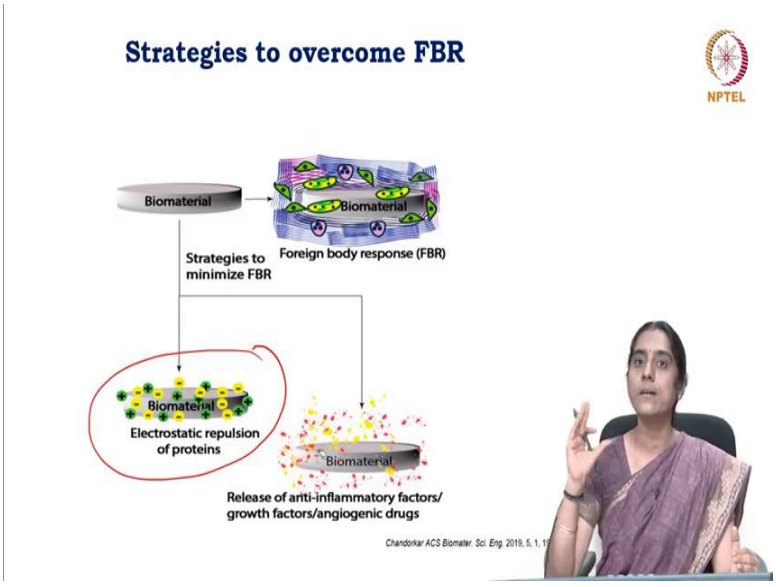

So, let us see how to overcome this extreme degree of foreign body reaction causing failure of implants? So, here is a broad way of doing it this strategy has been given, one is by electrostatic repulsion of proteins. So, you quote the biomaterial in such a way that it does not allow the protein to absorb on its surface this is one way.

Another way is to load the biomaterial with certain factors such as anti inflammatory factors or growth factors or angiogenic drugs which will be released ok sustainably released causing minimal immune reaction which will control the immune cells. So, these are the broad ways by which people are trying to overcome foreign body reaction.

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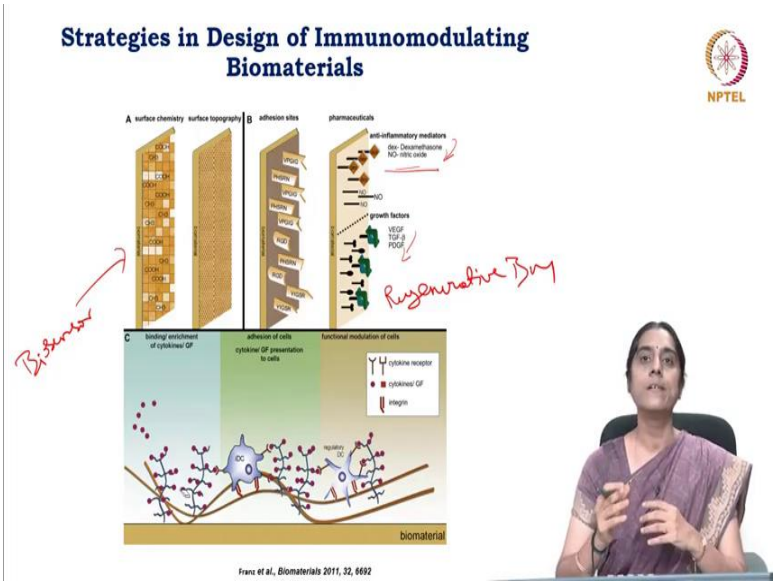

So, some of the strategies as I have told you even by tuning the surface chemistry, surface topography and by you know at making lot of using lot of drugs including certain peptides and pharmaceutics. We can tune in the immune response towards wound healing and to avoid foreign body extreme degree of foreign body reaction or inflammation.

So, in this case dexamethasone and nitric oxide which are anti inflammatory mediator are being conjugated to this on the surface of the biomaterial. Likewise, certain growth factors VEGF, TGF beta; so, what they will do is? They will recruit the appropriate cells, stem cells and other tissue cells towards the biomaterial and they will form a layer over it causing or inhibiting the ensue of foreign body reaction.

This is this will be helpful with regard to regenerative biomaterials ok. If we need for bio sensors then it is better to either quote it or to modify the surface or to modify the chemistry of the surface ok. So, here we can see that enrichment with cytokine and growth factor will cause adhesion of cells, appropriate cells ok, cytokine receptors are expressed in high quantity.

So, this is not going to allow the immune cells to directly bind to the biomaterial; instead you have a layer over it over which only the immune cells are going to interact and that interaction itself will help in controlling their response.

