Medical Biomaterials
Prof. Mukesh Doble
Department of Biotechnology
Indian Institute of Technology, Madras

Lecture - 12
Biofilm

Hello everyone. Welcome to the course on Medical Biomaterials. We will continue on the topic of Biofilm, which is most important topic in the entire biomaterials, implants and devices that is why I am trying to spend more time on this biofilm.

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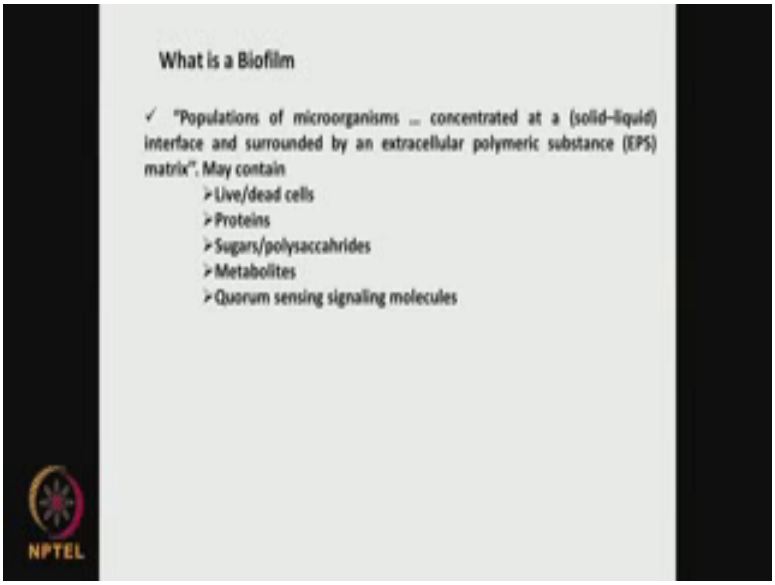

And as I said what is this biofilm, it is a population of microorganisms and the interface it may be surrounded by extra cellular polymeric substances EPS, it is called you may have live cells, dead cells, proteins, sugars, polysaccharides, metabolites, quorum, sensing signaling molecules, some of the cells may be will be at a different genetic level they might have stopped growing at a faster rate and so on actually, which may lead into persister cells or drug resistant cells. So, the formation of biofilm is a very serious issue on medical implants which can lead to chronic inflammation, infection, rejection of the material as well actually.

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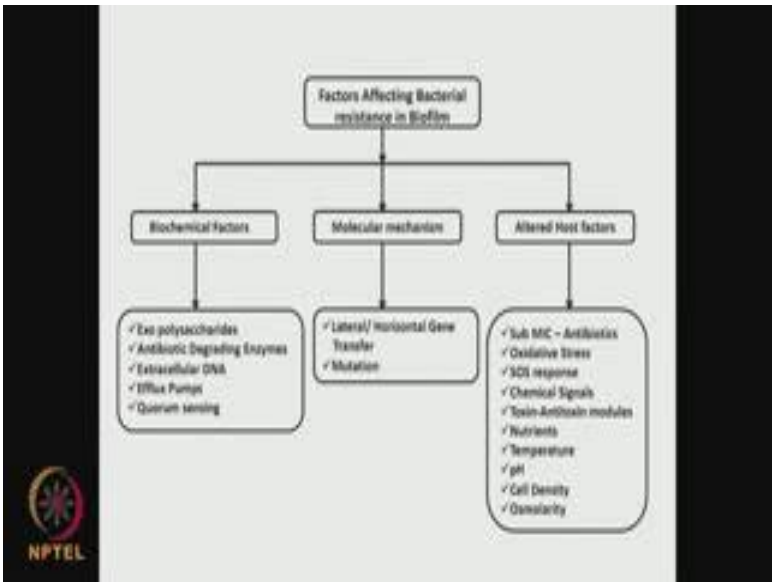

So, one needs to put in lot of effort in eradicating biofilms in implanted devices, which will be free of these type of attachment of a bacteria fungal species. So, what are the factors that affect the bacterial resistance in biofilm biochemical factors, molecular mechanism and altered host factors; that means, what happens on the surface or what happens in the environment and so on. The bio chemical factors include exo polysaccharides that are produced by the organism which sort of forms like a layer the bacteria may be producing certain antibiotic degrading enzymes which will degrade the antibiotic thereby making it inactive; the extra cellular DNA, which is also hiding the support of the bio film.

