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

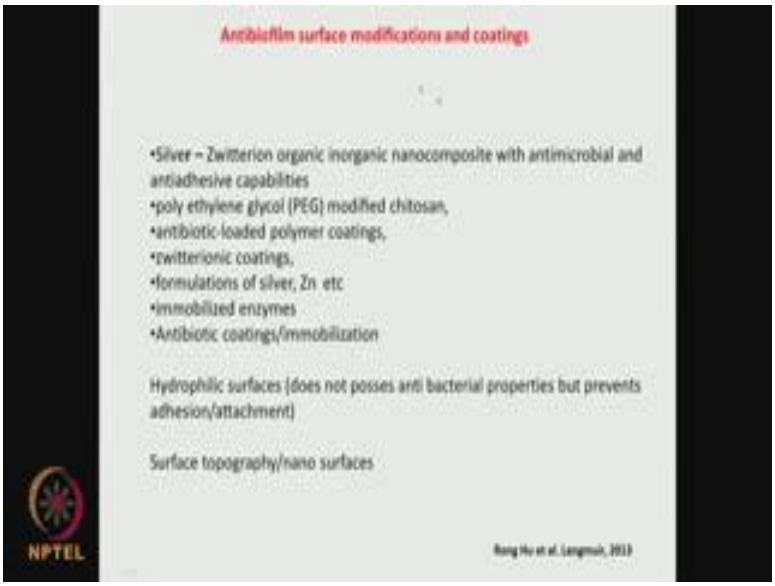
**Lecture - 13**
**Biofilm**

Hello everyone. Welcome to the course on Medical Biomaterials. We will continue on the topic of Biofilm. And, as I have been telling you that biofilm is formed on implants, devices, even if it is placed inside the human system for a few hours, leading right up to few years. And this biofilm could lead to inflammation, infection and rejection of the material. Biofilm can form on any type of material be it polymeric, be it metallic, be it ceramic; and it can be forming at any part of the body, it could be blood contacting device or it could be orthopedic implant or it could be urethral or it could be a drug delivery system.

So, we have been talking about biofilms and what are the various issues related to biofilms, what are the components of the biofilms, and what are the different techniques that have been tried out in research labs for eradicating these biofilms. Biofilm leads to lot of problems such as, the bacteria can slowly acquire antibiotic resistance because some of the bacteria which are right down inside the biofilm do not get the same concentration of the antibiotics or do not get exposed to the same concentration antibiotics as present at the surface of the biofilm.

The nutrient amount also varies the concentration of nutrients at the top as against the concentration of the nutrients somewhere in the bottom of the biofilm, the oxygen concentration also varies. So, there could be genetic modifications in the microorganisms which are present inside, because they are exposed to different environments, there could be altered in the growth pattern. So, they become persister cells. So, there are many issues, there are many reasons because of which biofilm are formed.

(Refer Slide Time: 02:20)




So how do you eradicate? There are different approaches; one is to develop anti bacterial antibiotic coatings you could have slow release systems you could have slow release polymeric systems you could have antibiotics immobilized on the surface or coated on the surface and so on. Silver is quite used because silver is anti bacterial in the form of nano particle in the form of ions Zwitterion organic inorganic nano composites which is known to have anti microbial properties and also anti adhesive properties what is this anti adhesive anti adhesive means it is that the material surface need not have anti bacterial or killing effect.

But it prevents the attachment of the microorganisms because of their altered surface characteristics like altered surface energy may be its more hydrophilic and so on; that is called anti adhesive use of polyethylene glycol modified chitosan antibiotic loaded polymer coatings Zwitterionic coatings formulations of silver zinc etcetera for example, on inorganic materials like alumina or hydroxyapatite immobilizing enzymes because some enzymes like protease or lipase or even papain have either esterase activity or amidase activity or combination of both. So, they may be able to kill coating with antibiotics or immobilizing antibiotics all these techniques help in killing the bacteria.

