Medical Biomaterials
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Lecture – 10
Biofilm

Hello everyone. Welcome to the course on a Medical Biomaterial. Now we are going to talk about Biofilm. Biofilm is a very important concept in biomaterial and it affects quiet a lot of biomaterial design and life of a biomaterial.

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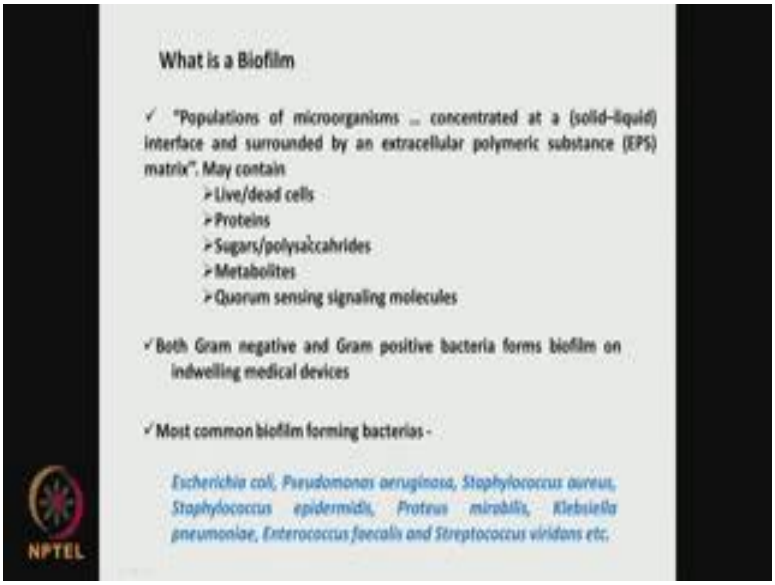

What is this biofilm? The definition is it is a population of microorganisms concentrated at a solid liquid interface, and surrounded by an extra cellular polymeric substance called EPS matrix. So, this biofilm is attachment on material surface. It could be a stainless steel or could be a polymer it be a ceramic it could be even the inside lining of vascular graft. It may contain life cells. It may contain dead cells it may con it will contain proteins sugars polysaccharides metabolites that is metabolites that are produced by the bacteria secondary and also quorum sensing signaling molecule. So, a biofilm may contain lot of these.

And they are attached in the early stages the attachment is reversible; that means, the materials leaves, but as time progresses the attachment becomes more and more difficult. Both gram negative and gram positive bacteria forms biofilms on indwelling medical

devices; that means, like I said it could be a short duration device or it could be long duration device biofilm can form. Even the urinary catheters the first few hours' bacteria can start accumulating which is reversible, but as time progresses the attachment becomes very strong and the bacterial biofilm starts growing.

So, large number of bacteria can form this, biofilm these are the common bacteria found like a E. coli pseudomonas staphylococcus aureus staphylococcus epidermidis proteus mirabilis klebsiella pneumoniae enterococcus streptococcus and so on actually. So, all these are bacteria found in biofilm, whether it is dental implant whether it is urinary catheters ureteral catheters and so on actually. All these bacteria can biofilms.

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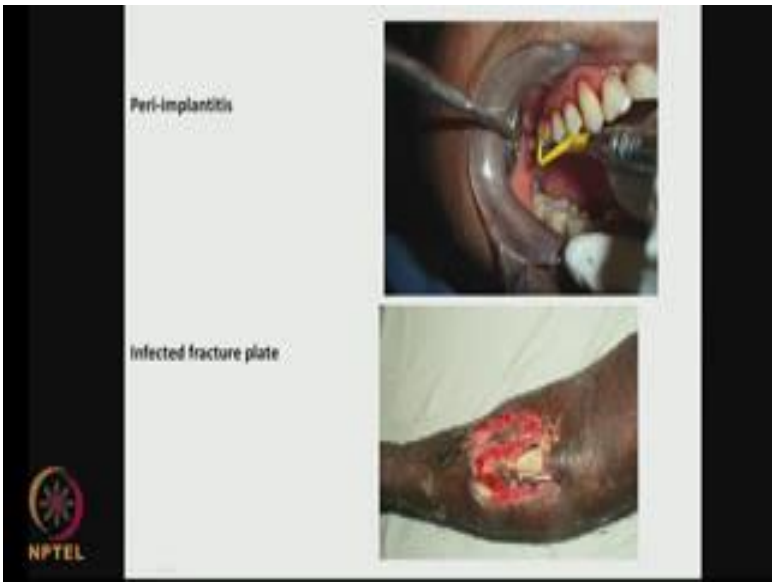

I just want to show some pictures. You can see the bacterial attachment and growth in some of the dental area. Look at this fracture plate, there is an infection in that you can see these where bacteria has settled down and formed. Biofilm and it is more difficult to get rid of this type of bacteria and compared to bacteria which are in the cell form or floating form.

It is been found that almost you need 10 times concentration of antibiotic to kill bacteria which are well established or surfaces. Like I said it could be a metal surface it could be a polymer surface it could be a ceramic and so on.

