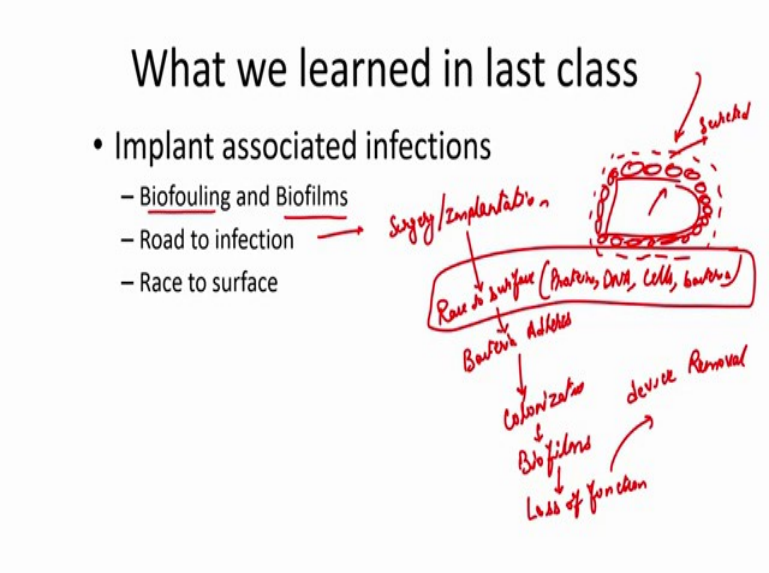
Drug Delivery Principles and Engineering
Prof. Rachit Agarwal
Bio Systems Science and Engineering
Indian Institute of Science, Bengaluru

Lecture - 34
Implant Associated Infections

Hello everyone, welcome to another lecture for Drug Delivery Engineering and Principles. I am Rachit and we are going to continue our discussion for this particular module. So, we are right now talking about Implant Associated Infections, just a quick recap of what we discussed in the last class on this topic.

(Refer Slide Time: 00:44)



So, again implant associated infections are a big problem, a lot of the implants that we put in, depending on what type of implants and what applications, quite a lot of them get infected we learned that anything between 1 percent to 50 percent can be the infection rate and some of them can lead to mortality because of the infections. So, it is a serious problem. We learned about some definitions so, Biofouling which is essentially coating of a biomaterial or a biomolecule over your implant. So, essentially that is called biofouling.

One of the problems that can happen in the biofouling is the molecules cannot really come in and out very well, because now there is a barrier layer that is protecting this movement in and out. So, this may actually impact the functioning of your device. And,

then we also discussed about biofilms, which is essentially nothing, but a type of bio fouling. And, what it essentially is this layer composes of small communities of bacteria.

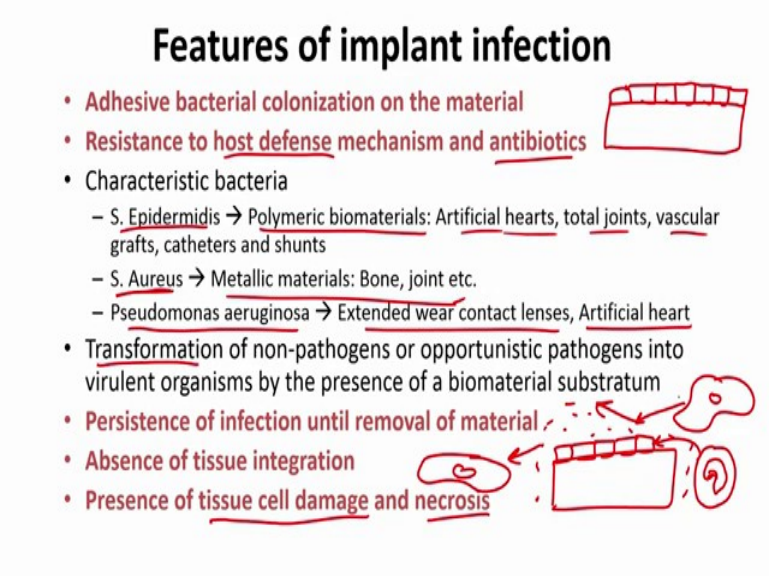
So, there will be bacteria and then some of the secreted material that these bacteria are producing and, then essentially forming a barrier layer, but this time only composed of the bacteria itself. The next thing we talked about was the road to infection. So, what typically happens? So, you have the first thing you do is do a surgery and implant your device. Once that is done there is interaction with all kinds of body fluid and now there is a race to the surface. And, when I say race to the surface; that means, all proteins, DNA cells, and the bacteria, or any other kind of pathogen are sort of racing to the surface to see which one comes first and I am going to talk more about this.

