**Drug Delivery Principles and Engineering**

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**Lecture – 33**
**Implant Associated Infections-I**

Hello everyone, welcome to another lecture for Drug Delivery Engineering and Principles, let us talk about what we have been discussing so far. So, in this course we have discussed quite a lot of things by now, more than half of the course is finished. We have talked about normal pharmacokinetics of the drugs that are currently used in clinics. So, how these things travel through the body, how do the clinicians decide what dose to give and different sort of parameters for the free drug. Then we talked about that we would actually not want the pharmacokinetics to be what it is currently, but to improve it further.

So, that the patient compliance as well as the patient comfort is increased, because if you look at the current drug delivery field, what we are doing we are basically giving tablets every 6 hours - 12 hours which is not ideal for a patient and so we talked about why not we have something that we give only once and hopefully that is going to help the patient not take any more tablets for another week.

So, then we discuss several ways we can do this, some of them are actually being used in clinics. So, we discussed about polymer drug conjugates we discussed about, various encapsulation strategies in various kinds of matrices, various scaffolds and we talked about why not we make them nano, so that we do not have to do a surgery. So, all of that we discussed and then now we were discussing the tissue engineering, protein adsorption part of it which we finally, finished in the last class. So, just quick recap what we finished in the last class before we move on to the next module.

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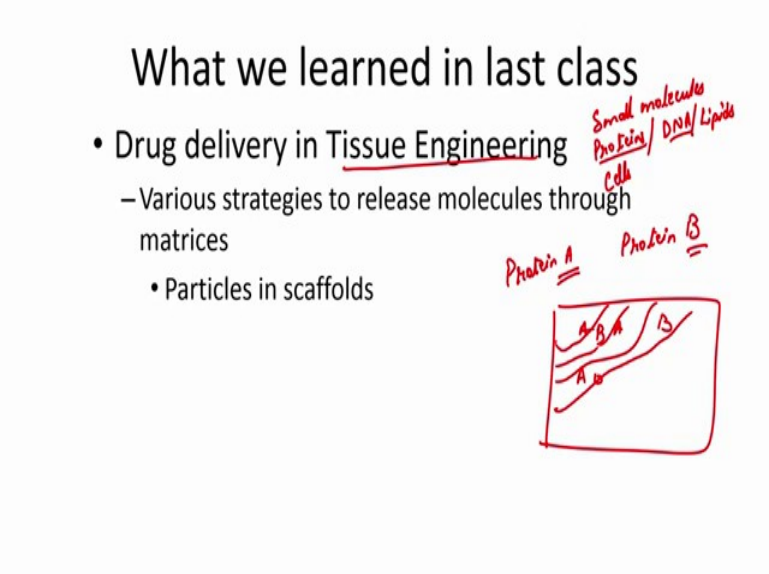


So, in the last class we talked about drug delivery in tissue engineering and the major part of tissue engineering involves some kind of delivery this could be either small molecules or this could be some biologic large molecule. So, this could be proteins and DNA, could be lipids as well, and then it could also be even cells. So, proteins would be growth factors, DNA could be a gene of interest that you are trying to deliver and all of those things.

So, basically most of the tissue engineering is related and goes hand in hand with drug delivery. And so again in this we talked about various strategies to release small molecules or large molecules through matrices and one thing we talked about is particles in scaffolds.

So, let us say if I want to deliver two proteins, protein A and protein B, but then I want to make sure that there is certain kinetics of protein A which is different from the protein B how do I do that, because otherwise if I just put it in my scaffold, both A and B, then what will happen is as this matrix will degrade slowly and slowly each and every of these molecules will start to come out, but then what if I want A to come out faster and first and then followed by B?

So, this could be desirable in some of the cases because in some of the cases may be growth factor A acts first and then only when the cells have reached the certain stage

then the growth factor B becomes useful. So, to do that what we talked about is we can make a system in which you can essentially put A in the matrix of your scaffold.