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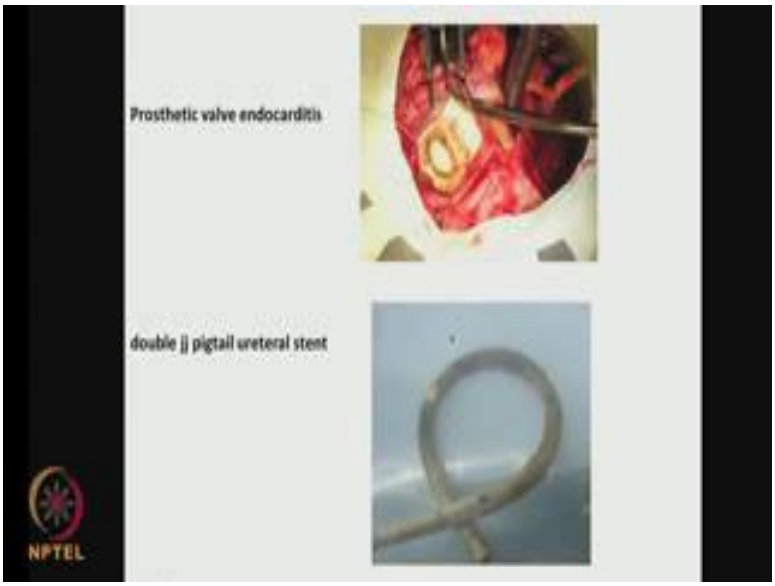

Some more pictures look at this this is a prosthetic valve you can see lot of biofilm growth. Look at this this is an ureteral stent this is called double j j pig tail ureteral stent. You can see bacterial infection. Generally, in the urinary region you may have a bacterial like E. coli proteus mirabilis there. So, biofilm is very common in medical implants. It could be even within a few hours, and it could last for years and years. So, addressing that biofilm is a very big issue in biomaterial research, because these biofilms can lead to infection can lead to immune compromising or failure of the biomaterial and so on actually.

There are strategies that are being practiced in getting rid of biofilm. Some of them are successful some of them still needs lot research. And you cannot have one single strategy to get rid of all the biofilms because depending upon where the material is introduced the type the nature of the bacteria.

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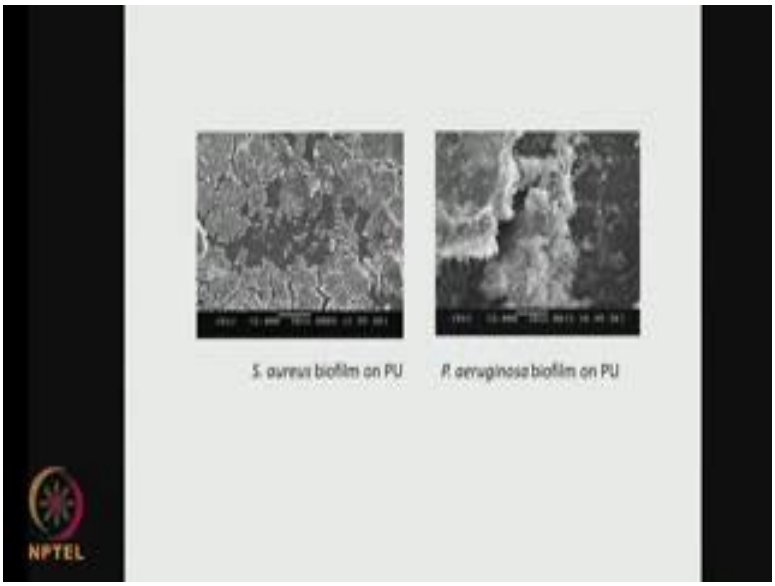

And the biofilm can vary quite a lot this are some more biofilm pictures these are scanning electronic pictures of staphylococcus biofilm on polyurethane surface, you can see this, and this is protein pseudomonas aeruginosa biofilm on polyurethane surface. So, you can see not only the bacteria the exopolysaccharides. So, it forms a thick layer.

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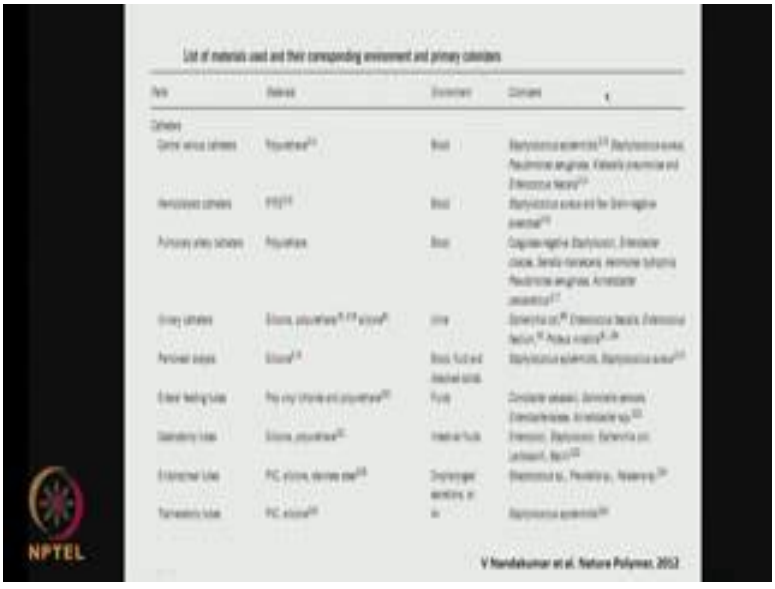

So, lot materials like I said in different environments end up having biofilm. For example, if you look at catheters central venous catheters made up of polyurethane environment is blood, but still you can have staphylococcus epidermidis staphylococcus

aureus pseudomonas aeruginosa klebsiella type of biofilm. Look at hemodialysis catheters polytetrafluoroethylene is used here. Again then environment is blood again staphylococcus aureus or gram negative anaerobes poly urethane is used in pulmonary artery catheters. Again then environment is blood sorry you can have coagulase negative staphylococci or enterobacter cloacae pseudomonas acinetobacter urinary catheters like silicon or polyurethane or silicone this is in the urine environment you can have E. coli you can have enterococcus you can have proteus mirabilis.