Efflux pumps bacteria produces certain efflux pump proteins which throws out whatever foreign material that enters the bacteria, these efflux pumps help in throwing out antibiotics or antibacterial, toxins, dyes and so on. Quorum sensing is a signaling molecule that is produced by bacteria especially when they are in a group, and it helps them to move from a sessile form or mobile form into a stationary form or a biofilm forming form. If you look at the molecular mechanism, there could be the gene transfer, the lateral or horizontal gene transfer, there could be mutations in the microorganisms all those could be causing the resistance in biofilm.

If you look at altered host factors because of the biofilm and because of slow diffusion, the concentration of the antibiotics may be very low at the bottom of your biofilm. So,

that could be sub MIC leading to resistance. Oxidative stresses the amount of oxygen present especially at the bottom of the biofilm could be very low leading to oxidative stresses. This could be because of SOS type of response. Chemical signaling, toxins-antitoxin modules that are produced; nutrients availability of the nutrients are very low, temperature changes in the biofilm, pH changes in the biofilm, cell density, osmolarity, all these are factors which are part of the host which affect the bacteria in the biofilm leading to resistance.

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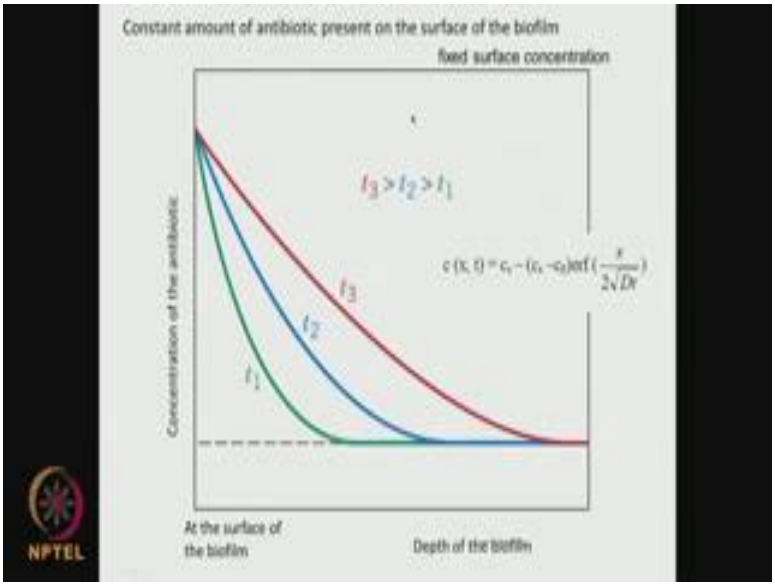

So, I also talked about the diffusion which plays a very important role at the surface of the biofilm that is at the external you may have certain amount of a antibiotic, but then as it diffuses inside based on Fick's first law and second law, the concentration of this antibiotic could be much, much low. For example if the concentration at these surface of the biofilm could be this much it may be falling drastically down and at the bottom it could be practically 0.

So, if you have a constant amount of antibiotic present on the surface that means, fixed surface concentration, these slowly as a function of time, this concentration graph may be increasing. This t 3 is greater than t 2 greater than t 1 that is this is valid only when we have a fixed surface concentration of antibiotic, whereas if you have a fixed amount of antibiotic that means, you place certain amount of antibiotic and that is it.

So, initially at time very low time at the surface of the biofilm you may have very high concentration, as you move inside the biofilm the concentration may drop drastically, but this amount also will start falling down like this. So, as a function of time, this graph will become like this and like this. So, in both these cases, as you can see, as you move inside from the surface of the biofilm the concentration of the nutrient or the antibiotic or oxygen will fall drastically, due to the diffusion processes hence the bacteria which are inside when compared to the bacteria which are much near the surface of the biofilm.

They are facing different environments, which is leading to changes in their growth pattern, which is leading to some gene changes, which is leading to antibiotic resistance and so on. So, that is a very serious problem which arises due to diffusions.