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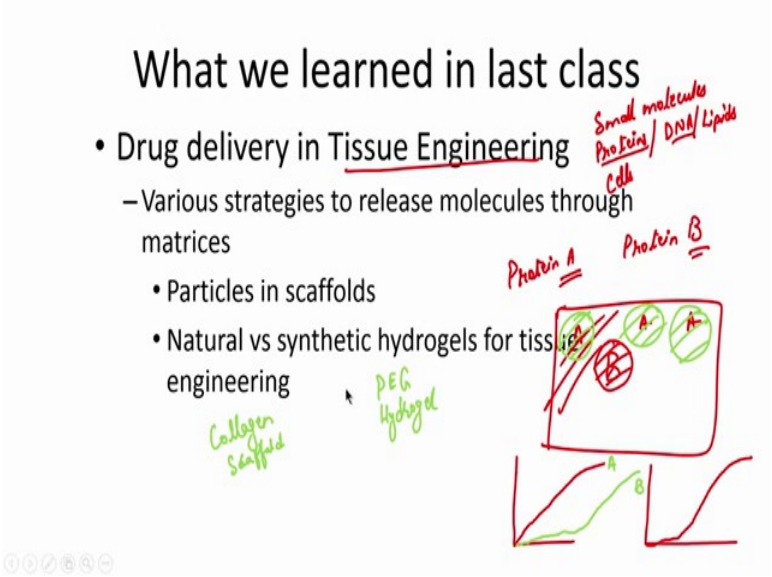


And then you can take another polymer make particles out of it and put B in there and so what will happen is as this outside matrices will degrade the A will release out and then only when the water is able to access B, or maybe the B degrades much slower than the polymer outside, only then the B will be able to come out.

So, this way you can tune, so that let us say if I have to plot release rate from the time of implantation maybe the A will come out at a certain rate whereas, in this case the B will be minimal at the start and only when a certain amount of polymer is degraded only then it will come out.

So, essentially if I overlap with this, so this is for A and if I overlap with this, I will have a more of a release of B closer to like this. So, that way I can get sequential delivery because A is mostly coming out first, its doing whatever it needs to do maybe getting the cells ready to a stage where B can start acting and its very efficient at that point and then the B is starting to release slowly.

And this is again I talked about 2 proteins, but you can envision a system that can have 10 proteins, 20 proteins and you can just encapsulate different kinds of things in different types of polymers that have different degradation rates and you can even, in this system,

this a does not need to be outside you can also have A in a second polymer. So, all of that, so this gives a lots of power and tool to play around with to get what you want to achieve.

And then another thing we talked about was major differences between natural and synthetic hydrogels for tissue engineering, in this particular case we took example of a collagen scaffold and a PEG hydrogel. And what we learn here that both of them have their own advantages and disadvantages PEG gives you a lot more control in terms of the properties, collagen is more mimicking of what the cell typically sees in the in vivo environment and there are certain properties. It is already bioactive, because the cells know how to deal with collagen.

It can manoeuvre, it can secrete more of it, can degrade it, so all of that is something that the cell knows how to handle; whereas, with the PEG hydrogel that is fairly alien to the cell, but then at the same time PEG hydrogel gives you a lot more control on let us say the mechanical properties, getting large batches of the PEG which are fairly uniform and all of this. So, there are certain advantages and disadvantages to both of these systems.

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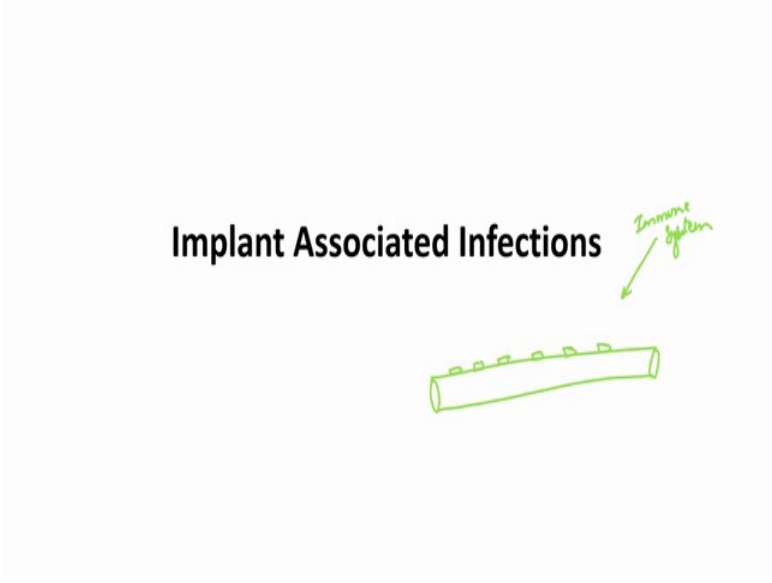


