Drug Delivery Principles and Engineering
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Lecture – 25
Protein Absorption – I

Hello, everyone. Welcome to another lecture for the Drug Delivery Engineering and Principles. So, let us just do a quick recap of what we learned in the last class.

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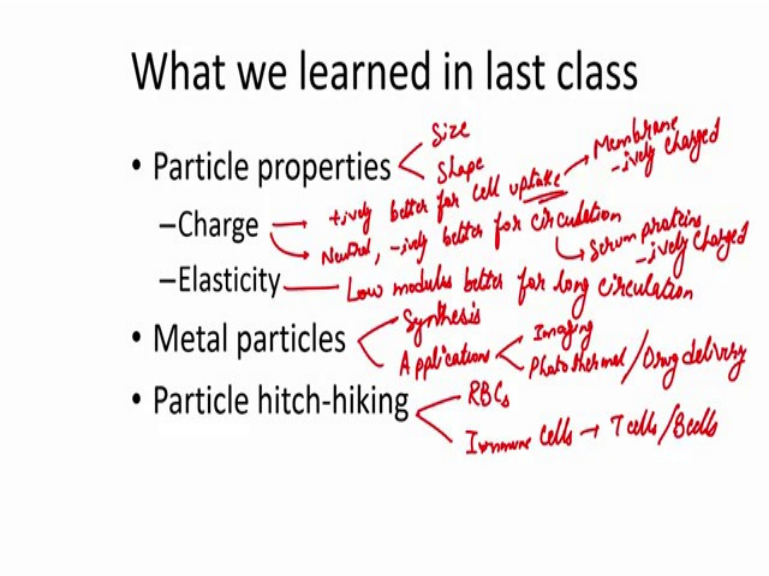

So, in the last class we talked about various particle properties; we had talked about size and shape before this and then we continued the discussion and further talked about charge. So, we said that typically positively charged particles are better for cell uptake, but if we want longer circulation and in-vivo applications, we want neutral or slightly negatively charged particles.

And what are the reasons for this? Of course, that the cell membrane itself is slightly negatively charged. So, there is a electrostatic interaction and that is why it will get closer to the cells for it to uptake it whereas, in circulation you have several serum proteins which are also negatively charged. So, because of that these serum proteins will adsorb onto your particles and will not let them flow longer because they might get recognized by some other cell.

Then we talked about elasticity and in general what we said elasticity that low modulus is good for low modulus circulation. We gave several examples of how to make these low modulus particles somewhat most of them trying to mimic RBC and then the reason we say that these low modulus is good is because spleen will clear any rigid particles if they are big enough. If they are above 200, 250 nanometer then spleen will be able to just clear these up. But, if they are they have low modulus they can squeeze through those spleen gaps and vessels and then they can continue to circulate longer.

Then we talked about metal particles. In this case we talked about both synthesis and applications. So, applications we are mainly talking about imaging or contrast agent we also talked about photo thermal therapy and then you can even conjugate the drugs on the surface. So, essentially drug delivery can also be done and then finally, we talked another sort of sidetrack about how you can use particles both particle hitch hiking. So, you can conjugate it to RBC's or you can conjugate it to other immune cells like T-cells and B-cells and because of that the body will not be able to recognize them and they can circulate or reach wherever these particular cells are growing.

So, this sort of concludes our major particle discussion for this course. We are going to come back to it in future classes and for different type of applications, but this was just a basic concept I wanted to give you on particles. Now, we are moving into tissue engineering and learn how various aspects of these polymers and these drugs can be used for better drug delivery for good tissue engineering and both of these things go hand and hand in - all tissues in application requires some sort of drug to be given for better efficacy and better retention of the tissue, better healing of the tissue.

So, let us talk about some concepts of tissue engineering, but before we do that we are going to talk about protein adsorption which is again an integral part of tissue engineering. So, that is what this class is going to be about. This is going to be about protein adsorption.