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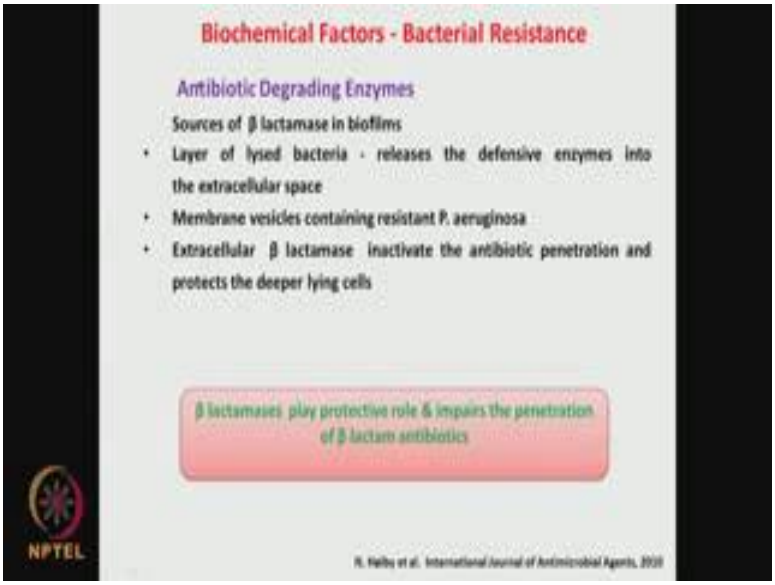

So, what are the biochemical factors, bacterial resistance? I also mentioned there are enzymes, which are produced by the bacteria and these are called antibiotic degrading enzyme such as beta lactamase. Now, these beta lactamase enzymes that are produced by bacteria can break antibiotics which have this beta lactam ring. So, these defensive enzymes in the extra cellular space can help in breaking certain antibiotics. Extra cellular beta lactamase inactivate the antibiotic penetration and protects the deeper lying cells that are cells which are right at the bottom, so that is one more problem.

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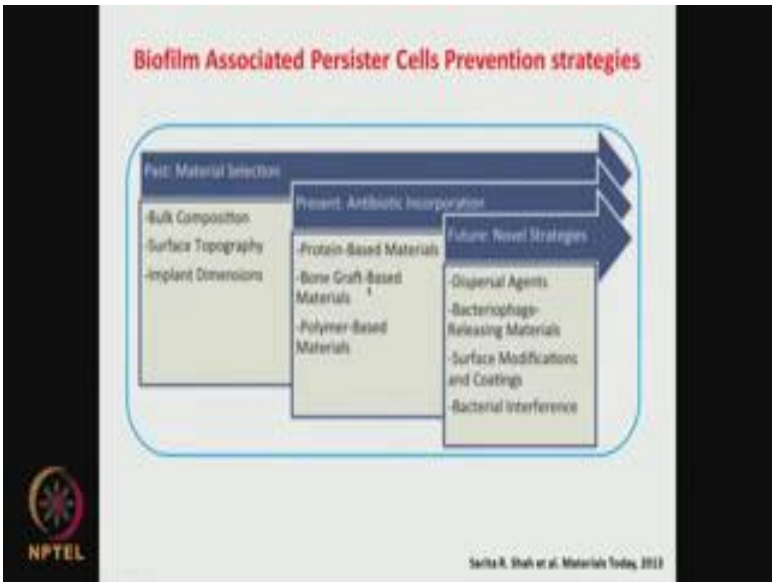

So, biofilm associated persister cells that are cells which remain even when you treat with antibiotic. How do we address them in original days they thought of having a material which is able to address these persister cells like composition, surface topography, implant dimension. And currently what do they do is they incorporate antibiotics in the material surface, so that they releases antibiotics or antibacterial, it could be protein based, it could be small molecule based, bone graft based materials, polymer based materials that is the current.

Future - one is thinking of dispersing agents, bacteriophage releasing material, surface modifications, surface coatings, interfering with the bacteria, so that is the strategy that is being thought of in the future to address these persister cells. So, but this problem of persister cells antibiotic resistance cells in biofilm is a very serious problem, and there is no single strategy to eradicate all of them, and there is no single strategy which can be used for different types of biomaterials in different environments.

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So, biofilm preventing strategies one can think about changing the surface of the implant such as coating with anti-adhesive coatings that means, you do not allow bacteria to adhere settle and bind. Releasing of bioactive coatings, inherent bioactive, materials, materials which are inherently bioactive nitric oxide releasing reactive oxygen species release photo activated materials nano structured materials these are all changes we can do on the implant surface. Developing inhibitors which will inhibit this quorum sensing, because I said quorum sensing is a group behavior and the quorum sensing molecules tell the bacteria that they have now a large group of bacterial population.

Can may have biofilm dispersions, can I have downregulating the expression of those (Refer Time: 08:55) which I talked about. Antibiotics, natural products, drug delivery carriers having biodegradable polymers which can release drugs such as chitosan, hydroxyapatite, dendrimers, hydrogels all these are different types of a natural and synthetic polymeric carriers which can release drug. And then there are other iontophoresis, vibroacoustic stimulation, bacterial interference bacteriophage, autologous platelet rich plasma, so these are all different types of approaches that are being practiced some of them are in industrial that means, in real life and some of them are still in the research stage.