So, today we are going to discuss another topic called implant associated infections. So, what essentially I mean by this is, you have now started putting foreign entities into the body, but then you have to make sure that these scaffold these implants and you are putting in the body are actually sterile and they do not start causing infections. So, this is

a big challenge the field is facing these days where quite a lot of time after something that you have implanted, in a weeks time, you realize that this actually has some contamination, has some infection with it. And what that means is, let us say, if I put a rod for my bone fracture. But then this rod could end up having bacteria colonizing on the surface and once that has happened the body is not going to heal because this is something that the immune system is going to continuously keep on attacking. So, immune system is going to keep on attacking, the healing process will not happen just because the environment is not conducive to it and eventually the patient will have to go back to the clinic, have to get this implant surgically removed and then get another implant and make sure that the area is now completely cleared of all this bacterial infection that has started at the site.

So, it is a very painful and a long process as well as extremely expensive and not to mention it basically worsens the disease that the patient was suffering with. We will talk about various aspects of these implant associated infection as we go along.

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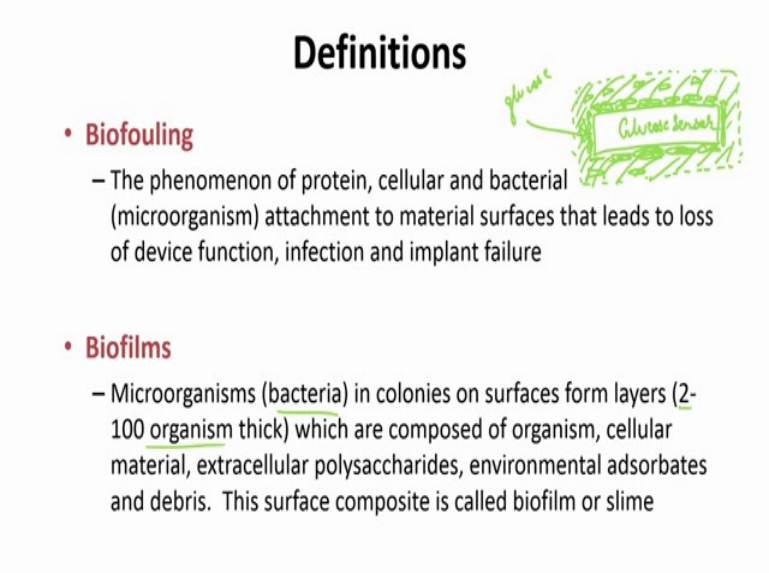


So, first few definitions, so let us start with biofouling and so what is biofouling? This is a phenomena in which a protein or cellular or any kind of biological things attach to medical surfaces and so as I said in protein adsorption we had talked about this quite a lot.

So, if I have an implant and I put it in the body fluid what typically happens is the first step? The first step is the protein starts to coat over it. And so this is essentially nothing, but biofouling where this surface is now being fouled by the bio molecules. This could be good, this could be bad, depends on their application, but this phenomena is biofouling and then the cells can come in and that is also a part of biofouling as well. And sometimes this could lead to loss of the device function.

So, if it is a thin layer usually it is not a big issue, but let us say if this layer of protein in cells gets coated to a quite a thick layer around it, quite a thick as well as compact layer around it, then what will happen? Let us say this was a glucose sensor.