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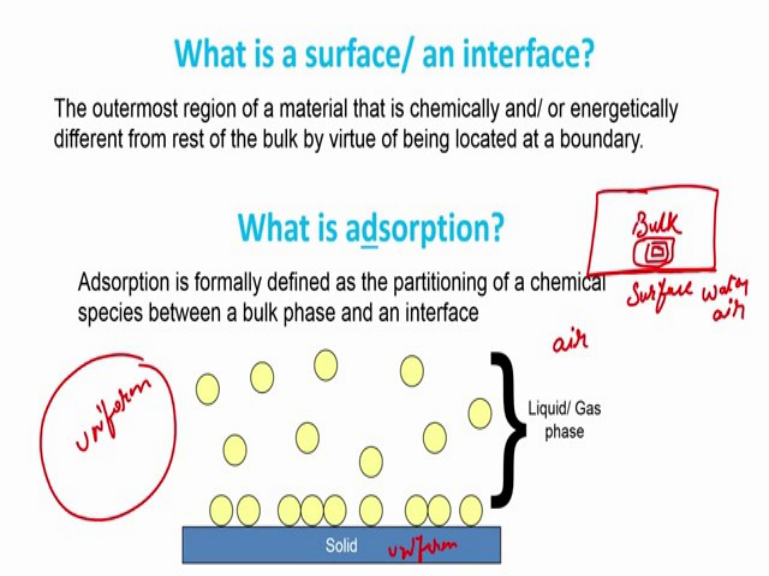

So, before we talk about adsorption let us talk about what is the surface and what is an interface? So, any outermost region of a material so, if let us say if I have this material. So, any material will have a bulk region and a surface region. So, when I say surface, the surface is a region which is going to be slightly different from the bulk because it is going to be exposed to different environment than the bulk- like if I take a bulk volume here, all sides of this bulk volume is exposed to a very same environment. But, as you get closer and closer to the surface, that is not true.

So, that is how you can distinguish between a surface and a bulk that the outermost region of the material will be chemically or energetically different from the rest of the bulk just because it is at the boundary. So, the interface, this could be water outside, this could be air outside or this could be some other medium outside but, the surface will be slightly different from the bulk.

Then what is the adsorption? So, adsorption is nothing, but it is formally defined as a partitioning of a chemical species between a bulk phase and an interface. So, let us say now I have this solid and let us say some air or some liquid here. So, how does this air or this liquid or any molecule in that air or that liquid partitions between the air and the solid at this interface?

So, for most purposes let us say we are talking about a very thin layer here. So, air in the surrounding area will be fairly uniform. The solid below the surface will be fairly

uniform, but right at the interface the air may like to sit or attached to the solid interface and that will create some sort of a difference in the gradient of the air it could be higher or it would be lower than the outside, but this will change and so, this physical adsorption or this physical partitioning of a chemical species between the bulk and the interface is termed as adsorption.

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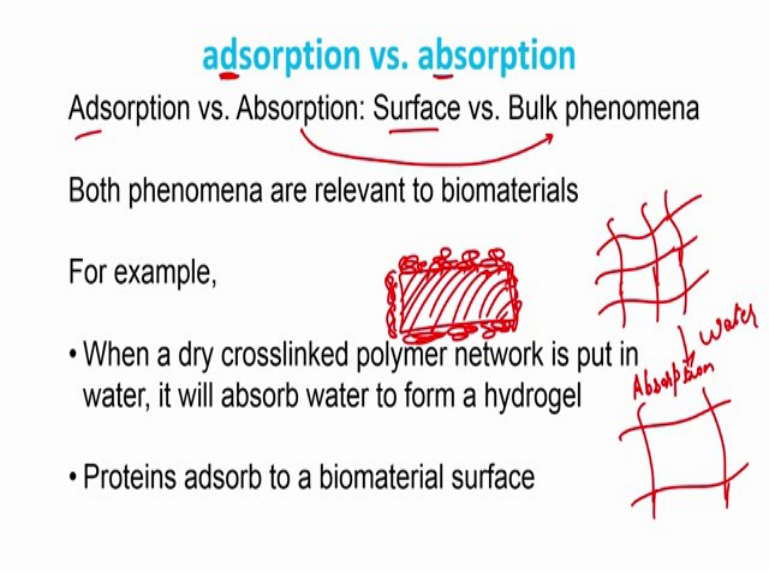

So, it should not be confused with absorptions remember the only difference is this one word here you have d and here the absorption is b. So, absorption is a bulk phenomenon. So, when I say adsorption that is surface whereas, absorption is a bulk phenomena. And both these phenomena are actually relevant to biomaterials and both of these will be widely used as we go along and so, let us quickly give an example.

So, let us say when I have a dry crosslinked polymer network. So, let us say a hydrogel for example; so, I told you right that there is a hydrogel which will have a certain network and where you have a very high tendency to absorb water. So, what that essentially means is the water is going to go throughout this network and will cause swelling of this network. So, this is absorption of water.