Look at peritoneal dialysis silicone it will have interaction with blood and fluids solids you can have staphylococcus epidermidis and staphylococcus. Aureus enteral feeding tubes like PVC or polyurethane, generally fluids again you can look at the type of bacteria here. Gastrostomy tubes made up of silicone polyurethane intestinal fluids again enterococcus staphylococcus E. colis. Again, endotracheal tubes PVC silicone stainless steel. Again we can have staphylococcus tracheostomy tubes these are made up of PVC and silicone. Generally, it is environment is air you can have staphylococcus epidermidis and so on.

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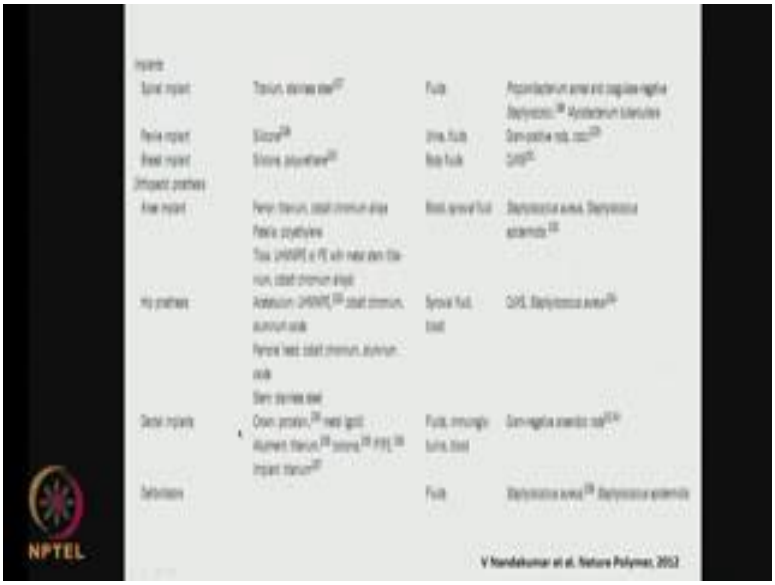

So, you can see the titanium stainless steel in spinal, it is contact with fluids; you can have a coagulase negative staphylococcus or mycobacterium tuberculosis. Penile implant silicon it is in touch with urine and fluids gram positive rods cocci. Breast implants silicone polyurethanes you can have again coagulase negative staphylococcus

orthopedic, whether it is knee implant hip or dental defibrillators different types of materials we are talking about mostly metals no cobalt chromium aluminum stainless steel gold is it and then inorganic material like zirconia aluminum oxide.

So, they all have different types of bacterial contamination like staphylococcus aureus coagulase negative staphylococci gram negative anaerobic rods and so on actually.

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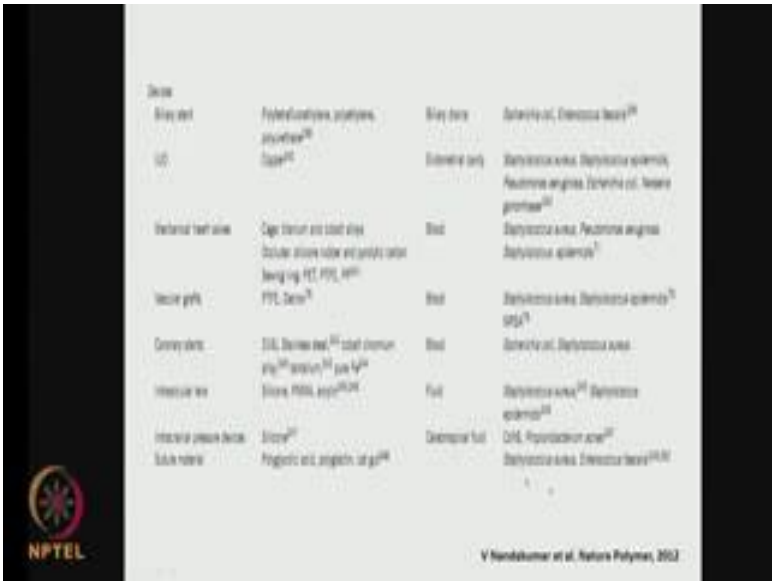

And finally, devices different types of devices biliary stent intrauterine device like copper vascular grafts coronary stents intraocular lens. They all can end up having staphylococcus aureus and epidermidis and e colli. So, these are the common bacteria which goes and as I showed you in past 2 3 slides the environment could be blood it could be urine it could be fluids different types of fluids like bacterial fluid cerebrospinal fluids and so on. So, we have metals nonmetals polymers and ceramics they have all these type of infection.

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So, how what are the possible entry points of infections perido notal disease, something related to the teeth catheters patients many times have urinary catheters inserted. So, infection can happen. Implant surgery a device is placed whether it is an orthopedic, whether it is dental, whether it is a knee, whether it is a stent ureteral stent or cardiova cardiovascular stent. Open wound, there is an wound which is open lot of bacteria has staphylococcus related bacteria can enter.

So, these are the points of entry and it is been found that most of the infections happen because of the implants surgery and almost 60 to 70 percent of rejection in the early days of implant is because of infection. So, if you can address the implant related infection. In the first 2 to 3 weeks then almost 70 percent of the rejection of the implant could be overcome.