So, now, that this glucose sensor is completely covered with a thick layer what will happen is, it required glucose to come and get sensed at the tip of this implant, this glucose now has a lot of diffusion problems because there is a thick layer of this protein and cell that is fouling the surface. And the diffusion of the glucose is going to be very different, so the parameters you optimized this glucose sensor to sense are going to be very different. So, maybe the readings are going to get from this glucose sensor is going to be completely wrong. So, this is the problem in such cases with biofouling.

Another term that is very widely used is biofilms. So, biofilms is one of the type of material that fouls implant that you are putting in. So, what are biofilms? Biofilms are colonies of some microorganisms typically bacteria, that are formed on the surfaces this could be several micron thick it could be anywhere between 2 to 100 organism thick or even larger for that matter. And this is composed of the live microorganism as well as other material that these things secrete and essentially form a thick slime layer over it.

So, it is very similar to what I have drawn here, but now in this case this is essentially formed by some kind of a microorganism which is also embedded in this. And its essentially doing the same function, if it is something foreign that has established on this implant, then our body is not going to accept it is going to continuously try to inflame the area try to reject this particular organism that has shown up.

So, that is a big problem with biofilms these days it is one of the very dreaded topic in terms of problems in clinics and patients and so lots of impedance has been given to try to avoid formation of bacterial infections in biofilms.

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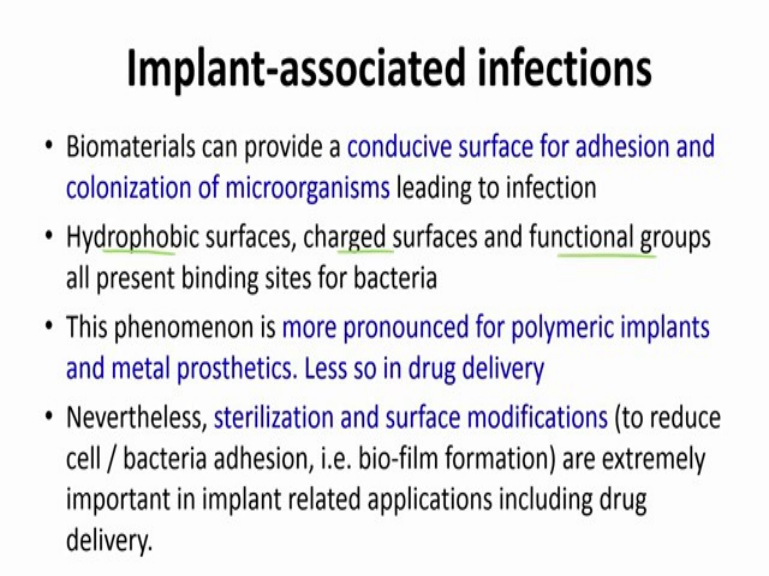


So, having now discussed these two terms, let us discuss what is implant associated infections. So, like all materials biomaterials can provide a conducive surface for adhesion and colonization of microorganisms. Basically I mean you will find that these microorganisms are very well adapted to grow on any kind of surfaces, you will even find them on your walls, on your objects which are completely not relevant to materials.

So, these things are very conducive to grow in any extreme environment, on extreme surfaces and the same thing also applies with biomaterials, where once they find that surface, they are able to colonize it. So, typically the hydrophobic surfaces, the charged surfaces, the functional groups that are present give a binding site for these bacteria.

So, I mean, if the surface is very inert, you will see less likelihood of that surface getting colonized by the bacteria, but if you have any of these properties where they are either hydrophobic or they are charged or they have some functional groups through which these bacteria can attach to it, then those surfaces become a lot more conducive for this implant associated infections.

And again we have discussed throughout this course that all of these are something that we use for whatever we are trying to achieve in different sort of circumstances like charged groups and functional groups are something that we deliberately put in. So, that we can modify our material the way we want it, but then the bacteria also uses those functionalities to colonize that surface.

This phenomena is actually even more pronounced with polymeric implants or metal prosthesis less so in drug delivery and the reason for that is most the time when you are trying to do drug delivery you are looking at, predominantly in major cases, you are looking at something that is going to be only there in the body for about 3 to 5 days. Its degrading it really is a very dynamic system for the bacteria to sort of establish itself. Even though it does happen in that system as well, but it is not as major of a problem as let us say, in tissue engineering where you are trying to put an implant for life or at least for a few years and few months.