Whereas, the other example is proteins will adsorb to the biomaterial surface. Let us say if I have a material which is solid in which the water cannot pass through and the outside water has proteins, the proteins will tend to aggregate on the surface and we will come to

the reason as to why they do that, but this is the property of only the surface. So, this is why it is called adsorption.

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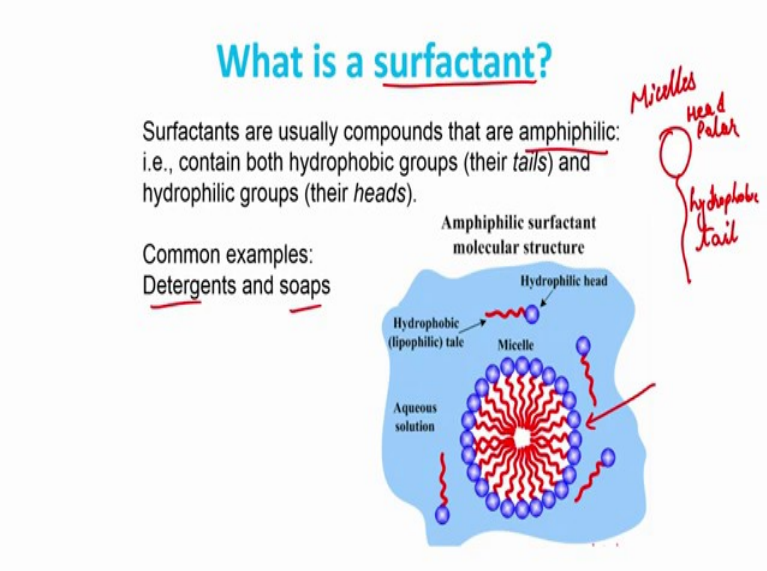

So, let us talk about a surfactant as well which is going to be important in this discussion. So, surfactants are usually compounds that are amphiphilic we basically talked about surfactants and we talked about micelles; essentially nothing, but they have a polar head group and a hydrophobic tail.

Some of the common examples of surfactants are detergents and soaps and what they are is essentially hydrophobic tails with hydrophilic head groups as is also shown here and they have a certain solubility in a aqueous solvent, but if you increase the amount that is present in, let us say, water, eventually they will start to precipitate out and form these micelle structure. So, that is why; that is why these soaps and detergents are very good in terms of cleaning out the dirt from your clothes. Let us say if you have a cloth and which has let us say some dirt which the dirt can be both hydrophobic or hydrophilic.

So, if you only use let us say water or molecules which are hydrophilic, it will only be able to dissolve any sort of drug contaminant that is hydrophilic, but the hydrophobic sort of impurities or the dust will still remain stuck to your clothes. But, if you have a detergent which is both hydrophilic and hydrophobic, then it will go and solubilize both parts of the dirt and that is why they clean the clothes lot better than the individual

components. So, essentially that is a surfactant that you use in your washing machines in at the time of washing your clothes and all.

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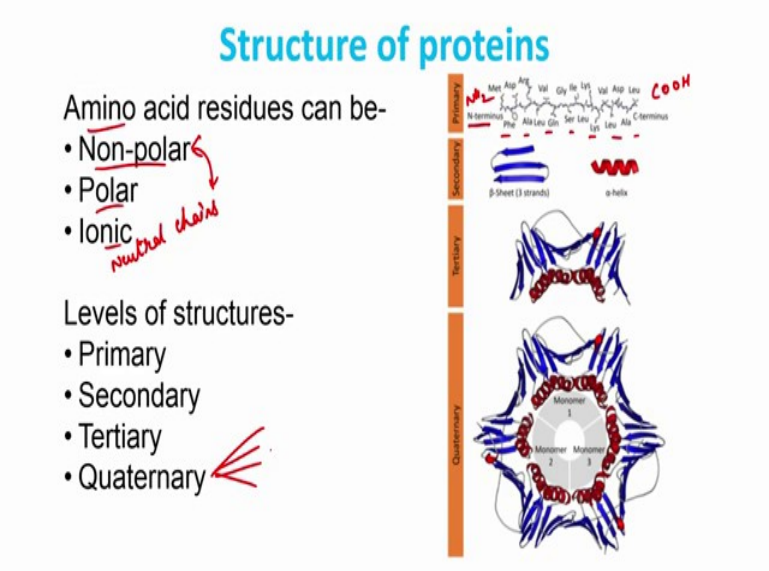

So, now we have that concept clear. Let us talk about proteins itself, since we are going to talk about protein adsorption predominantly. So, proteins again composed of amino acids they are nothing, but poly amino acids. So, as you can see here, several amino acids are being conjugated to each other. They have an N-terminus which is basically means the final protein which will have a primary amine at the end and a C-terminus which is the final protein which will have a -COOH at the end. And so, this is a primary structure which is basically all opened up.

