Introduction to Biomaterials

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Module No. # 01

Lecture No. # 11

Structures and properties of Protein; cell – material interaction

So in the last lecture I was describing this protein structure which is important to understand

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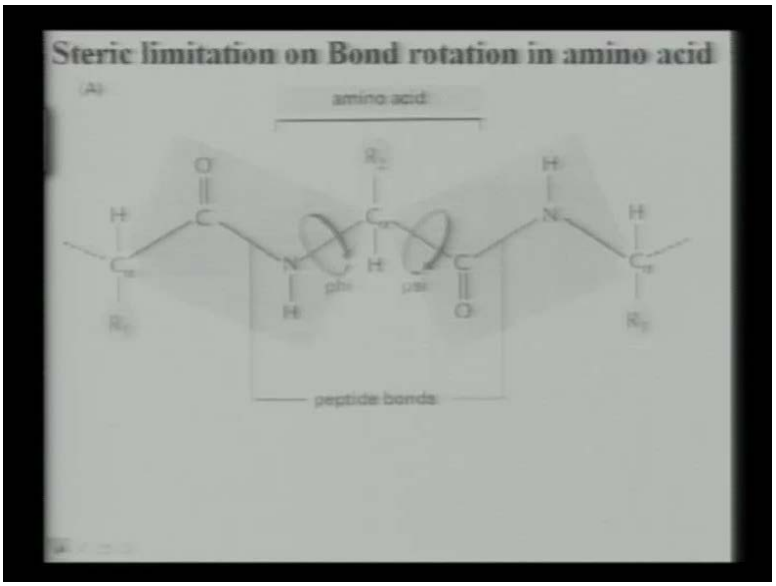

the molecular biology of cells structure as well as cell material interaction because protein adhesion first takes place on the material surface so one of the important thing is that that I have mentioned the protein molecules they have that peptide bonds that they can be rotated but, there is a limitation in the rotation of this peptide bonds.

So essentially this pi angle and psi angle so essentially this pi angle and then psi angle can vary within certain limits

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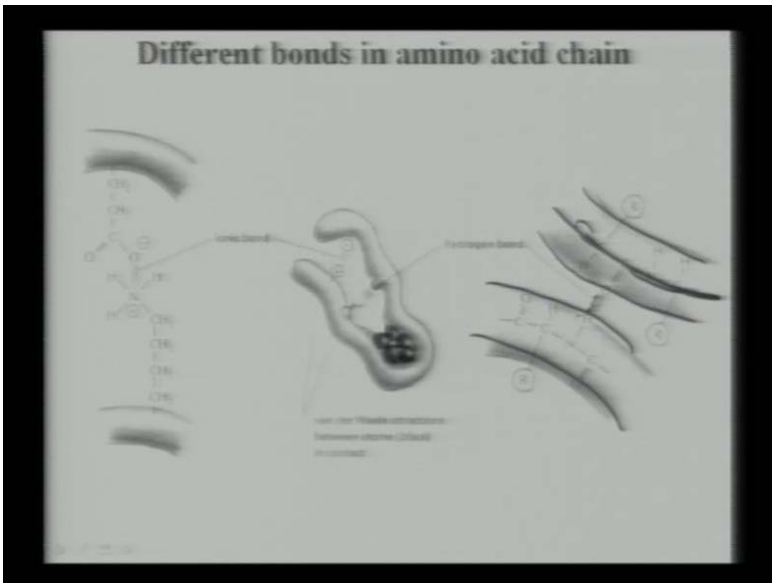

and other things is important in this proteins structure like you have this peptide backbone chain polypeptide backbone chain and then in this 2 proteins between this 2 protein molecules there is also a weak hydrogen bonds is there and because of this weak hydrogen bonds what will happen that this hydrogen bonds can be broken and can be reformed very quickly because of the much less energy involved in this hydrogen bond right.

So this is important because that can explain that 5 protein molecules can have very irregular type of shape I mean it can be squeeze together because there is a weak hydrogen bond or weak interaction between the 2 protein molecules other thing you can see that yellow things here yellow bonds these are like ionic bonds and these ionic bonds are taken place between the positive and negative ends.

Now positive and negative ends are the nitrogen end and the carbon end that I have explained earlier in this lecture