So, those things, if they get infected then the bacteria has enough time to sort of adapt itself to its environment and colonize its surface and so you will find that quite a lot in tissue engineering applications. But again of course, nevertheless there are some sterilization and surface modifications that you can do to prevent this and to make sure that this does not occur in any of the patients and so that is why it becomes very important for any kind of implant related applications including drug delivery and we are going to discuss them today.

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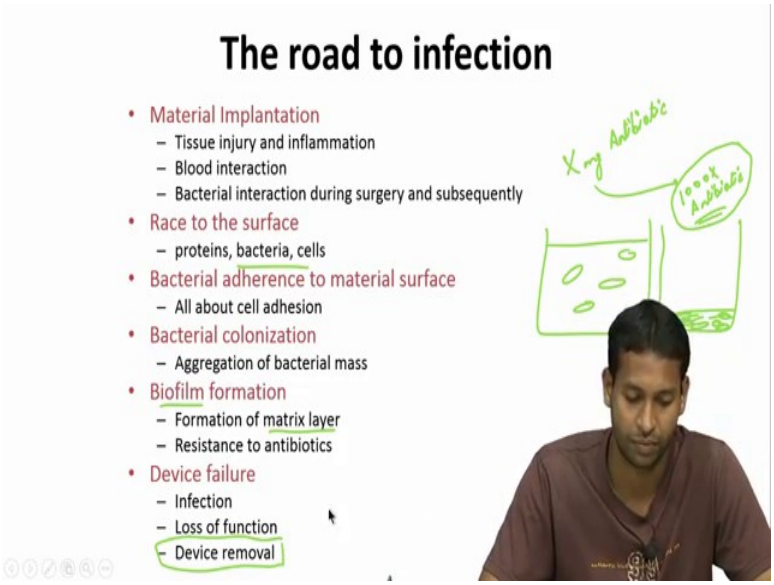


So, let us talk about how does this infection happen, what is the road that leads to this infection? So, the first thing is of course, you are putting in an implant, so there is some tissue injury that happens due to that injury there is some inflammation and the implant interacts with blood. So, if at the time of surgery let us say the implant was not clean or

the environment was not clean around it and bacteria are able to come and interact with your body.

So, that is where it all starts at the time of implantation and then it essentially becomes a race to surface. So, now, earlier I talked to you about when a new material is put in, first protein comes to it then the mammalian cells come to that particular area, but now we have another player here which is bacteria. So, now, these three components are sort of racing to get to the surface first and colonize it. So, whoever wins that race will essentially have major advantages in repelling anything else that is coming in afterwards.

So, let us say if the bacteria do win this race which happens in few cases then the bacteria, then adheres to the material surface which is very similar to cell adhesion, I mean whether its mammalian or bacterial; obviously, the receptors and the mechanisms are a bit different, but it is essentially talking about a having some sort of bond formed with the surface either through a protein layer or directly and that causes the bacterial to adhere to that material surface. Once the bacteria is adhered it can then colonize it can start to grow, it can aggregate, it can form this bacterial mass which we defined as biofilm in the previous slide. So, all of that can start to happen, so that is the next step.

And then finally, a more mature biofilm is formed, so this is essentially causing aggregation of bacteria as well as production of quite a lot of extracellular matrix from the bacteria such as polysaccharides, agarose is one of those example. And once this matrix layer and biofilm is formed the bacteria actually becomes quite robust and it is able to even repel antibiotics.

So, essentially what has been shown in the literature if you have planktonic bacteria which is basically meaning that the bacteria is free floating in a fluid and if you require a concentration of X milligram let us say of an antibiotic to kill this bacteria.

Then let us say now this bacteria has colonized the surface, so let us say this bacteria is here is colonized in an ECM matrix, then the amount of antibiotics you may require to actually kill this bacteria and stop from growing is could be all the way up to 1000 times of x.