You can have a secondary structure which means that these proteins or these amino acids will self align into some sort of a complex structure which may not be linear, it could be beta sheets, it could be alpha helix and these can then further self align into tertiary and quaternary structure which becomes more and more complex.

So, we know that all amino acids are non polar,polar and anionic. There are all kinds of amino acids as well as they are neutral chains as well and so, all of these properties are present in proteins. So, the proteins have nonpolar areas, they have polar areas, they have ionic and their neutral chains. So, a non-polar is essentially nothing, but somewhat related here, and their structures can also be primary, secondary, tertiary and quaternary.

And again all of these can have multiple configurations depending on the environment the protein is the quaternary structure can have infinite combinations of structure. So, all of this adds to the complexity of these proteins.

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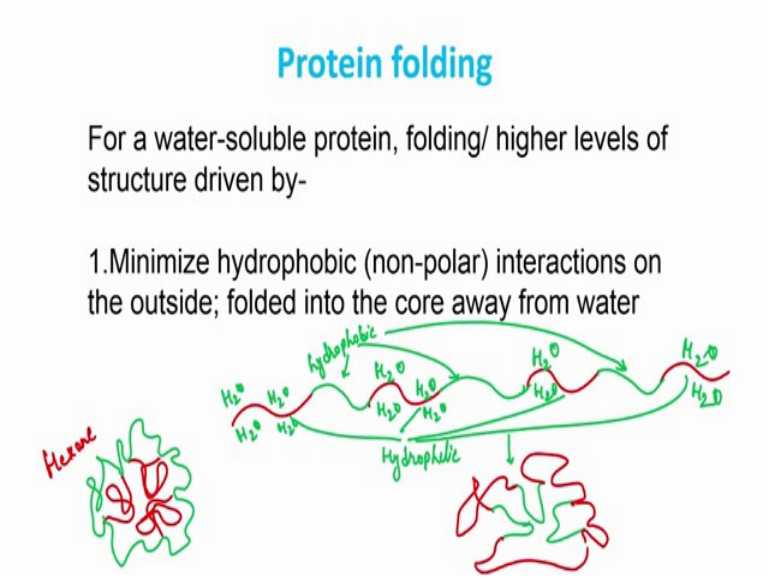

So, when we say it will talk about protein folding we are saying that for the water soluble protein, the folding is essentially driven by what is the medium in the surrounding. So, if it is water, it wants to minimize the hydrophobic interaction with the water. So, what will happen is let us say I have this protein. A long protein, in this case I am drawing a single chain, spreading it out. Let us say this is my protein where this is the hydrophobic domain and then these are all hydrophilic domains. So, this is hydrophobic, this is hydrophobic, this is hydrophobic and then all of these are hydrophilic.

So, now if I put this protein in water what will happen is the water will love to interact with the hydrophilic domain. So, it will go and start interacting with it will start accumulating near and the hydrophilic domain, but then this green domain will not want to interact with the water at all right because this is hydrophobic. So, it does not really like water. So, what will happen is then this green body will start to self assemble.

So, eventually what will happen is you will have the structure where all the green domains will tend to interact with each other because they do not really have anything else to interact to, whereas, all the red domains which are hydrophilic domains will tend to be away from the green domains as well as start to interact with water. So, I mean this

is one of the very simplest cases of protein folding that I have just described here. The protein folding is much much more complex because as I said they have all kinds of non-polar, polar, charged all kinds of moieties and hydrogen bonding being present.

So, all of this will play a role and will result in a structure which is very complex it is typically a quaternary structure for any large protein and so, that is what defines the protein folding. Of course, this is the protein folding in water, but if you change the environment in the surrounding then the structure will change right. I mean if the same folding was to happen in let us say an organic solvent. Let us say hexane- now hexane wants to interact with these hydrophobic domains, but does not want to interact with the hydrophilic domains.