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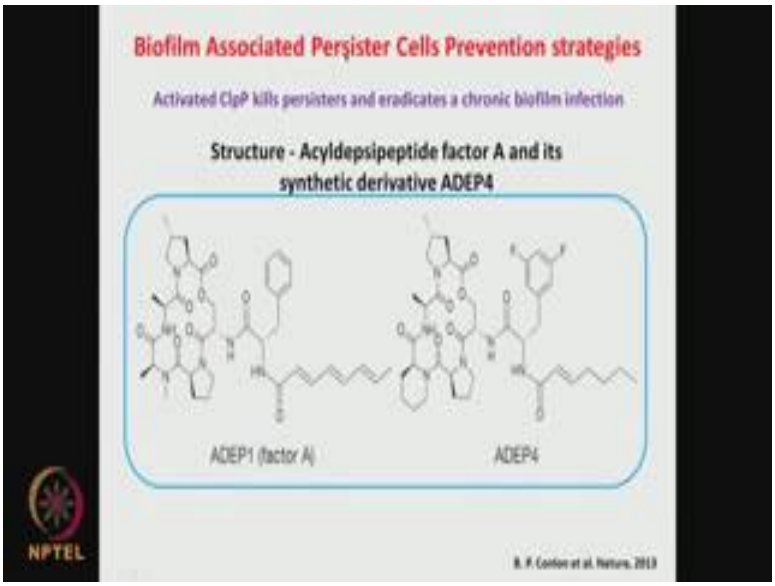

Here we will look at some of these examples of biofilm preventing strategy. For example, how can I prevent this persister cells. For example, if you look at this particular acyldepsipeptide factor A and its synthetic derivative peptide factor 4, now these are known to kill persister cells, these are small peptides, these are known to kill persister cells and also eradicate chronic biofilm infection based on this particular reference.

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So, combination of this particular peptide and rifampicin which is a well known antibiotic, it is been studied and found that they together can eradicate staphylococcus

biofilms in vitro and deep seated chronic infection in even animal models like mouse models. And then another compound like bromo bromomethylene methylfuran one and it sensitizes e coli persister cells to antibiotics. So, if I take this particular furanone and combine it with the antibiotics, I am able to kill persister e coli cells. So, brominated furanones seem to be a very good choice of a sensitizing antibiotic resistance cells and that has been shown in many examples in this particular reference.

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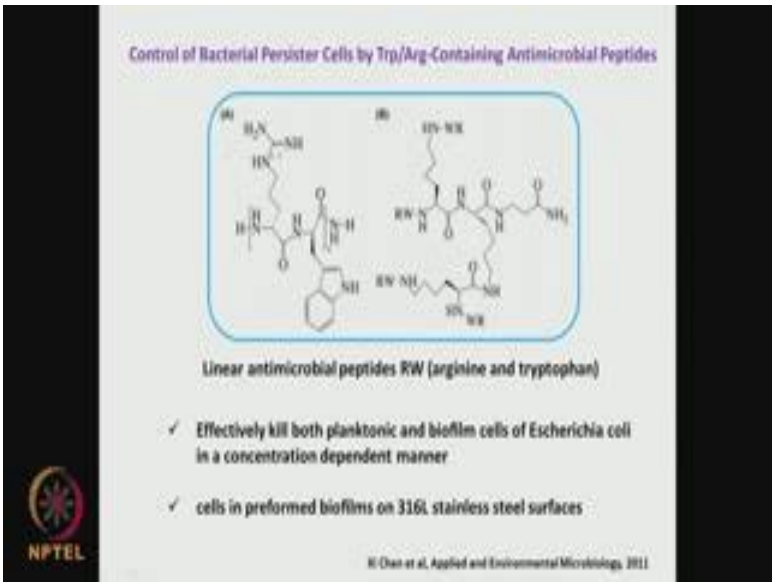

Again certain antimicrobial peptides, as you can see here which has got arginine and tryptophan type of peptides, these are known to be effectively kill both planktonic and biofilmic cells of e coli in a concentration dependent manner. That means, you have to increase the concentration, they are able to eradicate in a linear fashion. Especially the biofilms formed on 316L stainless steel surfaces these cell these surfaces are used especially in orthopedic implants.