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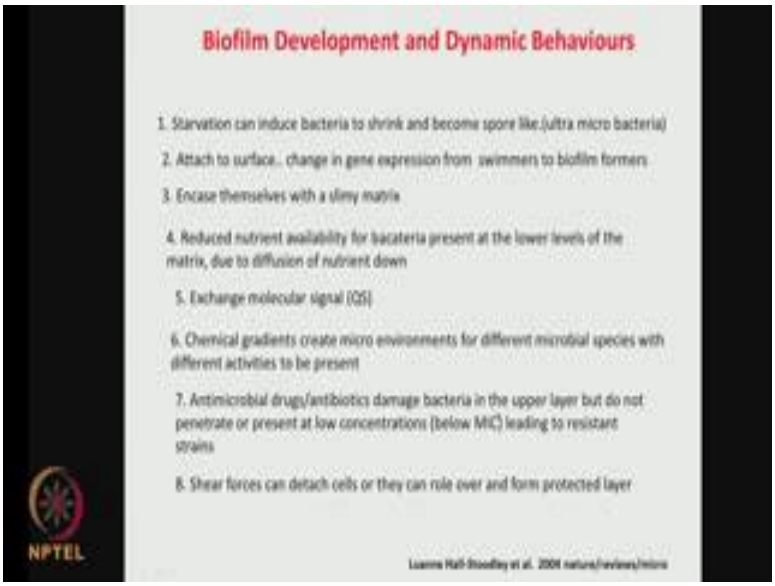

So, let us look at this biofilms development and dynamics. So, what happens is bacteria when it undergoes starvation, they can shrink and becomes spores. So, they become very small ultra-micro bacteria. So, they can attach to surface or they cannot be killed by antibiotics. So, the attaching to surface once they attach the change in gene expression from swimmers to biofilm formers; so there is a change in gene expression of those bacteria when it is in the sessile form as against in the biofilm form. They can encase themselves with a slimy matrix; we call it exopolysaccharides lot of sugar. So, there is a slimy matrix that is forming on top of it. They because of the reduced nutrient availability for bacteria, which are the lower level of the film nutrition diffusion also slows down. So, the bacteria stops growing and they reach this type of a starvation condition and hence there could be change in the gene expression of that.

Exchange molecular signal. Bacteria which are forming biofilms they produce a chemical called quorum sensing, and thereby they can change their gene expression and hence they can move from the sessile to the colonizer, and the bacteria can also identify that they are a large number of population which are in the colonizing stage chemical gradients. There could be because of the biofilm which is a matrix, there could be a gradient which can create micro environments for different microbial species, with different activities to be present.

So, they bacteria which are on the top of the biofilm which gets nutrients which get oxygen which gets chemicals may have a different environment. So, they will have a different activity and growth pattern when compared to bacteria which are right at the bottom of the biofilm, which do not get it of nutrient which starve much longer which do not get enough oxygen.

So, antimicrobial drugs antibiotics damage bacteria in the upper layer, but do not penetrate or present at low concentrations lead and the bottom. So, they are below the MICs when compared to concentrations of antimicrobials available at the top of this layer. So, the bacteria which are at the bottom, what happens is they become resistance to strains because they are getting drug concentration much below their MIC. So, shear forces can detach cells.

So, there are cells which are attached on the surface and there could be flow shear forces blood flow or urine flow it can detach the cells and they can go out and again attach somewhere else and again form a protective layer. So, you can see there are. So, many things happening it is a very dynamic process biofilm growth maturation detachment is a very dynamic process, and it is a very serious issue especially in the area of biomedical device.

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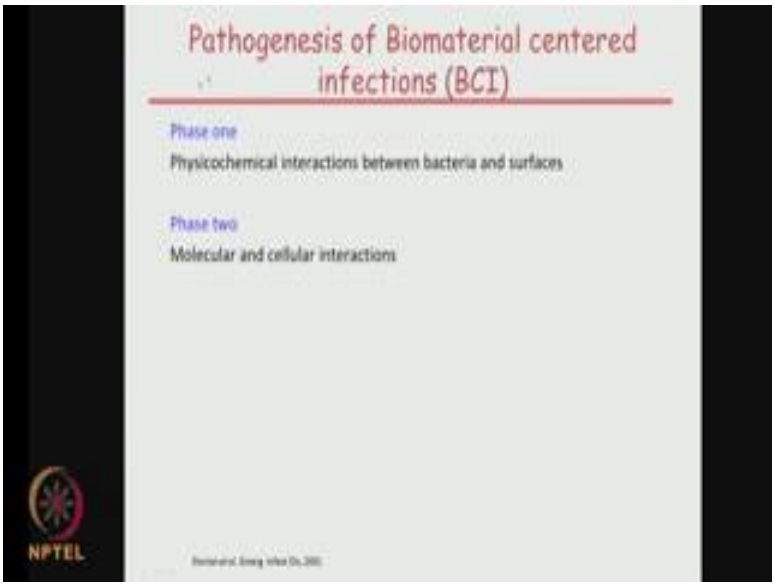

So, there are 2 phases of pathogenesis of biomaterial centered infection, physicochemical interaction between bacteria and surface molecular and cellular interactions. So, first is a

physicochemical interaction between bacteria and surface other one is a molecular and cellular interaction.