So, what will essentially happen is again all the red regions will collapse inside and then the green regions will be on the outside basically making sure that they are shielding all the red domains which are hydrophilic and not liking hexane. So, now you can see that the structure is completely changed. So, depending on the environment in which the protein folding is going to happen you will see these effects where the protein structure will change. So, it is fairly dynamic and it is actually very very sensitive to even just small perturbation to the local environment.

You can change the amount of salt in the liquid and that will change the protein structure. You can change the location of the protein from one part of the body to the other and that will slightly change the protein structures all of this is very sensitive to its environment.

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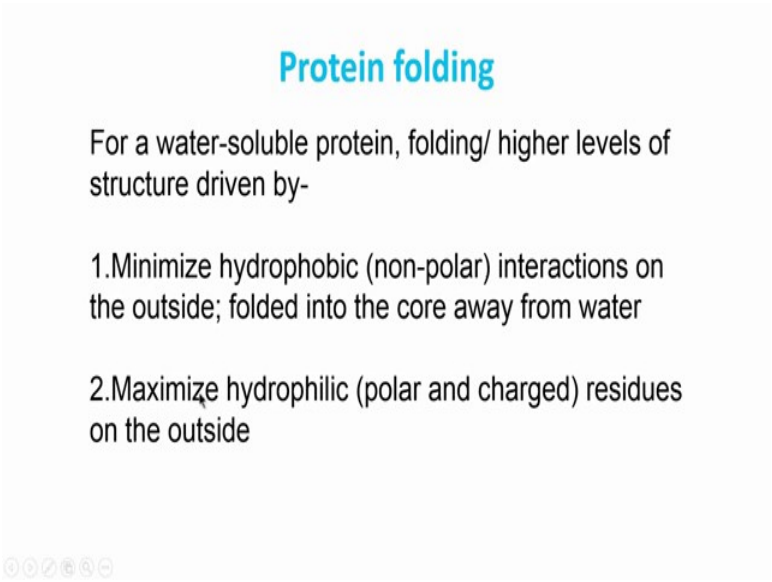

So, as I said they are trying to minimize the hydrophobic interactions. Hydrophobic domains folded into a core away from the water and they want to maximize the hydrophilic interactions - all the polar and charged residues are on the outside.

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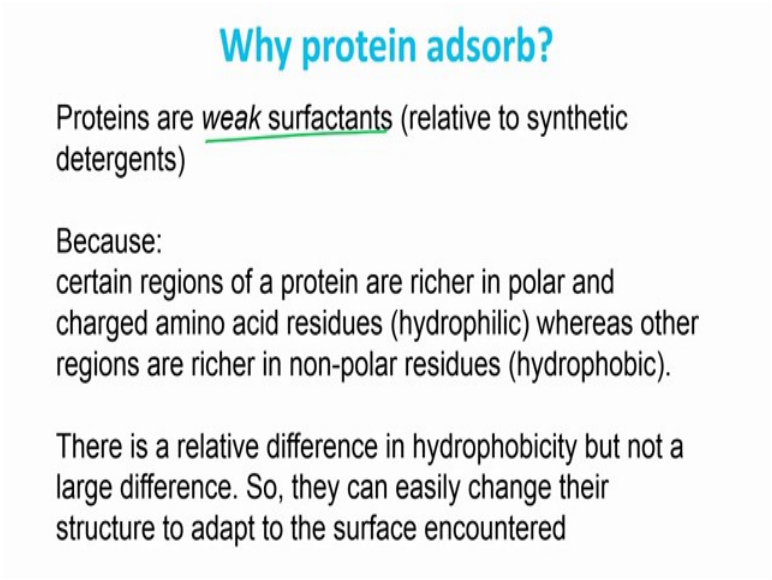

So, because of this now what we are essentially saying is that proteins are both containing a hydrophilic and hydrophobic domain and hence they are weak surfactants. They do not have a strong hydrophilic and hydrophobic domains, but they have a small small hydrophobicity and hydrophilicity in their individual amino acids and so, they are

weak surfactants and again because of this there is a relative difference in the hydrophobicity, but not a very large difference. So, they can then easily change the structure and adapt to whatever surface or whatever environment they are put in.