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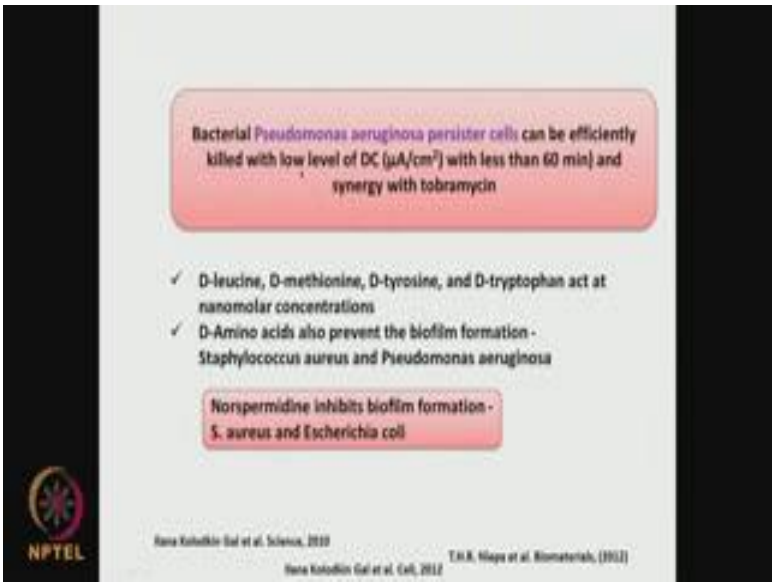

And interestingly even low levels of direct current micro amps per centimeter square are able to eradicate certain persister cells in combination with another antibiotic. This is an example where they have tested low levels of a DC and tobramycin that is an antibiotic they have found to kill pseudomonas aeruginosa persister cells. And again D-leucine, D-methionine, D-tyrosine, D-tryptophan act in nanomolar concentration. So, we can combine these D-amino acids with antibiotics to prevent the biofilm formation especially in staphylococcus aureus and pseudomonas aeruginosa there are many examples of that actually.

Again norspermidine inhibits biofilm formation of both staph and e coli. So, there are as you can see combination studies, where you can use a amino acid with antibiotic or even direct current with antibiotic or certain peptides and antibiotics, so that the persister cells can be sensitized for the treatment with the antibiotics.

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There are lots of anti biofilm surface modifications coatings that are being studied. There are lots, I am just showing you a few examples here, but there are many, many examples if one goes into literature. For example, silver is widely used as an antibacterial nanoparticle of silver, silver nitrate, silver ions and so on. So, is zwitterion organic inorganic non-composite with antimicrobial and antiadhesive capability. So, we can combine things to achieve both antimicrobial and antiadhesive surface. When you say anti adhesive; that means, the surface is basically not anti bacterial, but it prevents the attachment of bacteria.

Polyethylene glycol modified cytosine. So, polyethylene glycol is very hydrophilic. So, it helps in preventing the attachment of bacteria. We can even incorporate some antibiotic inside, so it also acts as a killing agent. Antibiotic loaded polymer coatings, so there are a quite a lot of drug eluting stents if you have read about where they coat metal stents with biodegradable polymers and it may contain drugs and drugs in the sense antibiotics or anti inflammatory. So, as the polymer slowly starts degrading the drug gets released. Zwitterionic coatings like you can have one NH plus type of zwitterion formulations of silver zinc copper. So, lots of other metals are also being tested and they have been shown to have antibiotic or antibacterial activity.

Silver is quite strong, zinc and copper they are much milder than silver and they have also been used as nano sized copper sets to prepare a materials. Immobilizing enzymes,

lot of enzymes immobilized like papain, like protease which has the antibacterial activity because for example, if you take protease it can clean the amide bond thereby it can kill bacteria, so immobilizing enzymes on material surfaces. Coating antibiotic coating immobilizing antibiotics on top of various surfaces, so lot of approaches where we are talking about a immobilization of proteins, immobilization of antibacterial, antibiotics or slow release of antibiotics or use of metals, metal ions such as silver nitrate or nano particles have been tested as an anti biofilm.

Another approach is to make the surface more hydrophilic. So, when you make the surface more hydrophilic, we are preventing hydrophobic organism settling down because bacteria contains quite a lot of hydrophobic patches, so the hydrophobic bacteria does not settle down. So, you are making the surface antiadhesive, you are preventing adhesion or attachment, it is not really anti bacterial, but it prevents attachment of the organism because the surface is very hydrophilic.