Another approach is developing hydrophilic surfaces. So, such surfaces may prevent attachment of hydrophobic organisms. So, it becomes anti adhesive how do the organisms acquire hydrophobicity or hydrophilicity on their surface that depends upon

the type of proteins that are present on the surface of these bacteria there could be lot of hydrophobic proteins present which will prevent attachment of these bacteria on hydrophilic surfaces, because the initial attachment is generally non-bonded interaction like electrostatic Vander Waals hydrophobic interaction and so on.

So, developing such surfaces or modifying the topography of the surface creating indents creating nanostructures by doing this you are preventing bacterial attachment because the question that is been asked is why does for example, the shells which are living all the time in water do not get biofouled or biofilm formation or if we take sharks and fish why there are no biofilms on them that is because they have lot of nanostructures present on them which prevents bacterial attachment which prevents large organism settling down and so on actually.

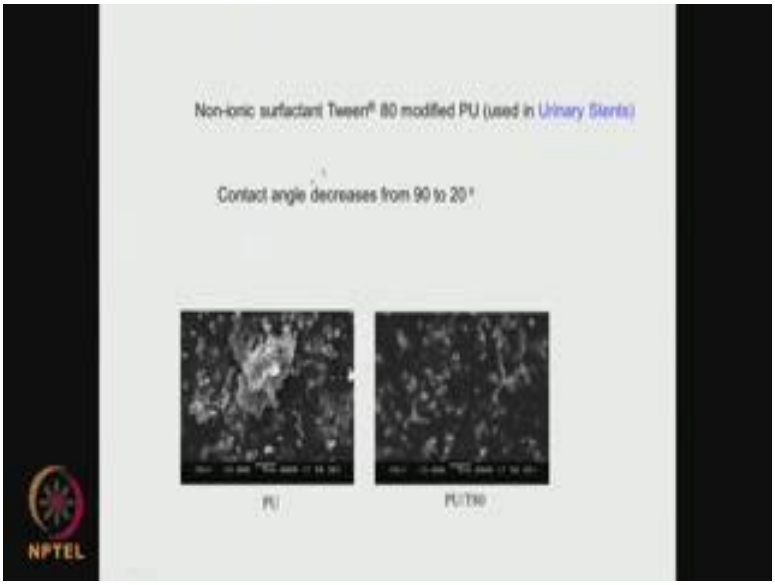
So, different approaches have been looked at and I have been giving you examples in the previous class of some of these approaches.

(Refer Slide Time: 05:30)


We will look at again a few more of these approaches for example, polyesters or polyethylene terephthalate. This is a polymer this is a terephthalic polymer this is quite lot used in large diameter vascular grafts suppose I immobilized sulphobetaine sulphobetaine has a n plus charge exactly Zwitterionic type of charge. So, we tested this modified these polyesters I am using this type of Zwitterionic material. So, this picture shows attachment of bacteria on pure polyester this is after modification. So, we can see

there is lot of prevention in the bacterial attachment. So, this shows this could be one technique for reducing the attachment of bacteria.

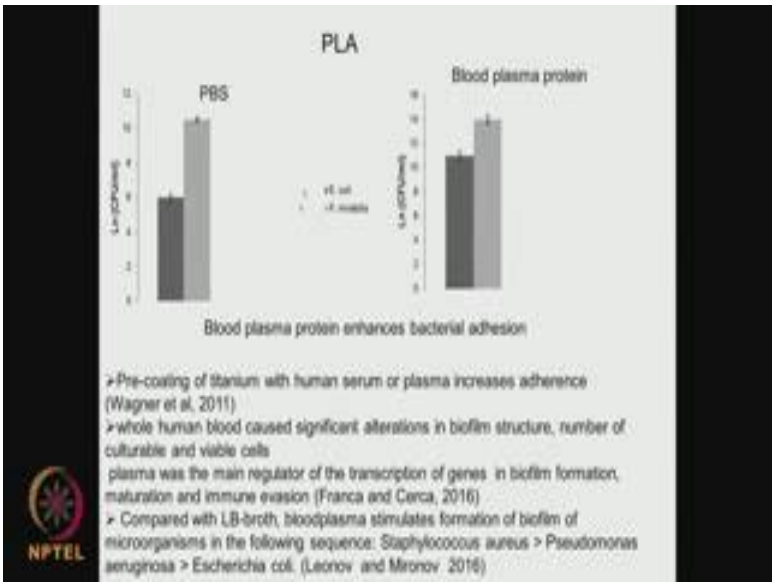
(Refer Slide Time: 06:33)