So, that is how resistant they become and that is how difficult it is because once you start giving 1000 times a dose you also have to then worry about the toxicity of the antibiotic

to the human body itself and not to mention we have several bacteria that are good to our body such as gut microbiota, lung microbiota, skin microbiota and all of that suffers with that heavy dose.

And then once this biofilm is formed, ultimately the device fails, this could be due to several reasons maybe the device is there as a sensor then essentially the sensing molecules are not able to reach to their target site. So, we gave an example already of glucose.

So, in that case we saw that glucose is now having difficulty in permeating through this layer, could be something else that is sensing maybe its calcium ions, maybe it is a concentration of a drug, maybe its a concentration of a solute. So, all of that leads to failure in sort of sensing scenarios and not only that I mean even if let us say this was a scaffold that you put in to grow a part of your liver and if its infected, then the environment there changes quite a lot because the body keeps on attacking that system because the body is not going to accept a bacterial infection.

So, it keeps or attacking, but due to this biofilm formation it is not able to clear it very well and we can talk about why that happens and eventually this is going to cause failure of your device because if the liver tissue is not going to grow in that new area, then that implant is useless. And in fact, not only that, because of the inflammation in all the surrounding area what we will start to see is even the liver that has attached to your implant which was healthy to start with will start to lose function.

So, at that point there is really no option, you can try some heavy dose of antibiotics to see if it helps, but most of the time what you will find is that the patient has to go back in and the device will have to be removed.

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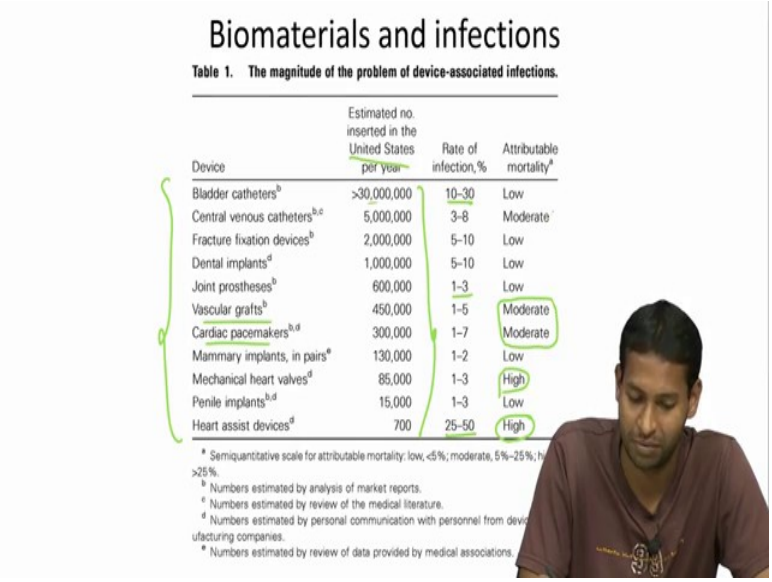


So, here is just some example of how grave this problem is, so we have biomaterials and related infections. So, what you can see here, so they are just mentioning some of the devices that are being used and this data is for the United States, but basically similar proportion can be found everywhere and what you see is how much of these implants are being used.

So, quite a bit as you can see some of the things like bladder catheters you are talking about in excess of crores of implants and similarly all of them are required in quite high number. And what you see is in some of the cases the infection is fairly high, I mean this is after following all kinds of state of the art practices you are looking at bladder catheters we are talking about 10 to 30 percent.

So, basically every third of the fourth patient will get infected and some of the cases I mean it is all the way up to 50 percent. So, I mean almost half of the surgeries that we will do you will have to come back and redo the surgery. So, that is quite a heavy toll on both the medical community as well as on the patient.

Some of them are also fairly low, but then even then that is a risk that still very high and then the worst part about all this is lot of this actually leads to the death of the patient. So, the implant related infection is actually causing the death. So, the patient came in to sort of get cured from a certain disease you try to cure that particular patient, but you ultimately end up having to kill that patient or you end up and killing that patient just

because this infection was, so massive that the patient could not handle it. So, as you can see especially with the heart assist devices you see that the mortality is very high.