Another approach that is being looked at nowadays quite a lot is surface topography. Can I create surfaces in nanoscale nano roughness, so that you can prevent bacterial attachment? Why it has if we take shells do not get biofilms or biofouling, why does not take whale for example, which is always found in water, shells are found in water, they do not have any attachment of bacteria, because they have certain rough surfaces which are called nano topographies which prevents bacterial adhesion and biofilm formation.

So, can we create surfaces like that? So, nowadays lot of interest in this area surface topography creating nano surfaces, nano indents, nanoscale indents different type of roughness and so on actually. So, they will be inherently antiadhesive. Can we create surfaces like that on say titanium, so they will be inherently preventing attachment of microorganisms?

So, many different approaches that are being looked at actually, and it is an exciting area for who want to do research. And can I combine these antiadhesive properties with antibacterial release and so on. So, the antibacterial may be released in early days, early stages, where the infection probability is very high, but when you have inherently antiadhesive surface then long term bacterial attachment can be prevented. Because the antibacterial release cannot be sustained for very, very long term, it can be sustained for may be 2 weeks, 4 weeks, 6 weeks, but after that period of time the inherently built in

anti adhesive surface will take over and there will not be any attachment of bacteria and so on actually. So, that is quite an exciting area to look at. I think anti biofilm itself is a very exciting area and one could spend lot of research effort in that.

Like I said the biofilm is an issue in whether it is an implant, devices, drug delivery system and various parts of the body environment is so different. So, the type of bacterial attachment the type of requirements for the biomaterial could be different depending upon where it is going to be located. In addition, if it is going to be located for say few hours going up to weeks, or months or years, then again the requirement of antibiofilm changes dramatically. So, there is always possibility of doing good research. Now, let me spend little time and show you some of those case studies where these types of strategies have been tested. This is based on some of our research our own research in my own lab.

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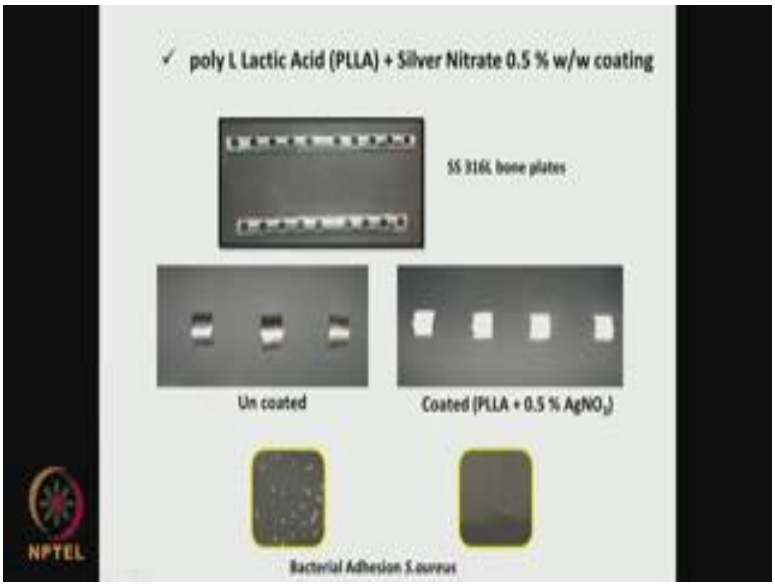

For example, if you look at these stainless steel 316L, these are bone plates used after and after breakage of the bone. So, these are grooves. So, attachment of bacterial infection biofilm formation is a very serious issue here. So, we looked at using poly L lactic acid mixed with silver nitrate, and we coated on top of it. Silver nitrate will be the antibacterial, this will be slowly degrading this will be the carrier for that. So, we could have antibacterial surface, so that was our idea. And as you can see here these are uncoated material this is after coating with a poly L lactic acid. So, the lactic acid can have the two forms the D and L form and so we have coated with the point five percent

silver nitrate. And as you can see here staphylococcus addition, this is uncoated surface, then after 24 hours and this is the coated surface. And silver is well known antibacterial material and silver nitrate silver ions really kill the bacteria. As you can see a big difference in the attachment of a microorganism on the uncoated surface as against the coated surface, so this is quite dramatic.