Once that has happened and let us say a bacteria does win this race, which happens in quite a few of the cases, then what happens is then the bacteria then goes ahead and starts to adhere to the surface. Once, the bacteria adheres this can go in and start colonizing essentially forming these biofilms. So, colonization, followed by biofilms and then at that point there is really not much you can do the device loses its function and then you will have to remove device. So, this is essentially the road to infection.

And, then there is also race to the surface that is more looking at this aspect of the process where all kinds of cells that are trying to compete and trying to colonize the surface first. So, mainly the race is between the bacterial cell and the mammalian cells, but what we learn is that the bacteria is much more evolved to be able to take care of this race to the surface and typically beats mammalian cell not only because it is adapted plus also it can divide very rapidly. So, it can increase its number at the site quite quickly.

And, once there is an establishment of either mammalian cell or bacterial cell it will be much more conducive to not let the other type to come and colonize it further.

(Refer Slide Time: 04:28)



So, let us talk further about some of the features of these implant infection. So, you have adhesive bacterial colonization on the material. So, let us say now that the implant has been infected and there are bacteria that are colonizing pretty much all of this implant, then this causes resistance to host defense mechanism. So, again as I briefly mentioned in the last class. So, there could be planktonic bacteria, which means free floating bacteria and there could be bacterial biofilms.

So, typically these bacteria together in a biofilm become much more resistant to any kind of stress. So, that could include antibiotics and we talked about that how some of the antibiotics may be required 1000 times the concentration to be effective, and this could also apply to host defense mechanism.

So, the host has several defences, it can produce some reactive oxygen species, it can come in with certain immune cells to engulf the bacteria that is in the surrounding, but once it is formed in the biofilm in these large communities it becomes quite or lot resistant to all of these mechanisms.

And so again we briefly talked about it, but there are certain characteristic bacteria for certain types of implants. So, S. Epidermidis typically you will see quite a lot of polymeric implants getting infected this, could be artificial hearts, this could be joints, this could be vascular graft and things like that. And, then you have staphylococcus aureus which is very heavily found in any kind of metallic implants. It can also infect

other implants, but this is just some of the predominant that is seen with terms of the use of material.

And, then you have Pseudomonas aeruginosa which is gram negative bacteria and this can again go in the artificial heart, on the contact lenses, even on the bone material. So, there is quite a lot of sort of promiscuity between these different applications with the different bacteria, but then this is just some general examples of what you predominantly see.

And, then sometimes there is a transformation of a non-pathogen or opportunistic pathogen into a virulent pathogen in the presence of a biomaterial substratum. So, maybe for an example, Staphylococcus aureus is something which is actually naturally present in our skin.

So, in some circumstances it is commensal bacteria, where it does not cause any harm to the host, but once the same bacteria starts colonizing particular artificial biomaterial that you put in they then transformed into a pathogenic bacteria, where they start secreting lots of ECM and lots of material that the body does not like, plus then the immune system starts to actually attack that particular bacteria as well. So, this transformation can also happen or it could be pathogenic right from the start, for example, Pseudomonas aeruginosa.

And, again then this bacteria is fairly persistent, especially in biofilms. This will persist till you remove the material. Because, once the metal is removed then the body can typically handle it, but till the material is there the body is not able to handle there is bacteria at all.

And, again just to give you further example as to what will happen let us say and this was a material that is again infected by the bacteria. First of all it is going to start repelling your own body cells from coming in so, the body cells cannot really attach to it. So, the tissue integration will not happen it cannot really secrete ECM, it cannot form good bonds with this material.