Let us look at another example Tween 80, these are surfactants these are nonionic surfactants that is neutral surfactants you can modify using them on polyurethane surfaces polyurethanes are widely used in urinary stents because of its flexible nature it shows some pictures of ureteral stents long time back. So, when you put these ambiphilic molecules the contact angle of the surface which was ninety degrees quite hydrophobic comes down to almost twenty degrees very very hydrophilic.

So, these ambiphilic molecules like Tween 80 reduces hydrophobic surfaces to hydrophilic and hence prevents the attachment of the bacteria these ambiphilics do not have antibacterial effect that mean they do not have killing effect, but they are able to prevent the attachment because they have reduced the contact angle from 90 to 20. That means, the surface energy increases or the material has become more hydrophilic once you immobilize these surfaces with this particular surfactant a nonionic surfactant that is another approach.

(Refer Slide Time: 07:47)



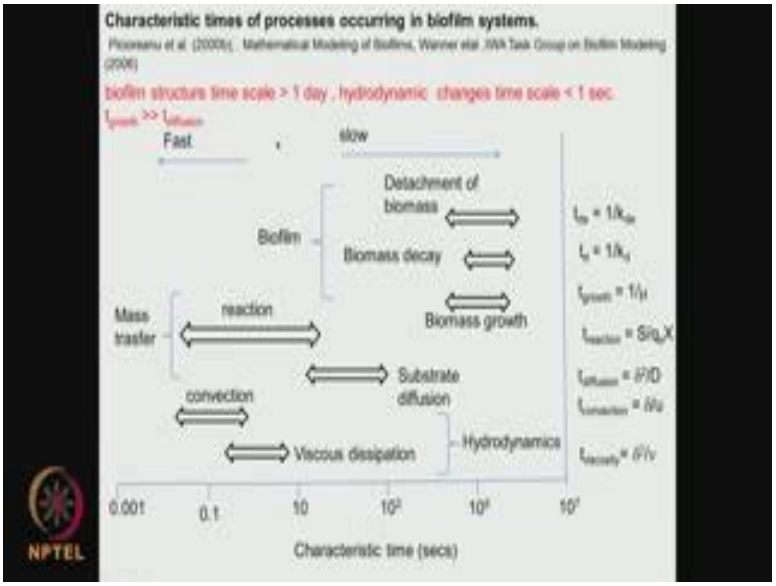

Let us look at another thing suppose you take polylactic acid and in the presence of PBS phosphate buffer, PBS is phosphate buffer you get certain attachment of say E. coil and or Proteus mirabilis about in 24 hours, we get about 10 power 6 CFU per ml of E. coil and about 10 power 10 CFU of Proteus mirabilis, but then in the presence of blood plasma protein when this PLA is coated with blood plasma protein E. coil attachment goes up dramatically.

You can see a 10 power 4fold increase in both E. coil and Proteus mirabilis increase 10 power 4 means almost 10,000 times increase in the bacterial attachment both types of bacteria E. coil as well as Proteus. So, the blood plasma protein enhances bacterial attachment. So, you have to be very careful when you are talking about having biomaterial in the presence of blood plasma blood plasma protein has been found not only in this polymeric system, but also pre coating of titanium with human serum or plasma increases adherence.