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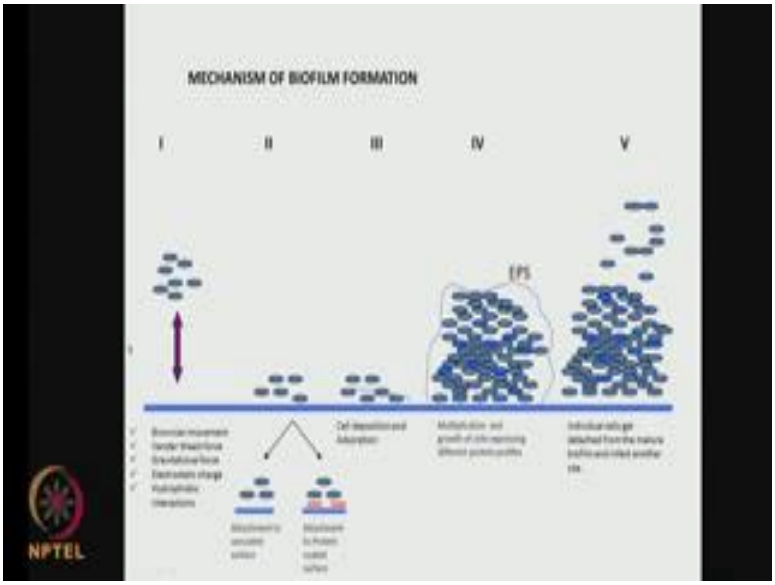

Let us look at it. So, initially bacteria in the Brownian motion they are moving around freely they are in the sessile form. So, they start getting attached because of non-bonded interactions like Vander Waal forces gravitational forces electrostatic forces hydrophobic interactions hydrogen bonds and so on. So, they attach either on uncoated surface or there could be already a protein layer which is formed on which these bacteria can be attaching, so either on uncoated or on a protein coated.

So, the cells deposit is adsorbed and slowly they may get multiplied exopolysaccharides are formed. So, they start forming a big layer of exopolysaccharides is called the matrix here. So, the bacteria which are on the surface getting of oxygen and nutrient bacteria right of the bottom do not get as much because of the limitations of diffusion of both oxygen nutrients and other chemical chemicals. So, they undergo gene genetic changes and compared to them. And the antibiotic flow is also controlled because of diffusion. So, the antibiotic may kill bacteria right at the top whereas, the concentration of the antibiotic at the bottom may be. So, little and the bacteria here are not killed. So, they get exposed to antibiotic concentration much below the minimum inhibitory concentration. So, they may start slowly developing resistance to antibiotics.

So, there could be antibiotic resistance bacteria of at these layers. Which are changed their gene patterns which are started growing slowly and so on. Now because of a shear forces because of thickness of the surface of the biofilms some bacteria may detach itself and start flowing downstream and again this process of biofilm formation can keep happening this is the process of biofilm. This can start in a hour and this can go right up to days and some of these matured biofilms can be in weeks and go on forever and ever.

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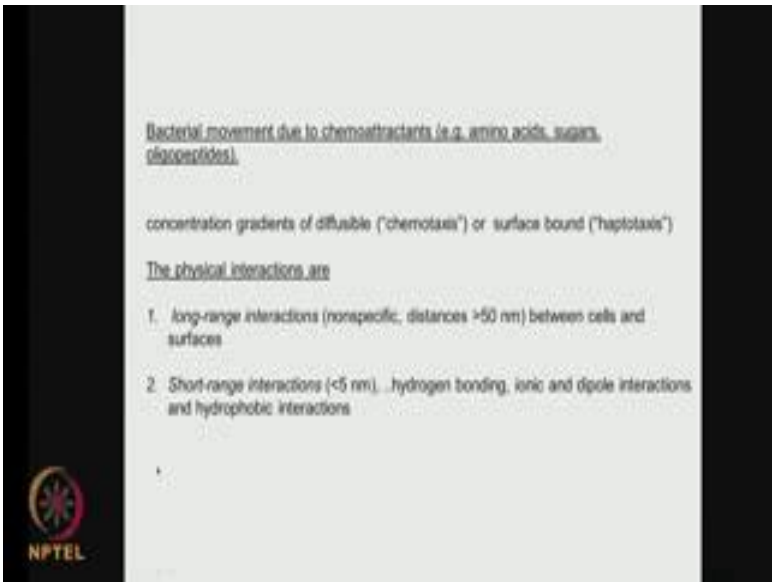

Now, bacterial movement due to chemoattractant; the chemoattractant could be amino acids sugar oligopeptides. So, this concentration gradient it could be chemotaxis or haptotaxis; chemotaxis which is diffusible. So, the concentration gradient happens because of the diffusion of the schema attractants, whereas there has some attractants which are bound to the surface. So, the physical interactions are long range interactions. They are not very specific it could be greater than 15 nanometers between the cells and surface for a short range interaction should be less than 5 nanometers hydrogen bonding ionic dipole interactions hydrophobic interactions, they are all short range. So, these are all long range. So, we have two types of gradients produced because of chemotaxis and haptotaxis. That is why the bacteria start moving towards surfaces.

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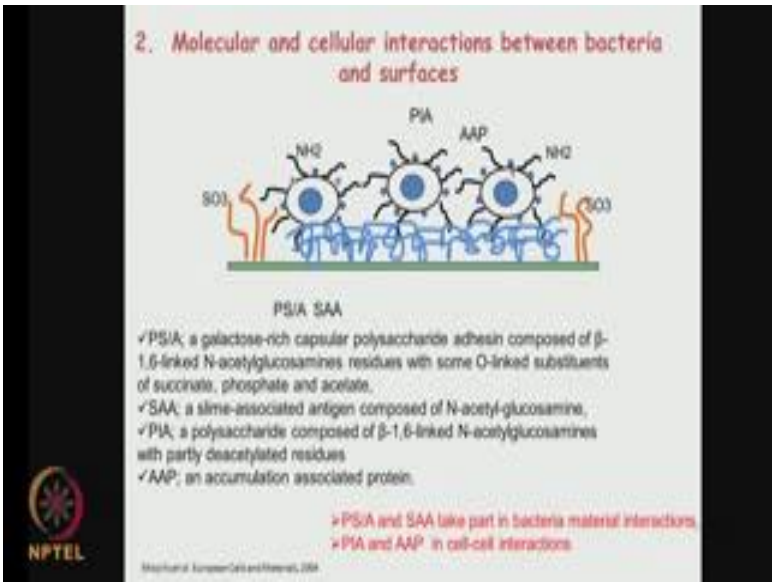