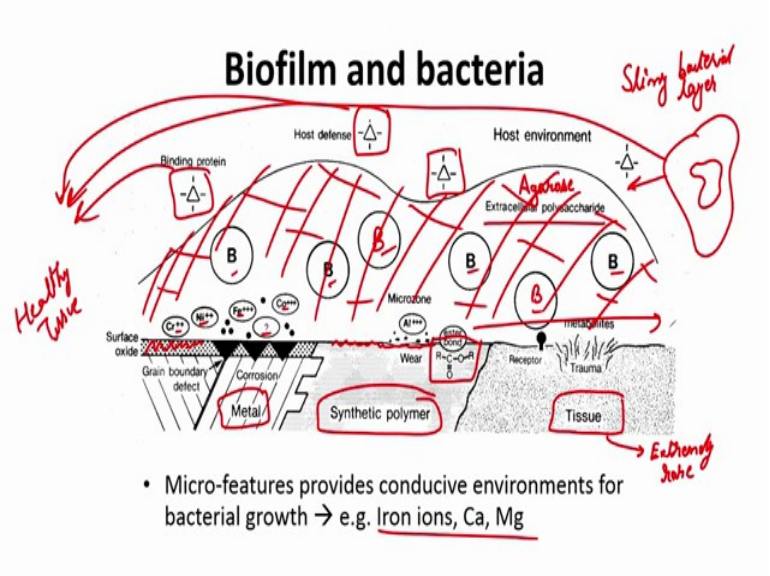
So, this material will be sort of just lying loose in the body. And, we actually discussed a paper on this as well, that stainless steel implants once you are putting or any kind of bone implants. Let us say if it is a bone screw or a bone plate you want them to actually

adhere much better to your bone. Because, otherwise what will happen is it would not be able to give you the structural strength to be able to support that bone.

So, that is very important and then because of not only this then there are all kinds of immune cells that are going to come in and try to attack this particular surface, because they continue to detect bacteria. They are secreting all kinds of proteins and reactive oxygen species.

So, even if let us say you had a healthy tissue or a healthy mammalian cell somewhere here it senses all these molecules and it starts to die. So, you are actually causing more tissue cell damage and essentially necrosis in that area, which is not good because not only now you are not able to restore the function for which you would put the implant, you are actually decreasing the function; because, it is destroying anything in the surrounding.

(Refer Slide Time: 09:28)



Ok. So, let us talk a little more about biofilm and bacteria itself. So, here is just a zoomed in image of how a typical biofilm will look like and there are again various aspects that are being shown here. So, as I mentioned before biofilm is the slimy bacterial layer. And, in this layer you can see there are several bacteria that are floating around this could be dead or alive. And, they have secreted lots of extracellular polysaccharides. So, Agarose is an example, which I am hoping all of you must be aware of. Agarose is a very widely used polymer to run your protein gels, DNA gels, and separate them out.

So, that is one example and there are several other types of extracellular polysaccharides that are also secreted, and they essentially form a gel like layer.

So, there is nothing, but cross linked either physically or chemically, layer which acts as a barrier. So, now, because these pore sizes could be fairly small let us say 100 nanometer or even smaller, you cannot have any bigger mammalian cell penetrate this. So, even if it is an immune cell which is trying to come and destroy the bacteria, that immune cell cannot destroy the bacteria, because, this bacteria is actually protected by getting encapsulated in this particular slimy layer. So, that is not going to work. On the bottom surface here we are just showing as to what are the different areas on which these things can metabolize.

So, here is an example of just a native tissue, the incidence of this infection is extremely rare, just because the body knows how to handle it? I mean this infection is not going to cause immediate biofilm, the body can essentially degrade this particular area, but once infection is established, that can start spreading to a normal tissue as well, just because the body is already overwhelmed and trying to fight your infection on your implant.

But, then typically that does not happen. Then you can have a synthetic polymer, something we have discussed quite a lot in this course and there could be again non uniformity on a surface to which a bacteria can adhere to, there could be functional groups, as we mentioned through which the bacteria can react there could be some metal ions that the bacteria like. So, all of this promotes bacterial adherence.

And, then similarly you have metals mostly for bone applications or some kind of devices that are put in to assist some function like heart devices. And, then here also you can have again some wear and tear on the surface, some of these ions that are released are actually very good and conducive for the growth of the bacteria.