So, whole human blood causes significant alteration in biofilm structure number of culturable and viable cells plasma is observed to be main regulator of the transcription of genes in biofilm formation maturation and immune evasion this is another reference and it is been found that LB broth blood plasma stimulates formation of biofilm of microorganisms and like staphylococcus Aureus greater than pseudomonas greater than E. coil this is another reference.

So, you see all these references tell that blood plasma protein enhances the biofilm formation of wide range of bacteria like staph Aureus pseudomonas E. coil. So, both gram positive and gram negative bacteria are affected tremendously in the presence of blood plasma protein and they enhance the bacterial adhesion. So, environment has a very serious effect on the attachment of bacteria. So, we looked at different types of case studies where different approaches have been tested to see how the bacterial attachment could be reduced.

(Refer Slide Time: 10:29)




Now if we look at this entire biofilm formation, there are many things happening, you are having a flow, you are having mass transfer; that means, movement of substrate and then we are having bacterial attachment detachment now these bacterial attachment detachment they may be having longer characteristic time where as the mass transfer diffusion mass transfer kind resistance or mass transfer coefficient may be happening fast. So, they may be having lower characteristic time.

So, if you look at the biofilm growth biofilm maturation attachment of bacteria detachment of bacteria biomass growth they all operate at different time frames and this is a very interesting picture which I have developed based on these references. So, the mass transfer reaction substrate diffusion convection they are all happening very fast they are happening around 0.1 to say 10 seconds, where as if you look at the biomass

growth biomass decay detachment of biomass this is happening in terms of 1,000 or 10,000 or 100,000 seconds they are much slower.

So, if you look at the biofilm part the mass transfer part the hydrodynamics part, the mass transfer the hydrodynamics are much faster when compared to the biofilm formation which is much slower. So, we have a situation where the transfer of material the mixing is faster where as the attachment of bacteria the detachment of bacteria the biomass growth is much much slower almost by 1,000 to 10,000 times. So, let us look at the characteristic times for each one of these process.

So, let us look at this mass transfer the time the diffusion time the characteristic time diffusion time is delta square by capital D; D is the diffusion coefficient, this is the thickness, now if you look at the convection this is D by u the characteristic time convection time again this is thickness u is velocity the viscosity which happens because of the viscous dissipation and hydrodynamic delta square divided by kinematic viscosity this is the thickness. So, these are things related to mass transfer and related to hydrodynamic now if you look at the biomass growth that is reaction substrate utilization and then biomass decay biomass detachment.

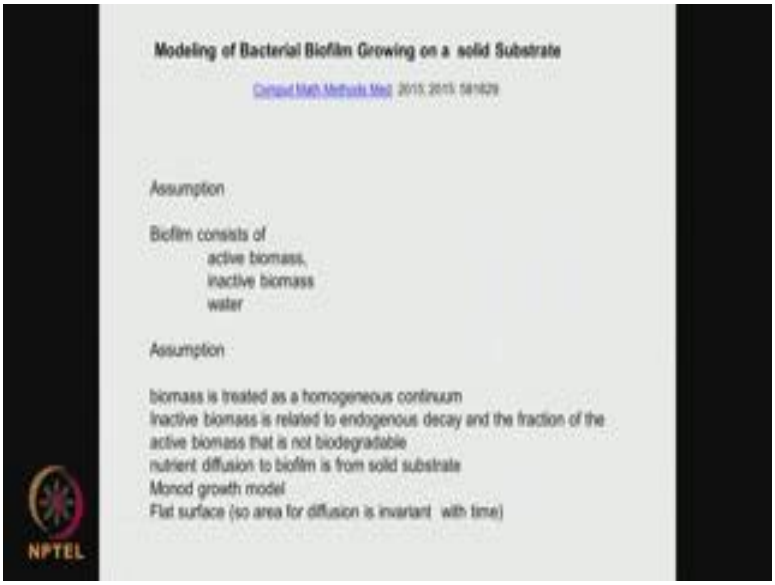
So, the reaction happens because of presence of substrate because of the biomass that is the characteristic time the reaction time the growth is 1 by mu; mu is the monod term, T D is biomass decay is 1 by K, D K D is the constant related to the biomass decay and T D E that is detachment is 1 by K D E. So, all these terms contains reactions decay terms monod terms and so on which are much slower when compared to the mass transfer diffusion and viscous hydrodynamics which are much much faster.