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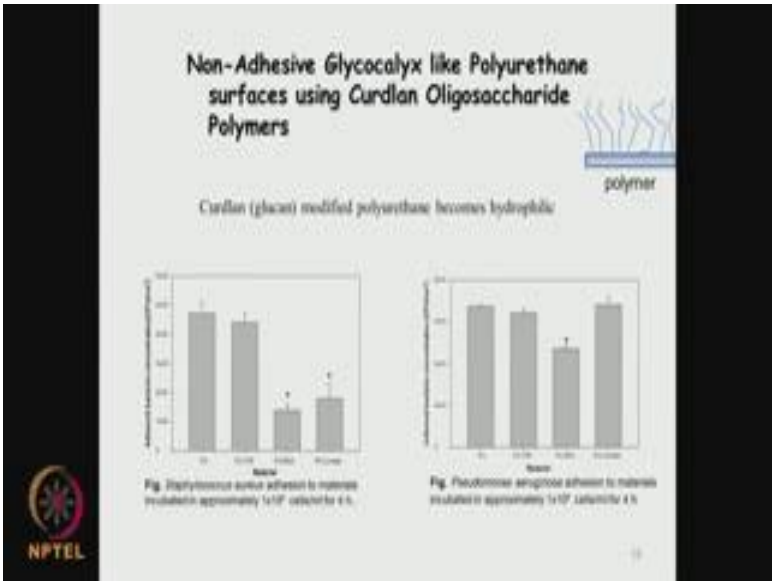

Let us look at another example. So, there we are talking about having an antibacterial material like silver and doing the job. Now, here what we did was we have a polymer material, this is polyurethane. Polyurethane is used quite a lot in medical ureteral stents or a even guide tubes and so on, because it is very flexible and it is almost like rubber, but bacterial attachment is a problem. So, in this example, what we did was we tried to immobilize a glucan which is called a curdlan, it is a oligosaccharide. So, it becomes a non adhesive glycocalyx type of surface. So, the polymer itself polyurethane has a very contact angle and it is very hydrophobic.

So, as we immobilize this then the hydrophobicity goes down, it becomes very hydrophilic. So, the attachment of a organism as I said on hydrophilic surfaces is much less when compared to the hydrophobic surface. So, here the cyclic sorry the glucan or the oligosaccharide does not have any antibacterial property, but it makes the surface hydrophilic. So, what happens the bacterial attachment goes down quite a lot. As you can see here this is the polyurethane, this is the polyurethane with this immobilized

oligosaccharide glucan. So, bacterial attachment goes down by a factor of 2.5. This is with the bovine serum albumen. If you recall I said if you have a albumen, albumen prevents bacterial attachment that is why we here also we see less bacterial attachment. This is the staphylococcus aureus, after 4 hours, and these experiments are where pseudomonas aeruginosa both are bacteria, which are found in many infectious situation.

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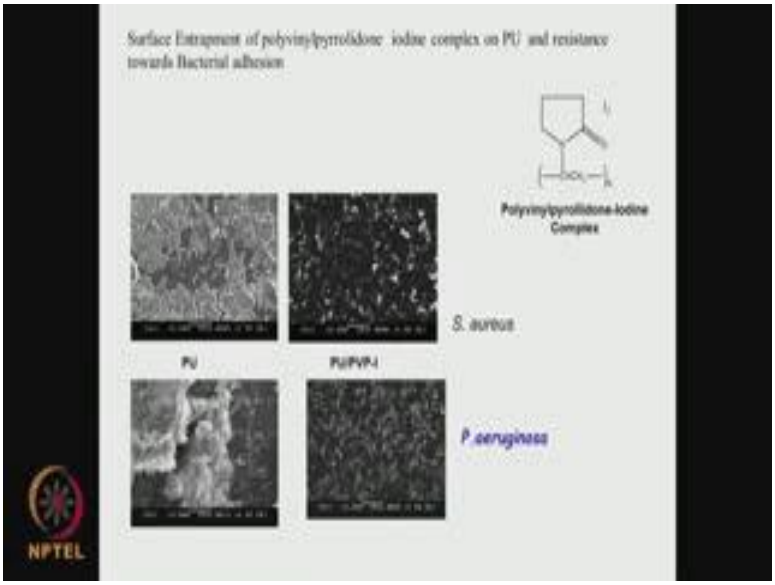

Now, if you look at pseudomonas with bovine serum albumen I am able to reduce the bacterial attachment, but with the glucan, we do not see much reduction in the bacterial attachment. Again it is do with the hydrophobic, hydrophilic nature of the surface of the microorganism as against staphylococcus aureus. Staphylococcus aureus is very hydrophobic surface becomes hydrophilic, so the bacterial attachment is less. Whereas, here there is not much difference in the hydrophilic, hydrophobic nature of pseudomonas aeruginosa, so the bacterial attachment remains the same. So, this example tells you I can achieve changes in the hydrophilic, hydrophobic nature of the surface thereby I can prevent attachment of hydrophobic bacteria, relatively when we talk about hydrophobic hydrophilic we are talking relatively here, please remember that.