The molecular and cellular interactions between bacteria and surfaces there are many proteins on the surface of the bacteria which may be doing this type of a job. So, the PS a this is galactose rich capsular polysaccharide adhesion composed of beta 1 6 linked n acetylglucosamines, amines residues with some oxygen link substituents of succinate phosphate and acetate. Then we have the SAA these are slime associated antigen composed of n acetyl glucosamine. We have PIA this is a polysaccharide composed of beta 1 6 linked acetylglucosamines with partly deacetylated residues. Then we also have AAP this is an accumulation associated protein.

So, we see the proteins of the surface of the bacteria and the protein on the surface of the material surface there is an attraction. So, the bacteria may have lot of NH 2 groups' proteins and the surface proteins may have lot of SO3 groups. So, there could be attraction. So, this is the molecular and cellular interactions between bacteria and surface whereas, the physicochemical interactions could be because of chemotaxis because of non-bond that interactions and so on. So, PSA SAA take part in the bacterial material interactions whereas, a PIA and AAP in the cell interaction. So, PAA is a polysaccharide consist composed of beta 0.6 linked n acetylglucosamines, whereas AAP is an accumulated accumulation associated proteins. So, that leads into interaction between cell to cell whereas, PSA and SAA leads to interaction between the cells to surfaces.

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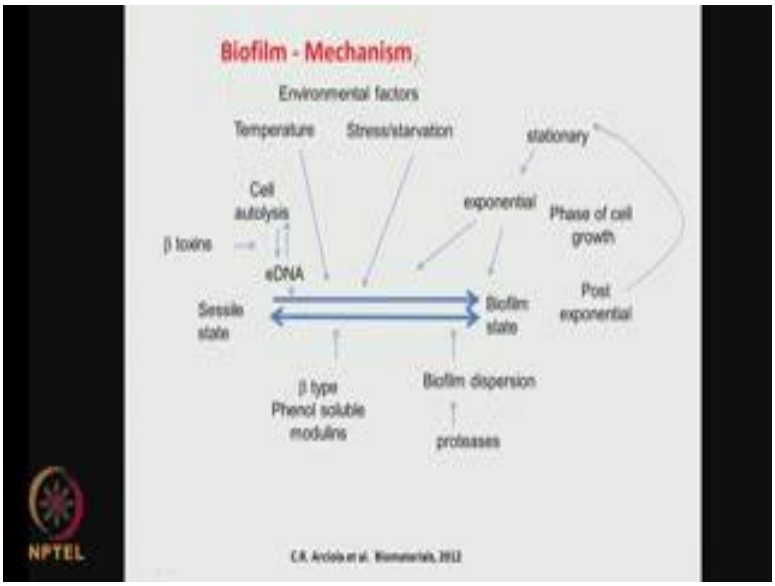

So, look at the biofilm mechanism there could be environmental factors there could be toxins and this could be cell growth process, and there could be something related to the surface proteins. So, environmental factors could be temperature stress or starvation because like I said bacteria at the bottom of the biofilm or almost under starvation condition. So, bacteria are at sessile form it moves into biofilm state and there could be a reversible process also biofilm dispersion because of enzymes like proteases which breaks the bond between the bacteria and the surface.

We also have something called beta type soluble moduli's these are supposed to be very toxin produced during the biofilm formation which can lead into lot of infection. Now phase of the cell growth. So, the bacteria grow and some sometimes it reaches into stationary phase and inside the biofilm they may be in the stationary phase. They we also have the beta toxin which are involved in the cell autolysis through EDNA which is also a reversible process. So, all these are in the mechanism of biofilm formation.

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So, what are the problems associated with the biofilms. Lot of clinical challenges chronic or recurrent infections a patient is a bio biomaterial is placed inside the patient and the biomaterials gets infected. So, the patient gets into chronic infection or recurrent infection is given antibiotics, I does not get cured they are given stronger antibiotics and so on actually. Chronic inflammation infection later there could be inflammation in the surrounding tissues rapidly accruing antibiotic resistance, because as I said the bacteria which are attached to the surface inside the biofilm slowly start acquiring antibiotic resistance. So, they are not able to be killed whereas, bacteria which are on the surface gets killed spread of infectious emboli; so the infection stats spreading on remaining parts of the surfaces. So, all these are problems associated with the bacterial biofilm.

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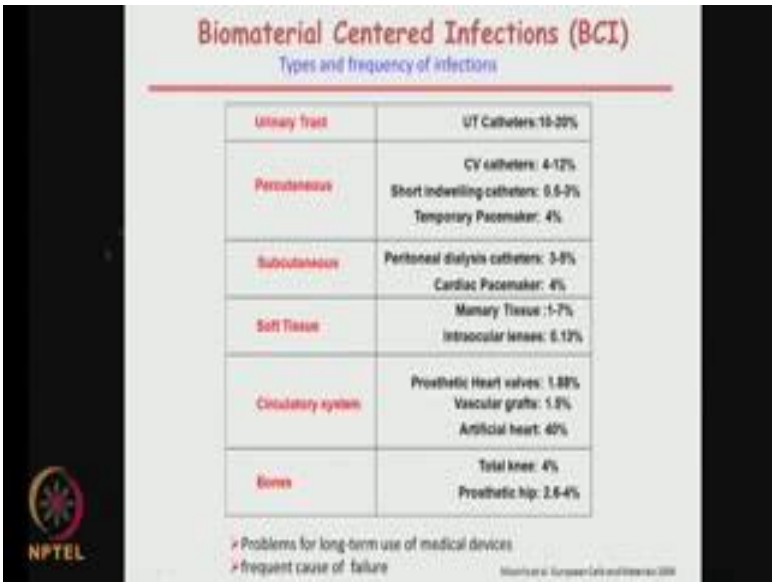