So, in a typical biofilm growth we are having all these things happening very fast processes that is the mass transfer hydrodynamics very slow processes which is the bacterial growth bacterial detachment and bacterial decay it is very important to know this scenario because we although we are predominantly interested in the biomass growth we need to understand when this mass transfer does not play a role in biofilm and when the mass transfer or movement or convection plays a role in the biofilm growth.

But then there is a vast difference in this time which is of the order of almost thousand seconds I would say this is a very useful slide to get a feel of the characteristic times we have fast processes and slow things happening actually. So, if you are looking at biofilm

growth we can completely ignore because we can say the mass transfer happens very fast the movement of material from the bulk on the surface also happens very fast and so on. So, biofilm structure we are talking in terms of days whereas, hydrodynamic that is less than 1 second. So, the time for growth is much, much, much larger than the time for diffusion. So, we generally we ignore that because diffusion times are much smaller.

(Refer Slide Time: 15:51)



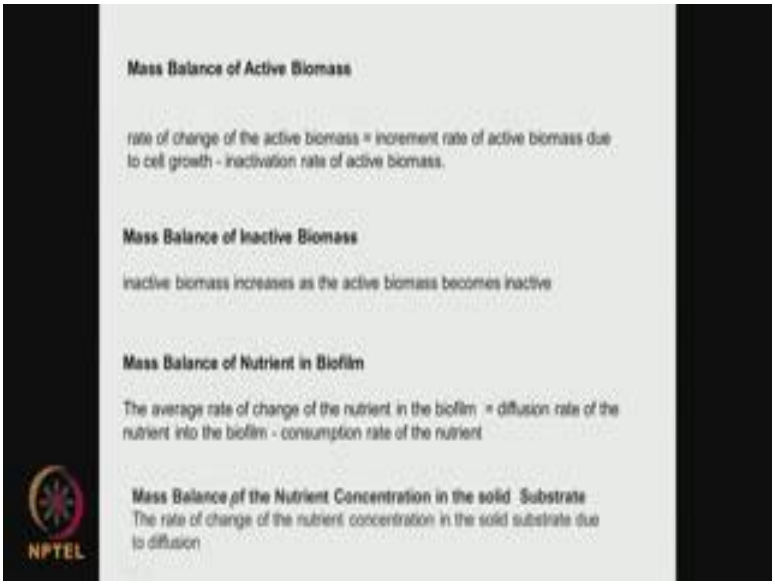

Now, there has been some attempts to model the bacterial biofilm we are not going to spend much time in modeling these bacterial biofilms models could be quite complicated and we can have heterogeneous type of models and, but then at least, we will look at what are the assumptions on which the models are based what are the terms the literature has considered while trying to model this and it is based on this particular reference. So, what are the assumptions they have assumed biofilm consists of active biomass inactive biomass and water.

So, active biomass which is not bio degradable where as inactive biomass is related to endogenous decay and it is a fraction of this active biomass what are the assumptions biomass is treated as a homogenous continuum. So, you have to remember that although biomass is heterogeneous you may have live cells dead cells proteins and so on, it is considered as homogenous. So, inactive biomass is related to the decay and the fraction of the active biomass is inactive biomass and that is not biodegradable.