Let us look at another example. When you look at another example, here we are immobilizing a material called polyvinylpyrrolidone-iodine complex on a polymer. So, it releases iodine continuously. And as you know iodine is a antibacterial, so we have a polyurethane surface, and we have immobilized something called a

polyvinylpyrrolidone-iodine on that and as it keeps releasing iodine which is an antibacterial then we looked at the biofilm. As you can see this is the polyurethane without any changes, this is the polyurethane which has this PVP iodine complex entrapped on it or coated on it. So, a big difference in the bacterial attachment, there is practically no bacteria.

This is staphylococcus aureus and this is pseudomonas aeruginosa. As you can see the bare polyurethane there is lot of attachment of the pseudomonas, whereas once I modify this surface with this iodine complex attachment is practically 0. And you may wonder what these are these are more of a surface modification after attachment of these. These are not biofilms here and here. So, this is a different example. Third example, I would say where I am talking about having a coating, which slowly releases antibacterial material. So, again we see we have very good reduction in the biofilm and attachment.

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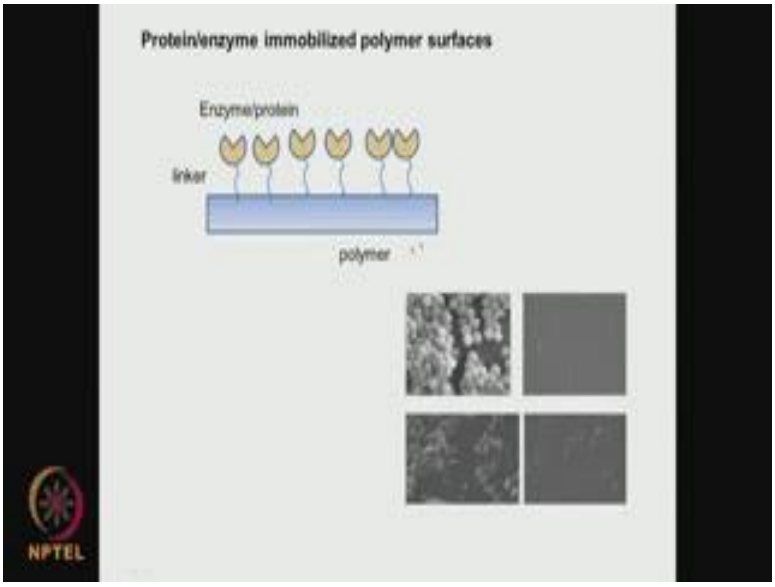

Let us look at another example here I have immobilized something called an enzyme here, enzyme or a protein, here it could be a protease papain and so on which has a certain effect on amide bonds that means, they can kill bacteria. So, I have a linker here. So, this is a polymer surface I have a liquor and I attach a protein. Then I am trying to see whether this type of design can reduce bacterial attachment. So, as you can see in this picture, this unmodified polymer bacteria two types of bacteria staphylococcus and different type of bacteria here, whereas when I modify you can see complete eradication

of biofilm, absolutely no biofilm on the surface. So, in this design what I have I have immobilized proteins on top of it using a linker, the advantage of having this type of design, so I have a covalent linkage. So, the surface life will be stable for very long period of time unlike the drug eluting where after a few hours or few weeks, the drug will be completely washed out where as here I am forming a covalent bond. So; obviously, these are very strong bonds; the protein will be always present on the surface.

So, this type of surface can always be antibacterial and these are examples as you can see the bacterial attachment on unmodified surface. And once this surface is modified with immobilizing this particular protein, you can see practically no bacterial attachment at all. So, this is very fascinating. So, we looked at so many different examples where I talked about slow releasing of iodine which can prevent a bacterial, and then I talked about immobilizing a protein or an enzyme which can act as an antibacterial, it can kill both the gram positive and gram negative bacteria. Because some of the; for example if you take papain, it acts on both the amide bond as well as the ester bond. Or we can modify the surface, so that the surface becomes more hydrophilic thereby you prevent attachment of hydrophobic organisms that is another approach.

Then you have another approach, where we can have a antibacterial surfaces built in to the polymer, so that biofilms can be prevented. So, there are so many different strategies in my lab, we are also working on those strategies and I showed you a few examples of a these strategies. We will talk more about these biofilms in the next class as well because as I said the biofilm is a very important topic which needs to be addressed and different strategies are being practiced. And hence, I thought I will cover some of these strategies as we go along.

Thank you very much for your time.