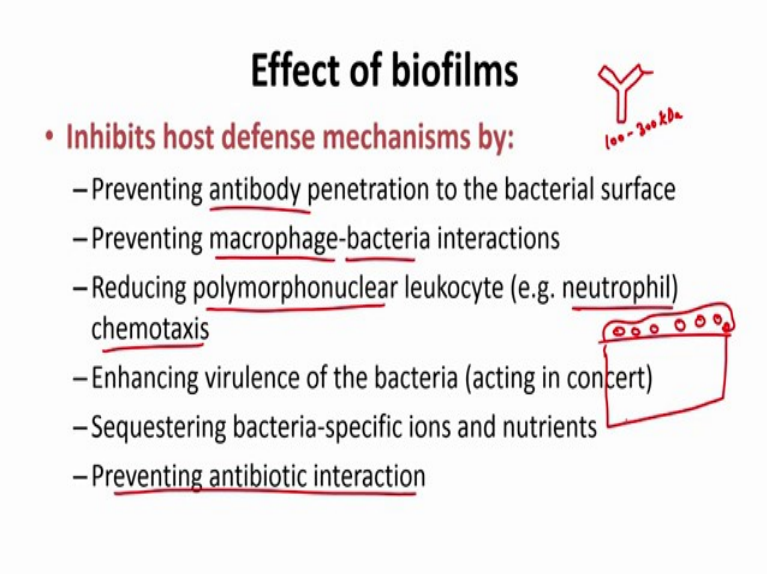
So, some of them are also unknown, but essentially and these can lead to attraction of the bacteria as well as survival of the bacteria on the surface. You can have corrosion happening. So, that gives you again very large surface area for the bacteria to start colonizing that surface.

So, again here just the same thing mentioned here some ions like iron, calcium, magnesium, these are extremely good for bacterial growth. And, those can then lead to

the promotion of the bacteria to come in and again these host defense molecules or cells they are extremely big compared to the pore size of this biofilm.

So, they are not able to go and degrade or kill this bacteria off, but then here your healthy tissue is also experiencing all these proteins coming in and including the cells to come and kill your healthy tissue, so major problem there.

(Refer Slide Time: 13:41)



So, what are the different effects of biofilms? So, first is of course, we have discussed in few slides now, that it inhibits the host defense mechanism, it can prevent antibody from penetrating. We know that antibody is a fairly large molecule, we are talking about anywhere between 100 to 300 KDa. So, it is not able to penetrate through this slimy layer and this is one of the major mechanism the host uses to detect bacteria and get it cleared from the system through immune cells, but if the antibody cannot even go through then immune cells are not able to go through either.

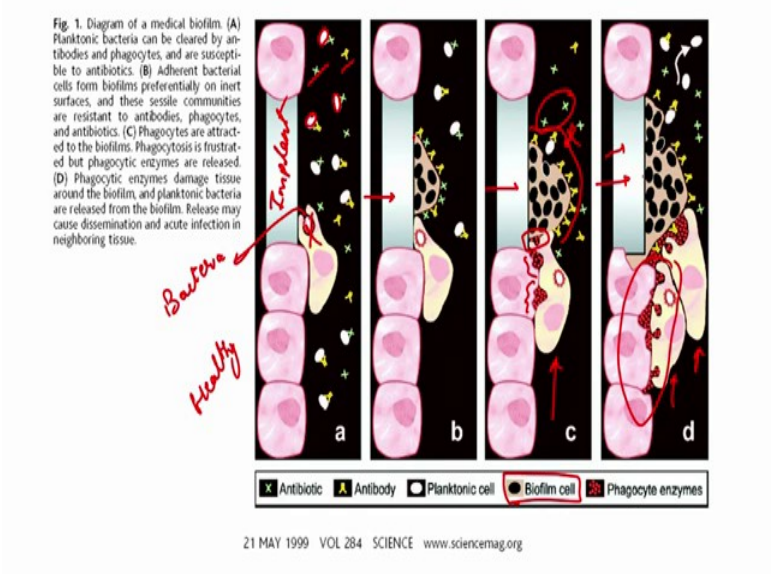
Then, as I said the immune cells majorly macrophages, they are neutrophils, they are not able to go and interact with the bacteria. So, one of the mechanism that these immune cells take is to eat up this bacteria. So, take it up or just release lots of molecules in the vicinity of the bacteria which are toxic to the bacteria, but then if these macrophages cannot really interact cannot really go close to the bacteria, then you cannot really have this mechanism either.