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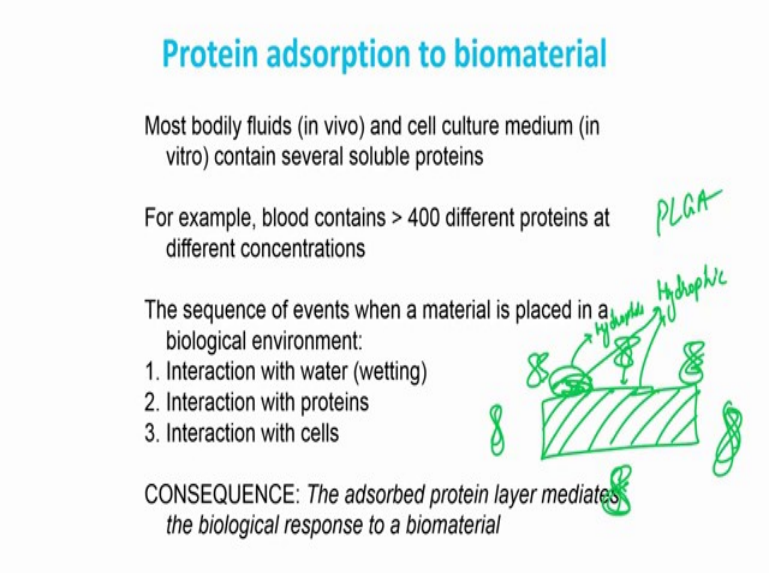

So, hence most bodily fluids which contain several proteins will result in protein adsorption onto any foreign substance that the body sees. So, we have several types of protein. For example, a blood contains almost about 400 different proteins at different concentrations that is flowing through our blood circulation. And so, what will happen is if I let us say put an implant for example- a pen in my body and then what will happen is let us say this is an implant. For the purpose of this particular slide let us say that this implant is non-porous, nothing can penetrate in - it is a solid pen. So, what will happen is we will first interact with water. So, water is everywhere. So, water will come in contact with this implant. Now, this water which in this case is serum contains several proteins which are folded into a certain structure.

So, let us say this implant is hydrophobic maybe let us say a PLGA implant then what will happen is when this protein comes in contact comes in contact with the surface these outer domains these are hydrophilic right. So, they want to stay in the water, they do not really want to interact with the surface and neither does the surface want to interact with them. So, what will happen is the protein will open up and refold such that the hydrophobic domains are more than contact with the surface. So, this protein is going to

reopen such that all these regions are hydrophilic and all the regions directly in contact are hydrophobic.

So, all of this and because of these hydrophobic-hydrophobic interactions there is a strong bond that is formed or the number of small weak bonds, but there is so many of them that the whole interaction is very strong, so, this protein absorbs very strongly on do these surfaces. And once these proteins adsorb, then the cells which are in much lower quantity than the proteins will come and start sensing the surface and most of these cells well actually also start to see these proteins that are absorbed. So, most of the time the mediation of the cell attachment to the implant is being done through these proteins that are adsorbing through either serum or some other body fluid wherever the implant is put in.

So, the consequence is the adsorbed protein layer mediates the biological response. So, if I say that the cell is the main unit that is governing whatever response we are going to get then the proteins that are adsorbing onto it determines how the cells are going to come and attach to it, what sort of signals the cells will get and hence essentially define what sort of biological response the body will give to a certain biomaterial.

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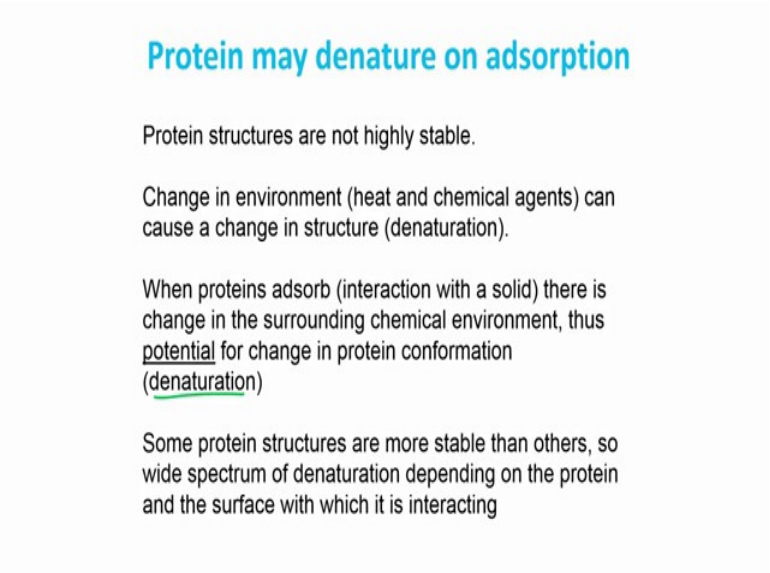

And as I said the proteins may denature on adsorption. So, the protein structure is not very stable. So, once the environment is change the heating, the chemical agent and they will all cause denaturation of the structure. So, when protein adsorbs, the interaction with

the solid there is a change in the surrounding chemical environment and this potential change causes the protein confirmation change as well which you can call denaturation which essentially means just change from the original structure. This does not necessarily mean that they are going to completely open up. They just means that whatever their natural state was, that got denatured.