Similarly, in anything related to a heart is extremely critical just because it is not feasible to continue to manoeuvre around with it is a very sensitive organ if it stopped beating for a few even few minutes the patient is essentially dead. And in some cases, the mortality is again moderate which is again as you will see is related to some sort of blood and something like that can be very serious.

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**Table 1.** Partial list of human infections involving biofilms.

| Infection or disease | Common biofilm bacterial species |
|---|---|
| Dental caries | Acidogenic Gram-positive cocci (e.g., *Streptococcus*) |
| Periodontitis | Gram-negative anaerobic oral bacteria |
| Otitis media | Nontypable strains of *Haemophilus influenzae* |
| Musculoskeletal infections | Gram-positive cocci (e.g., staphylococci) |
| Necrotizing fasciitis | Group A streptococci |
| Biliary tract infection | Enteric bacteria (e.g., *Escherichia coli*) |
| Osteomyelitis | Various bacterial and fungal species—often mixed |
| Bacterial prostatitis | *E. coli* and other Gram-negative bacteria |
| Native valve endocarditis | Viridans group streptococci |
| Cystic fibrosis pneumonia | *P. aeruginosa* and *Burkholderia cepacia* |
| Meloidosis | *Pseudomonas pseudomallei* |
| Nosocomial infections | |
| ICU pneumonia | Gram-negative rods |
| Sutures | *Staphylococcus epidermidis* and *S. aureus* |
| Exit sites | *S. epidermidis* and *S. aureus* |
| Arteriovenous shunts | *S. epidermidis* and *S. aureus* |
| Schleral buckles | Gram-positive cocci |
| Contact lens | *P. aeruginosa* and Gram-positive cocci |
| Urinary catheter cystitis | *E. coli* and other Gram-negative rods |
| Peritoneal dialysis (CAPD) peritonitis | A variety of bacteria and fungi |
| IUDs | *Actinomyces israelii* and many others |
| Endotracheal tubes | A variety of bacteria and fungi |
| Hickman catheters | *S. epidermidis* and *C. albicans* |
| Central venous catheters | *S. epidermidis* and others |
| Mechanical heart valves | *S. aureus* and *S. epidermidis* |
| Vascular grafts | Gram-positive cocci |
| Biliary stent blockage | A variety of enteric bacteria and fungi |
| Orthopedic devices | *S. aureus* and *S. epidermidis* |

Again this is just again for your reference, so here is a partial list of human infections that involve biofilms. So, actually one thing that I want to point out in the previous slide here, is if you notice here most of these implants these are some things that stay for life. I mean you are talking about heart assist devices, so as long as the patient is alive they will need heart assist devices, you are talking about bladder catheters, dental implants. So, all of these are some things that I am going to stay in patient for life and typically what you will find as these are non degradable implants.

So, the surface that the bacteria is presented with on which the bacteria started to grow is going to remain. So, this particular surface is going to remain there and then the bacteria will happily colonize it once it adapts to it, it is not a dynamic implant that the bacteria is looking at which is typically seen in cases of drug delivery.

So, going back, here is a partial list of human infections that involves biofilms. So, the previous case we were just talking about that there was infections, now you are talking about infections that are involving biofilms and there is quite a long list here that you are seeing and some of the bacteria that are quite often seen is streptococcus is one of them, hemophilius is another one of them, you have E. coli, some fungal species also come in, so all of these are there. And then they are few which are actually very common, so one of them is Pseudomonas aeruginosa, another one is Staphylococcus aureus.

So, these are very rampant and actually you might have heard about them in newspaper and various news around that these are also now becoming antibiotic resistant, so that then compounds the problem. So, not only you have a problem that these bacteria are coming in, forming biofilms which have more tolerability against a particular antibiotic you also have bacteria that are forming powerful there are already antibiotic resistant.