And, again the same point here that polymorphonuclear leukocytes such as neutrophils, which are again a major player in terms of controlling any pathogenic bacteria, they are not able to go and penetrate into these slimy layers and they are not able to act either.

Now, what also starts happening is now these bacteria which is sort of happy in these slimy layers they start interacting with each other. And, let us say if a particular bacteria has developed a mutation that causes it to become a pathogenic, it can start to acquire those mutations, it can start to multiply, it can start to degrade the surrounding environment to get more nutrients, more growth factors for it is growth. So, that becomes a major problem again. They start to sequestering bacterial specific ions in nutrients too.

And, then again finally, any kind of external intervention that we might try to do and one of the major one is giving antibiotics and they can prevent antibiotic interaction. So, as we discussed already the antibiotic amounts that are required to kill bacteria growing in a biofilm is orders of magnitude higher than one which is not growing in the biofilm.

(Refer Slide Time: 16:14)



So this is just sort of pictorial representation of how these biofilms once they are formed, they are able to tackle the immune system. And so, as an example let us say this is your implant that you have put in here is your healthy tissue, here is your implant.

Now, let us say this one bacteria here. So, if this is bacteria, our immune system let us say macrophages, dendritic cells, they are very well capable of going there finding the

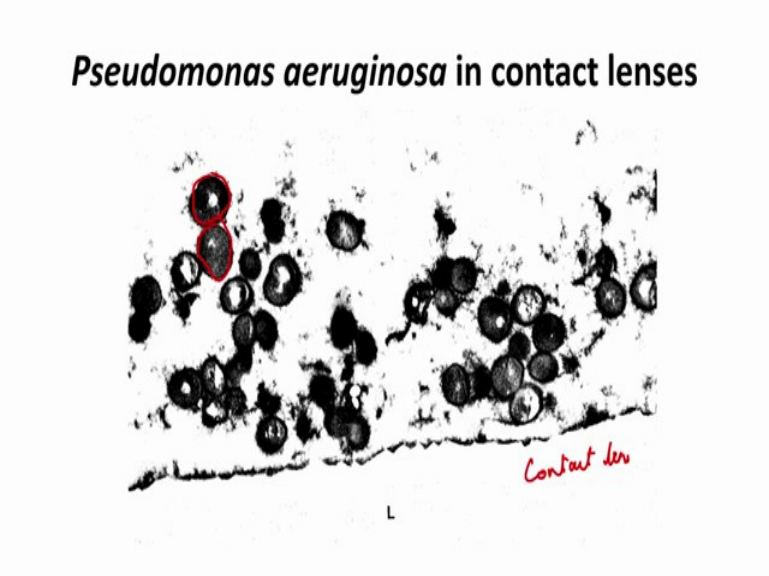
bacteria engulfing them and then essentially killing it. So, it is very easy for them to be able to kill of a single bacteria it is detected early enough, but again what does the bacteria do and again you have all these antibodies that are binding to the bacteria, which is planktonic, which is floating around. So, you can have antibiotics, you can have antibodies, all of these are able to kill the bacteria.

However, once this bacteria starts to colonize and rapidly form a biofilm so now, you have this biofilm being formed. So, here is your biofilm cell. Now, that this biofilm cell is being formed, now this is too big for this immune cell to take up. So, it can it can take up one or 2 bacteria, but then it cannot take up a whole community which could be even bigger than the size of the cell. So, at that point all it can do is, it can secrete some molecules that are antibacterial.

But, then again because of the slimy biofilm layer, even these molecules have a hard time diffusing into the biofilm. So, all that ends up doing is actually start degrading your healthy tissue. Now, your healthy tissue is getting damaged, because all these harmful toxic chemicals are being secreted in the vicinity to kill that biofilm bacteria which is not being able to do, and you can see both your antibiotics and your antibody are not able to penetrate through.