So, they may take up either a completely open chain or they may have some other conformation that is not typically found in the nature. And again there are different types of proteins depending on the composition of the amino acids some structures are more stable than the others. So, the magnitude of response that you are going to get on a material for different proteins is also going to be different.

So, some proteins are very liable to denaturation. They will completely open up everything in the structure, while some proteins are not really that liable in terms of changing their structure. So, they may still maintain their activity, they may still maintain the natural structure that was present originally.

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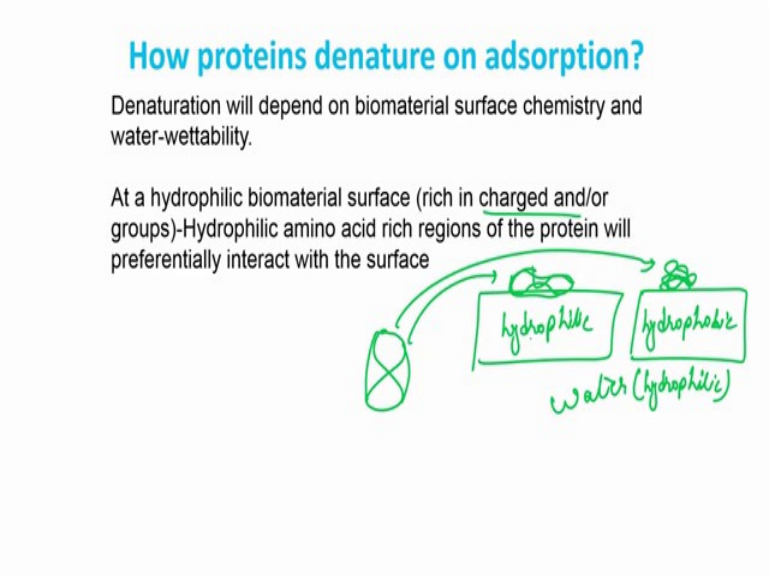

So, how proteins denature on adsorption? We already covered a bit of it, but the denaturation will depend on biomaterial surface chemistry and water wettability. So, how much is hydrophilic; how much is hydrophobic; what sort of surface chemistry is there; what bonds can form between the proteins and the surface all that we will determine it. So, typically at hydrophilic biomaterial surface, which is rich in charged groups or

charged amino acids, these hydrophilic amino acid rich regions of proteins will preferentially interact with the surface.

So, if I have let us say two surfaces - one is hydrophilic and another is a hydrophobic and of course, I am doing this in a water environment which is hydrophilic and I have a protein structure which is let us say like this. Then in when it comes in contact with the hydrophilic surface, the protein structure may change a bit, maybe it is going to become slightly elongated, but more or less the structure is going to be similar. Whereas, when it comes in contact with the hydrophobic domain, it is going to become completely inside out.

So, the structure will change quite a lot more compared to a hydrophilic surface just because originally the protein was in a water environment which is fairly hydrophilic. So, there is not much of a drastic change that is happening. However, this hydrophilic surface can have a lot of functional groups that are reactive and that can further cause changes. So, there is all magnitude and various degree of response that we will get.