So, once that happens there is really no way you can treat them because all the antibiotics that we are currently using some of these bacteria are extremely resistant to all of those. So, how do you kill that bacteria? So, there is only one way at that point, you'll have to remove the implant. And again there is quite a bit of them and pretty much anything that you are putting in the body you find that there are all kinds of infections. So, you have sutures even those little sutures that you put in, they get infected, create a lot of pus you have to remove them, then you have these contact lenses they get infected with pseudomonas.

So, forming a layer over your contact lens which is again not very good. you have endotracheal tubes, vascular grafts we talked about in the previous slide, mechanical heart valves again we talked about in the previous slide, orthopedic devices which are quite heavily used for any kind of fracture and these implants are essentially metal implants and S aureus is one of the major organism that sort of infects these bone devices. So, this is a major problem and again we need to take precautions when we are handling these things.

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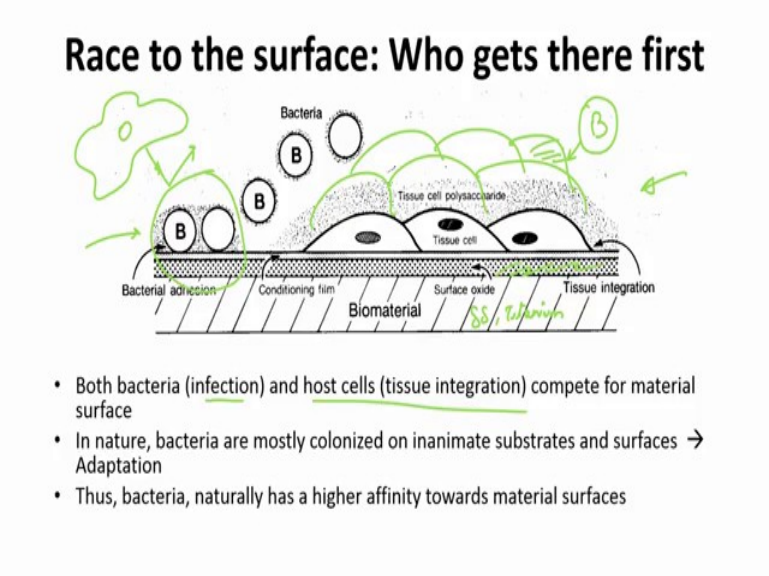


And again as I talked about, so there is a race to the surface. So, let us say here is a biomaterial that you are looking to implant in the body and it essentially boils down to who starts colonizing the surface first because it could be the mammalian cells that are coming in or it could be the bacterial cells that are coming in and depending on what it is, whoever comes in first, then gets an advantage because that particular cell can then adhere to the surface and let us say now if a mammalian cell is trying to come, these cells can essentially is repel it.

Because there is no conducive surface for this mammalian cell to come in attach to and the same thing happens with the bacterial cell, once the mammalian cells are there if a bacterial cell is trying to come in and attach to it and the tissue ECM, these tissue cells they do not let this bacteria to come and colonize its surface, the body is well adapted to take care of anything natural that is present so that it does not get infected with bacteria, but then anything unnatural that is there the body really has no control.

So, let us say for example, if a bacteria does come in adhere to this multi cell layer surface, but then what will happen the body can essentially just kill this particular cell and along with that this bacteria, so that the bacteria is not able to do essentially stay in the body. But then in the case where is residing on a surface the body has nearly no option if this is, let us say a stainless steel rod or a titanium implant, and the body cannot degrade it.

So, at that point the body is really helpless against such bacteria. So, as I said both bacteria the infection causing agent as well as the host cell compete for this material surface, typically the bacteria in the nature is well adapted to colonize inanimate surfaces. So, you will you will see in your pipes may have layers of bacteria, your walls have layers of fungus and bacteria.

So, the bacteria is actually very well adapted in terms of colonizing such inanimate surfaces much more so than the mammalian cell, not to mention the bacteria divides much rapidly in the mammalian cells the numbers can increase very rapidly and so that way it has some advantages over colonizing a material. And so this bacteria has a naturally high affinity towards material surfaces, so, which can then again lead to implant infection and essentially fouling of that surface. So, we will stop here and we will continue rest in the next class.

Thank you.