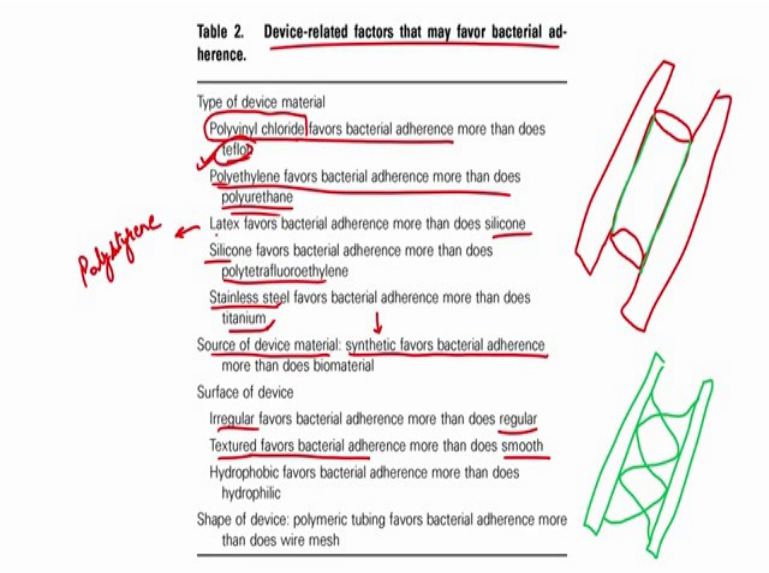
So, this biofilm can then start growing as you can see it is now growing further and it is causing more tissue damage, more and more immune cells are coming in, but these immune cells are not able to do much, in fact, the more they are kept coming in the more they are doing damage to the tissue. So, at this point the only option that remains is to go back take this implant out make sure all these biofilm and bacteria is gone and only then you can then have some other intervention that can help patient from whatever they were suffering.

(Refer Slide Time: 19:07)



So, here is just an example this is this is a contact lens, zoomed in image. And what you can see is there are these bacterial communities that are being formed, which are able to then colonize the surface and protect it from any kind of response of the body might be generating through the immune system or through some other phenomena so, a major problem.

(Refer Slide Time: 19:39)



So, here are some device related factors that may favour bacterial adherence. So, you can have and these are just some general some of them are empirical some of them are

known mechanism, but there is several characteristic would have been observed now at a period of a few decades that these things have been used. So, you have we find that the polyvinyl chloride typically favours bacterial adherence more than let us say any other material like Teflon. Similarly, polyethylene favours bacterial adherence more than polyurethane.

So, again this is just to give you an idea that since we have this huge library of polymers to choose from, we can then decide to choose a certain polymer for a certain application also keeping in mind that which is more susceptible to bacterial infection. So, as I was just saying, if you are trying to choose in Teflon and PVC maybe Teflon is better, just because it has a lower adherence to the bacteria.

Similarly, if you are trying to choose between polyethylene and polyurethane, polyurethane might be better only purely in terms of the bacterial infection and then you can see whether other properties are similar or you can work around with them.

Similarly, latex which is polystyrene, also favours bacterial adherence more than let us say silicone. So, or you can make best implants out of silicone maybe latex may not be a good material just because you need them forever and silicone might be a better way to go about it.

Similarly, now you start comparing silicone is not as good of a surface let us say as PTFE, if you are looking at the bacterial adherence. Similarly, stainless steel adheres to the bacteria more than titanium. So, these two are the major material that have been used for bone. So, if you are looking for bone and you know that typically in bone infections you will see staphylococcus aureus being quite a lot maybe you can try to look into using titanium over stainless steel, purely because they might be infection that is more prone on stainless steel than on titanium.

And, then the source of the device material is also important. So, as I just mentioned synthetic surfaces favour bacterial adherence much more than anything which is natural. And, the whole point behind that is the body knows how to handle the natural material, it can degrade it, it can remodel it to make sure that if the bacteria is even adhered to it maybe the bottom surface is going to completely degrade and all the bacteria is then going to become planktonic.