Nutrient deficient biofilm is from the solid substrate. So, in this particular example they have assumed that the solid surface is giving the nutrient; that means, from the bottom rather than nutrient coming from the liquid side of it then you can assume a monod growth model for the biomass growth, you remember you must have done in long time back in bioprocess where you have mu is equal to mu max divided by K plus S and so on actually then you have flat surface. So, area of diffusion is invariant with time.

So, there is no change in the area of diffusion, but in a real situation as the biofilm grows area that is exposed to the movement of the nutrient can change as the biofilm becomes bigger and bigger the area per volume can become smaller and smaller and so on actually.

(Refer Slide Time: 17:55)




So, we have different terms we can have a mass balance of active biomass rate of change of the active biomass that is you may have a D by D time term increment rate of active biomass due to cell growth minus inactivation rate of biomass active biomass. So, some active biomass is becoming inactive and the biomass is growing because of the cell growth this is these 2 in the left hand side we have rate of change of the active biomass let us look at the mass balance for inactive biomass where does it come from it comes from the active biomass a fraction of it becomes inactive; inactive biomass increases as the active biomass becomes inactive.

So, a fraction becomes inactive that is quite simple mass balance of nutrient in the biofilm the rate of change of the nutrient in the biofilm that is accumulation of nutrient diffusion rate of the nutrient into the biofilm. So, some material is coming in substrate is coming into the biofilm due to diffusion and consumption rate of the nutrient because biomass or the bacteria makes use of this nutrient. So, this is plus this is minus that is very obvious. So, that is the rate of change of the nutrient in the biofilm mass balance of the nutrient concentration on the solid substrate. So, this happens because there is a diffusion that is taking place from the solid state in this particular example the nutrient is coming from the solid surface. So, you have to remember that.

So, all these terms come into the picture we have the mass balance of the active biomass part of the active biomass gets converted into inactive biomass. So, this there is no decay of the inactive biomass. So, that remains constant then you have the nutrient that is coming in and due to diffusion from the solid surface then the nutrient gets consumed because of the as bacteria grows. So, the consumption rate we can put in a monod term monod type of rate equation. So, the left hand side will be the accumulation of the nutrient in the biofilm as the function of time then we have the nutrient concentration in the solid substrate rate of change of the nutrient concentration in solid state due to diffusion basically this is happening because of the diffusion at the interface.

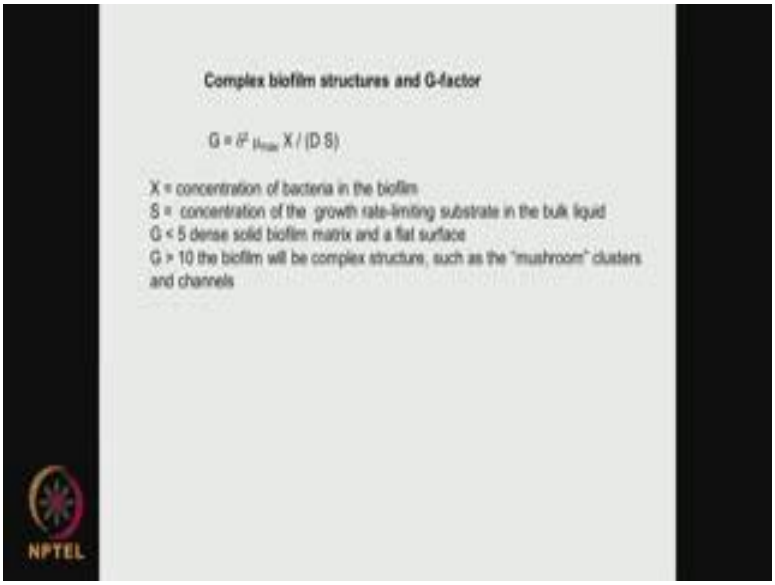
So, all these terms; so, we can have a differential equations it can be a non-linear differential equation because when you talk about monod equation you can have a non-linear monod term here then we can have fraction of the active biomass going into the inactive form and then for the mass balance of nutrient in the solid we can put in something like a fixed law term here. So, all these equations can be put together and they can be solved. So, we can get a biofilm that is the amount of live biomass change as the function of time the amount of dead biomass or inactive biomass as a change function of time the amount of nutrient concentration inside the biofilm as a function of time.