So, for example, if you look at the frequency of occurrence, urinary tract infection when you place urinary catheters like 10 to 20 percent of infection is because of this biomaterial centered infection. Percutaneous cardiac catheters short indwelling catheters temporary pacemakers we are talking in terms of almost 10 percent max subcutaneous like peritoneal dialysis catheters cardiac pacemakers around 4 percent; soft tissues memory tissues intraocular lenses. Circulatory systems like prosthetic heart valves vascular grafts artificial heart. Bones total knee placement around 4 percent prosthetic hip 2 to 2 to 4 percent.

So, if you are thinking about having a long term indwelling biomaterial like hip prosthesis or knee replacement or cardiovascular stents or cardiac vascular diaframe valves, then long term use of biomaterials. Medical devices you have to address this infection. And it is frequent cause of failure. This numbers where obtained from this particular reference.

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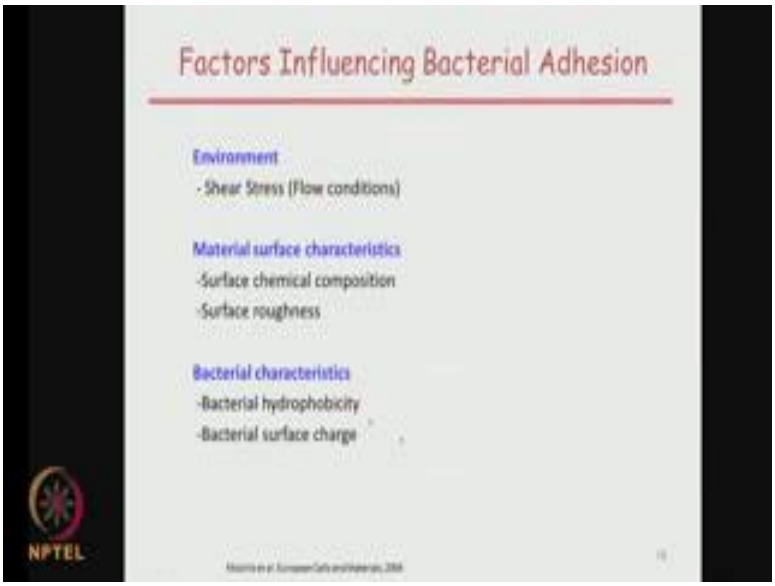

So, what are the factors influencing bacterial adhesion environment, if there is continuous flow or there is a stagnant flow. If there is a continuous flow shear forces can prevent biofilm formation, whereas if there is a stagnant then this can lead into a biofilm attachment. Material surface characterization like surface chemical composition surface roughness hydrophilicity hydrophobicity. So, on bacterial characteristics, bacterial hydrophobicity, hydrophilicity bacterial surface charge; so all these 3 factors affect the biofilm and bacterial adhesion.

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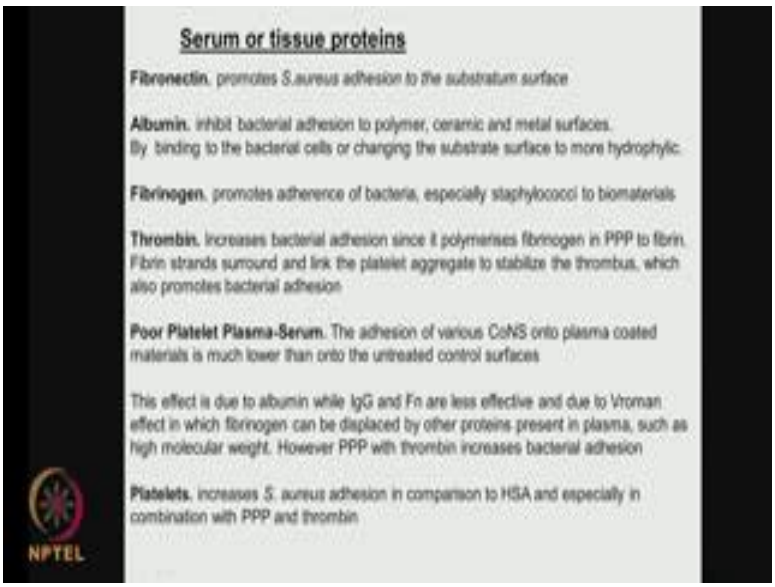

So, if you look at serum or tissue proteins there are many proteins like fibronectin which promotes staphylococcus aureus adhesion to the substratum surface. We have albumin which inhibit is bacterial adhesion to polymer ceramic and metal surface. Because blood will contain all these proteins by binding to the bacterial cells or changing the substrate surface to more hydrophilic. So, if you have a albumin it will inhibit bacterial adhesion whereas, if you may have fibronectin it promotes staphylococcus aureus adhesion because albumin is very hydrophilic, when the surface becomes hydrophilic then bacterial adhesion is reduced. Fibrinogen it promotes adherence of bacteria especially staphylococci to biomaterials. So, fibrinogen also promotes.