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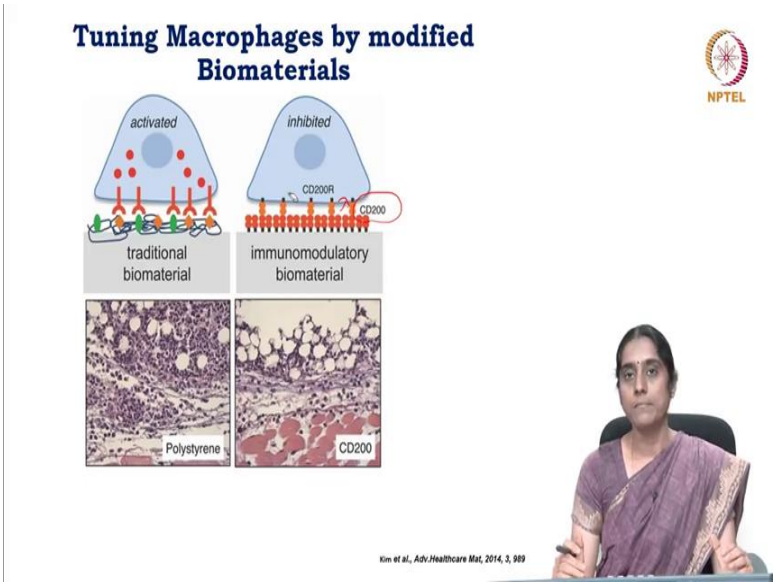

So, finally, this is a an interesting way I as an immunologist I saw that they have modulated this material biomaterial by means of conjugating it with molecule called a CD200. CD200 is a molecule which will modulate the macrophages into M2 phenotype. So, this CD200 will interact with CD200 receptor of macrophages and it will stop the activation of M1 phenotype and switch it to the M2 phenotype. So, this is again another way by which you can modulate the immune response ok.

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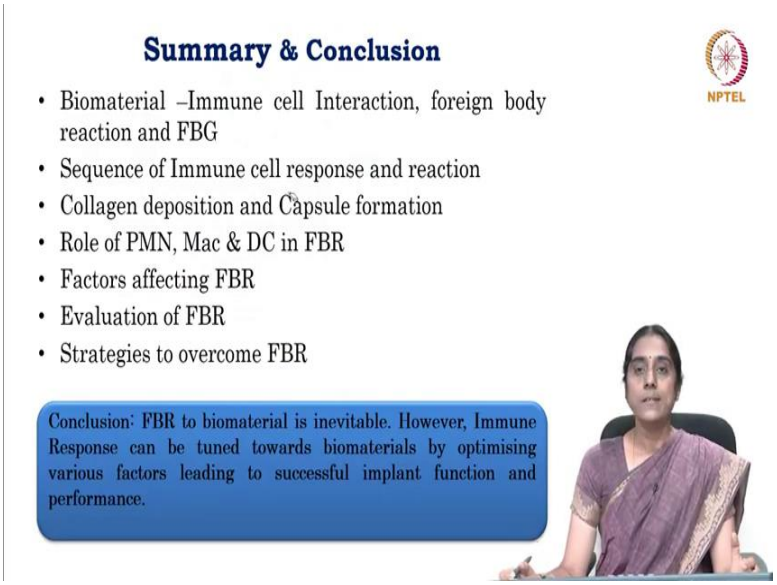

So, we saw until now that the biomaterial and immune system are of course, going to interact, this interaction leads to foreign body reactions. And we saw the major players of this interaction mainly the neutrophils, macrophages and dendritic cells. So, this is what we saw how they are playing different roles either the reaction and the response of all these cells.

If they are reacting it leads to degradation of the biomaterial as well as injury at the site of implantation leading to either implant failure or rejection of the graft. Whereas, if it is being responded response is created and if it is tuned, then we can have a successful implant or biomaterial.

Likewise, we need to we saw that we need to evaluate the foreign body reaction. We saw how it is being done the various strategies to mitigate the foreign extreme foreign body reactions. And we in we also saw that these the factors which influences the foreign body reaction. So, when we know the factors which influences the foreign body reaction; obviously, we can strategize against it to mitigate it ok.

So, in conclusion I feel that foreign body is inevitable. However, immune responses can be tuned or modulated towards biomaterial by optimizing various factors for successful implant function and performance. So, hope this lecture would have given the young minds and the budding researchers to work on various biomaterials. If I expect that more

of clinicians and dentists are also into research because, they know better what is the requirement.

And these aspects should be address from both point of view, from the material science point of view as well as from the clinical point of view.

Thank you.