So, it is quite a simple model because we consider it as a homogenous type of situation. So, it can be modeled without much difficulty what is the use of this type of model we can see how the biofilm grows how much nutrient is getting consumed we can see what is the rate of conversion of active biomass into the inactive biomass we can look at the accumulation of the inactive biomass. So, all this can be done. So, there are different approaches by which you can model the biofilm growth biofilm maturation and nutrient

consumption, but I just showed you superficially how one can go about it this is the philosophy of it let us not go too much into the biofilm modeling, but this is how it is.

So, as the biofilm grows you are going to have very complex structures you can have a very uniform flat biofilms or you can have biofilms with lot of interstitials for the nutrients to flow.

(Refer Slide Time: 22:05)




So, we have very complex structures that are possible that depends upon the rate of nutrient diffusion that depends upon the rate of oxygen diffusion and that depends also on the rate of consumption of the nutrients by the bacteria and the rate of growth of the bacteria. So, there is a factor called G factor which is defined like this G factor is equal to delta square mu max x divided by D S delta.

Delta is the thickness of the biofilm mu max is your monod term maximum x is the concentration of the bacteria in the biofilm D is the diffusion coefficient S is the concentration of the substrate that is growth rate limiting substrate in the bulk of the liquid. So, if we calculate this. So, biofilm thickness I know the mu max that is the bacterial growth monod kinetic term I know the concentration of bacteria in the biofilm I know the concentration of the substrate in the liquid side of it I know the diffusion coefficient if G is less than 5. So, G is less than 5 then you will get dense solid biofilm matrix and the flat surface. So, if small g; the g is small; that means, this term is very large.

So, the diffusion is diffusion of the substrate from the bulk on the surface of the biofilm is very fast. So, it is more of reaction limited small g means this term is large, this term is small. So, this term relates to reaction this term relates to the diffusion. So, small g means the diffusion term is much larger than the reaction term; that means, the diffusion is not controlling the reaction is controlling. So, we get very dense solid biofilm now if the G is large; that means, reaction is much larger than the diffusion.

So, reaction is limited by the diffusion of the substrate from the bulk on the surface of the biofilm that is when large G then the biofilm will be complex structures such as mushroom clusters and channels. So, the growth is very fast the substrate is not enough for the bacteria to growth. So, the diffusion is the limiting factor; that means the diffusion of the substrate from the bulk on to the surface of the biofilm then you are going to end up with very complex structures of biofilms with mushrooms clusters channels and so on actually.

Whereas, if the reaction is slow; that means, diffusion is much faster. So, you are going to have very thick flat solid biofilm matrix this is also very useful term to have this factor it tells you which is controlling the biofilm processes is it the diffusion of my substrate the limiting substrate which is helping the bacteria to growth to grow the diffusion of that from the bulk on to the surface of the biofilm that is controlling or is it the rate at which the bacteria grows the biomass increases and the biofilm starts building up which is controlling this or that. So, based on that and we can also tell what type of biofilm structures you are going to have whether you are going to have a very flat biofilm structure or whether you are going to have a mushroom or channel like biofilm structures.

So, that completes what I wanted to talk about on biofilm. We spent quite substantial time on biofilm, because as I said it is the most important topic especially in the area of biomaterial, implants and devices. And many materials fail in early days because of formation of infection. Biofilm development of persister cells which cannot be easily eradicated by simple antibiotics the concentration of antibiotics required may have to go up by 10 times to kill the bacteria that is present and well developed inside the biofilm matrix which contains exopolysaccharides and proteins and so on. So, we will talk about other topics in medical biomaterial in the next class.

Thank you very much for your time.