Thrombin it also increases bacteria adhesion since it polymerizes fibrinogen in PPP to fibrin. Because fibrin strands surrounds and link the platelet aggregate to stabilize the thrombus which also promotes bacterial adhesion. Then we have this PPP poor platelet plasma serum the adhesion of various coagulant negative staphylococcus onto plasma coated materials is much lower than onto the untreated control surfaces. So, generally many of these serum proteins expect albumin increases bacterial adhesion like fibronectin fibrinogen thrombin. So, what is this? So, this effect is due to albumin while igG and Fn are less effective and due to vroman effect in which fibrinogen can be displaced by other proteins present in plasma such as high molecular weight; however, PPP with thrombin increases bacterial adhesion because thrombin enhances bacterial adhesion fibrinogen enhances bacterial adhesion fibronectin also enhances bacterial adhesion.

So, platelet us increase staphylococcus adhesion in comparison to human serum albumin especially in combination with PPP and thrombin. So, platelet us also increases staphylococcus. So, as I said generally PPP serum an albumin inhibit is whereas, all other proteins in the serum sort of enhances in different ways with bacterial adhesion. Now you may ask what is this fibronectin. It is a 450 440 kilo dalton glycoprotein of the extracellular matrix, that binds to membrane spanning receptor proteins is called integrin. Similarly, if you look at what is albumin serum albumin is the main protein of human blood plasma and it water soluble. What is this fibrinogen it is also a protein produced by the liver and helps in the blood clotting? Thrombin it is a serine protease that converts fibrinogen into fribin fibrin in blood clot coagulation.

So, serum also plays very important part in adhesion of bacteria to material surfaces. So, if you are having implants which are exposed to blood the you have to consider these aspects also because the blood protein serum contains tissue proteins contains lot of different types, which may enhance or retard bacterial adhesion to various material surfaces.

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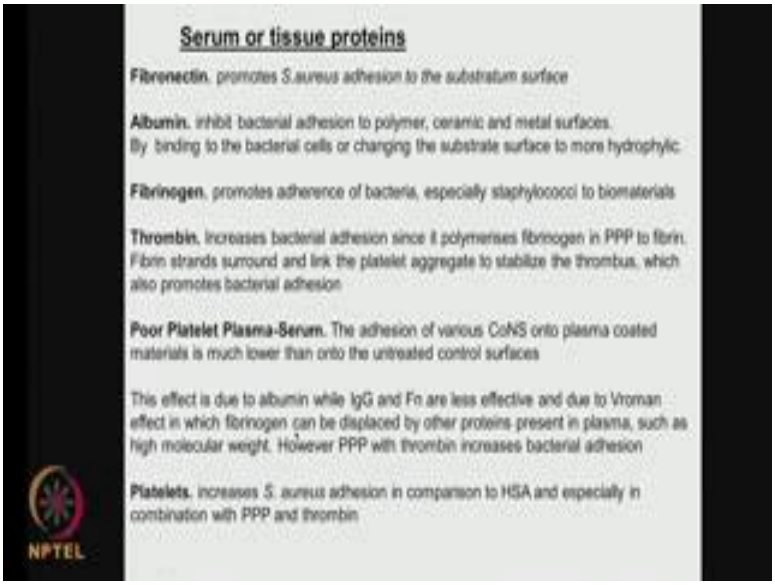

There are some bacteria which become persistent once they form biofilms, so this persistence or persisters.

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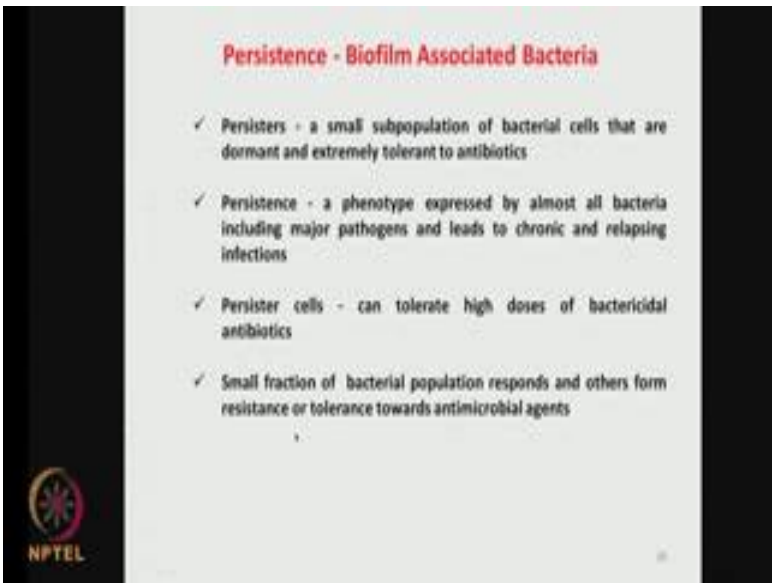

These are a small sub population of bacterial cells that are dormant and extremely tolerant to antibiotics. Because you have ethic extra polymeric extracellular polymeric surface that is formed on the material with the bacteria live and dead cells antibiotics penetration becomes more problematic. So, the antibiotics right at the bottom of the biofilm concentration is so low much below their minimum inhibitory concentration. So, they become those bacteria become tolerant to antibiotics they also become persister they are called persister cells.

So, this persistence is phenotype expressed by almost all bacteria including major pathogens, this can lead to chronic and relapsing infection. These persister cells can also tolerate high doses of bactericidal antibiotics. Small fraction of bacterial population can respond and other form resistance or tolerance towards antimicrobial agents. So, that is one of the main reasons in bacteria in biomaterial related infection, because of the formation of these persistent cells.

We will continue about persistence of bacteria in the next class in more detail.

Thank you very much for your time.