So, some of these things become important. Synthetic surfaces are more prone to bacterial infections than natural material. And, then what kind of surface it is also makes an impact. So, if it is irregular then the bacteria has a lot more surface area and it will adhere much better than on a regular surface. So, a smoother surfaces are better. So, same thing here, textured surfaces favours bacteria adherence; or however, more and more research has gone into this. And, now they are coming up with extremely textured surfaces which have certain characteristic and they are able to kill the bacteria, but in general if you have more textured surfaces without any specific research based application, that end up getting more colonization then let us say a smooth surface.

Then, similarly hydrophobic surface, we have mentioned this before already, hydrophobic surfaces tend to favour bacterial adherence more than a hydrophilic surface. And then again the shape of device can be important. So, the more surface area there is for the bacteria adhere to the more chances that the bacteria will colonize it.

So, let us say if I am just putting a tube and if the only major function of the tube is to just support let us say this vessel. So, let us say this is a strength we talked about to push away a plaque; then all of this surface, then all of the surface is actually prone to bacterial infection right.

But, then if I instead of using this, if I use a wire mesh and let us say again if this was a vessel. So, if I only put a wire mesh let us say then the area of contact between the bacteria and the material that you are putting in is much lesser. So, the chance of the bacteria adherence is low. So, these are some of the factors that have been observed as well as something that can be kept in mind if you want to reduce bacterial infection.

(Refer Slide Time: 24:37)

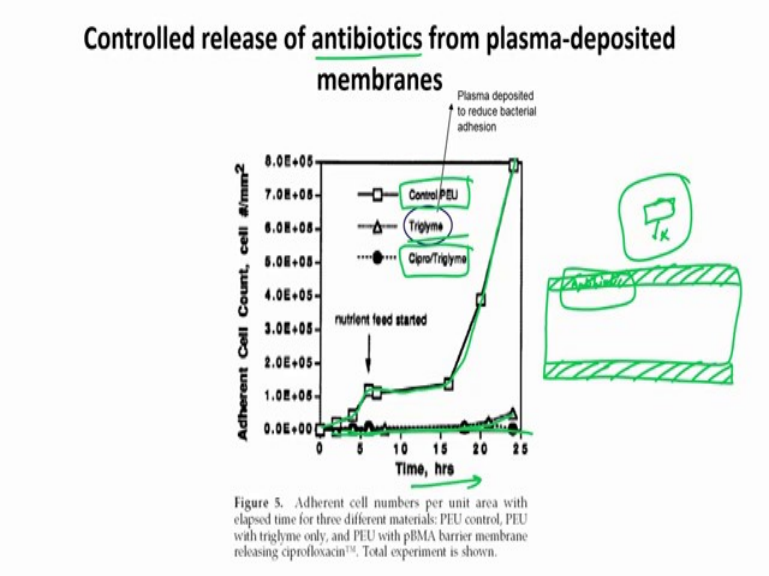


Figure 5. Adherent cell numbers per unit area with elapsed time for three different materials: PEU control, PEU with triglyme only, and PEU with pBMA barrier membrane releasing ciprofloxacin[34]. Total experiment is shown.

So, there are another ways that you can reduce these further. So, one thing we already talked about is making materials hydrophilic. So, hydrophobic surfaces we have said that end up taking quite a lot of your bacteria. And then the other way we talked about was antibiotic release.

So, essentially so, this is what you can do is you can prophylactically release some antibiotics from the surface. So, let us say if this is my surface and I can have a layer of antibiotic on it. And, then the first bacteria that is coming is actually planktonic, it is still very susceptible to antibiotic and it does not like the surface.

So, this bacteria is not going to come because it will either get killed off or it will not adhere to the surface and go away because of this continuous release of antibiotic. So, that is another strategy that people have adopted to prevent any kind of infection from happening. So, this is essentially you are relying on the fact that if there is an infection, then this antibiotic which is a low dose and typically is fairly inert to your body.