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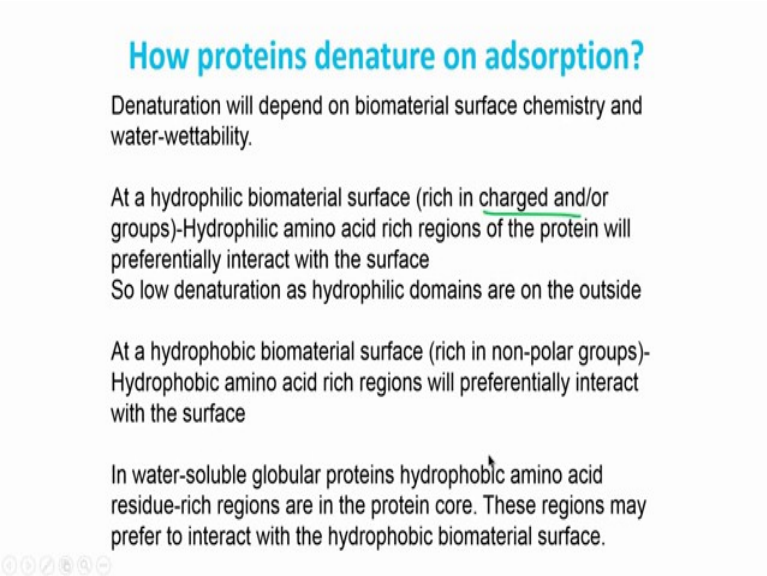

So, typically there is a low denaturation as there are already hydrophilic domains present outside when you are talking about a hydrophilic surface. That is why typically when you talk about tissue engineering or talk about implants. The major emphasis is to make the surface fairly hydrophilic so that you do not start denaturing lots of proteins that may cause some toxicity.

At the hydrophobic biomaterial as we talked about which is rich in non-polar groups, the hydrophobic amino acids will tend to preferentially interact with the surface. So, these hydrophobic domains were initially buried inside the protein structure. So, they will have to then come out and that is going to cause a lot more change to the protein structure than let us say a hydrophilic surface.

So, in water-soluble globular proteins hydrophobic amino acids are in the protein core. Thus, these will try to interact with the hydrophobic surface and change the structure quite a bit.

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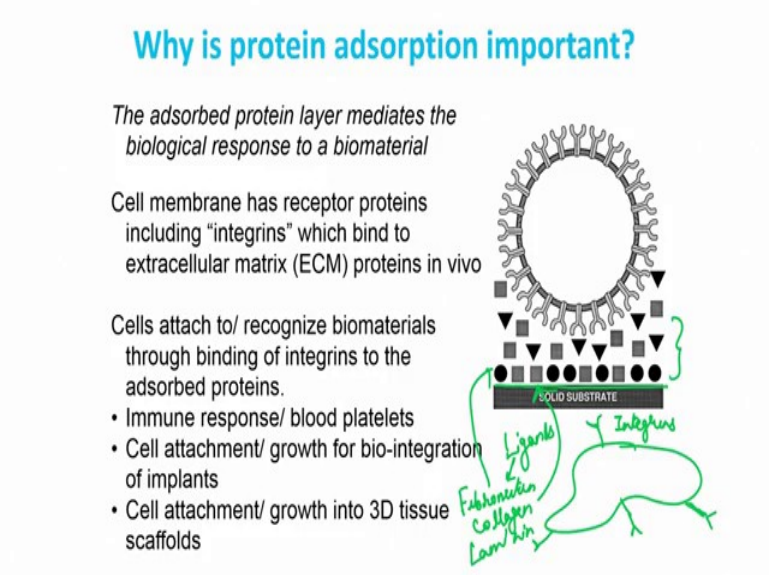

So, again why is all this important? So, we again briefly already we have talked about this, but if you have a solid substrate, the first thing that is going to interact is the proteins and they will adsorb on the surface and then when the cell comes it will actually not be able to see the solid substrate surface right. This surface is all hidden by this layer of protein.

So, the cells will only be able to interact with whatever is present on the surface which is in this case protein that is adsorbed and that is going to lead to any biological response it is going to happen. Of course, the protein that is absorbing is dependent on the surface itself. So, you can argue that you can sort of control it and anyways, but it is still becomes very important to study the protein adsorption.

So, cell membrane has receptor proteins, including integrins which bind to several of these proteins that are found in the serum and that is how they will bind to the surface, that is how they will attach to it, that is how they will start functioning on that surface and so, the cells that attach will recognize this biomaterial through these integrin molecules.

So, in this case what we are talking about is these cells. When they attach to the surface they have a special class of molecules which are called integrins and most attachment and spreading of these cells on these surfaces will happen when these integrins bind to their receptors or their ligands. So, these could be proteins like fibronectin, collagen, laminin and several others.

So, when these proteins get adsorbed onto the surface only then the cells can go and bind to the surface before that the cells will not be able to attach to those surfaces and actually grow. So, as I said any kind of immune response will also be driven by these protein that are adsorbed. So, everything is sort of controlled by the protein adsorption.

We will stop here and we will continue rest in the next class.

Thank you.