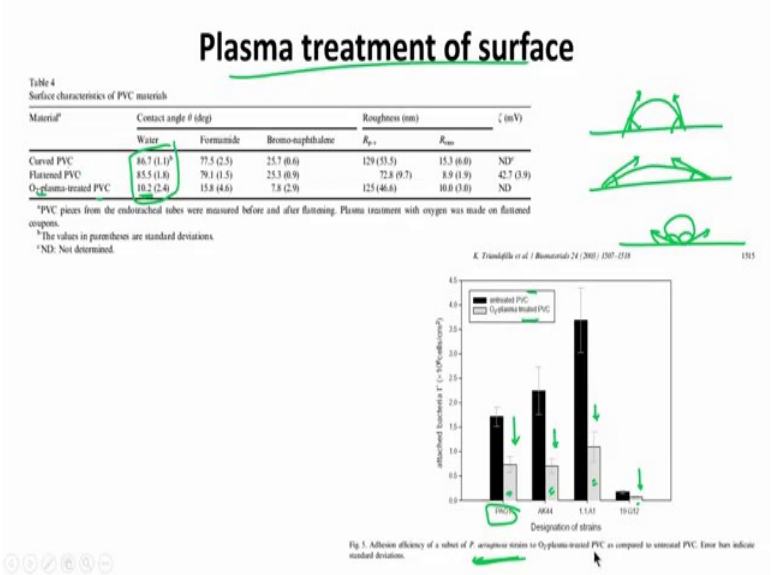
However, it will cause some damage to your commensal bacteria, some of your beneficial bacteria, but even then this might be a small cost to pay, if you want to make sure that patient cases where 25 to 50 percent of these implants get infected this will ensure that your implant is not getting infected.

However, obviously, the issue here is you do not know what type of bacteria is coming as we have already discussed there at least 8 to 10 which are very heavily seen in the literature and in clinics and some of these antibiotics that you putting in may not be effective against few of these bacteria.

So, in that case you will have to put a cocktail of your antibiotics, maybe a mixture of antibiotics that are effective against all of these normally seen. And, that way it will ensure that this bacteria does not come in. Here is some of the data to support that. So, there at this point they are looking at how much bacteria is able to adhere to an implant. In this case they have used a polymer called PEU. So, here is your control graph. So, you can see that the adherence is actually increasing quite a lot as time increases.

However, what you can do is you can have an antibiotic called trigiyme release from this. And, you do not really see much adherence even after 18-20 hours after which is starts to increase a bit, maybe the antibiotic has released or maybe the bacteria has adapted to it, or you can have 2 antibiotics release from it ciprofloxacin and trigiyme. And, in that case what you can see is even after 25 hours, you do not see any kind of bacterial growth over that surface. So, this is a strategy where you can prophylactically release these antibiotics, coat these antibiotics to prevent bacteria from adhering to a surface.

(Refer Slide Time: 27:46)



And, then here is another example which is plasma treatment of the surface. So, this is to make it more hydrophilic. So, when you treat a surface of the plasma it becomes oxidized. And, the hydrophilicity of the surface increases, the hydrophobicity goes down and this is what it is showing here. So, you have 3 different surfaces you have a curved PVC, you have flattened PVC and you have O2 treated PVC. And, here they are showing about the water contact angle.

So, what is water contact angle? So, water contact angle is - let us say if I have a surface and if I put a drop of water, then at the interface the angle that this drop makes with the water this angle is essentially the water contact angle. And so, what will happen if a surface is very hydrophilic, when the same drop is going to spread a lot more. And, because spreading a lot more your angle is now much lesser whereas, if it is extremely hydrophobic then what will happen is the water will try to prevent any interaction and it will just sort of ball up and so your angle will be extremely high. In this case even above 90 degree.

So, what you see is for the normal PVC you see angles up to 90 degrees, which is still fairly high, but when you treat with the oxygen plasma it becomes extremely hydrophilic and this angle has come down to 10 degrees. So, that is one way to change this and then what they have seen is so, they have untreated PVC versus the O2 treated PVC, and then they have come in with different strains of bacteria this PA01 is a pseudomonas aeruginosa strain.

And, what they say is using various different kinds of strains; they find that the adherence of this PA01 or pseudomonas is much lesser depending on what strategy you are using to coat these, make them hydrophilic. So, if you are treating them with plasma, they are more hydrophilic and they have lesser bacterial adherence. So, combining these few things may be treated with plasma, coating with an antibiotic layer, release antibiotic from the system, all of these strategies can be combined to ensure that your implant is not getting infected.

Once it is there and it is not infected, the body will take care of it, but if it gets infected initially, that is where the major problem lies. So, again we will stop here and we will continue further in the next class.

Thank you.