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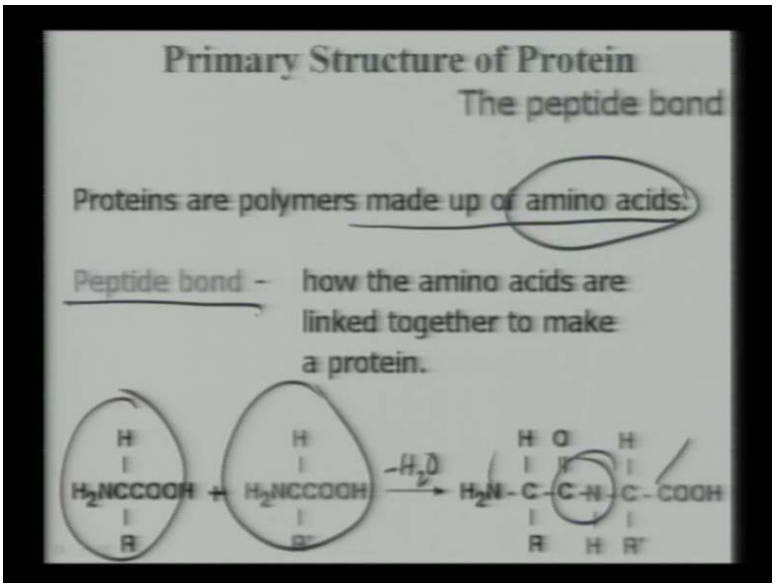

so essentially if we if I can summarize this protein structure proteins are polymers which are made up of amino acids so essentially proteins is a polymeric macromolecular structures proteins are polymeric macromolecular structures and it is made up of amino acids amino acid is the mar unit polymer means number of mar units with joint together to form a macromolecular structure and this amino acids when they join together then they release 1 molecule of hydrogen hydrogen that is the water molecules and this once is water molecule is released so then they make this peptide bond here carbon and nitrogen and essentially you have the carbon end and you have the nitrogen end peptide bond is formed when the 2 amino acids are link together.

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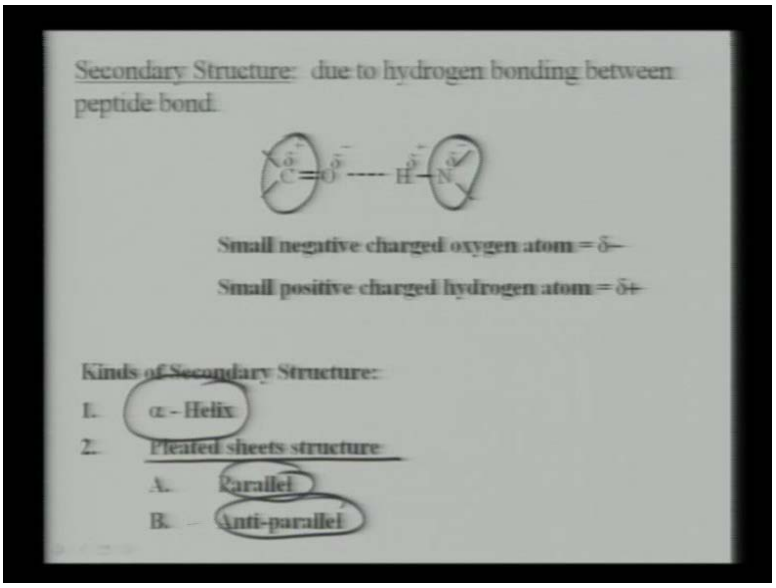

And the secondary structure of the protein can be described as follows you have a nitrogen end and you have a carbon end so carbon end is mildly positively charged and nitrogen end is you can mildly negatively charged and then kind of secondary structure in the protein that is observed like alpha helix structure and also plated sheet type of structuralized parallel and anti parallel structure

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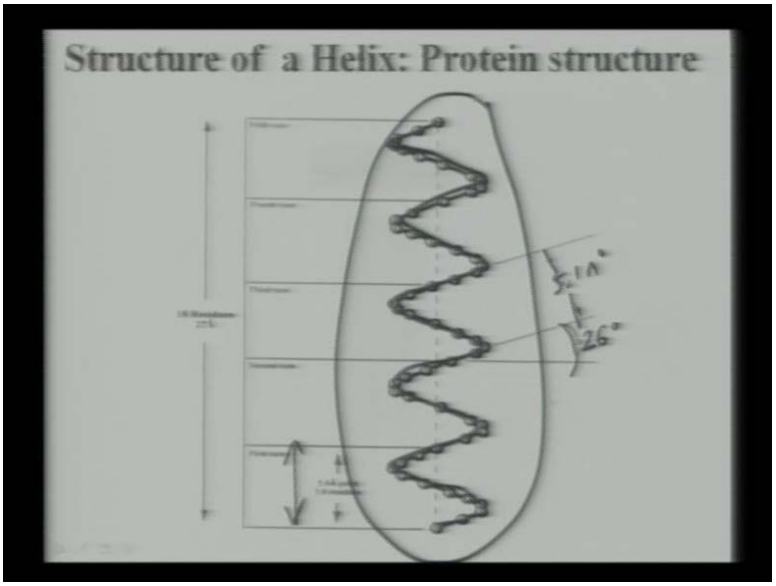

and this is like a typical helix pattern that helical pattern the protein structure what you can see that this as a 5 typical turns we explains that typical helical structure of the protein.

Now what is important in these helical structure is that each turn is roughly 5.4 angstroms and 5.4 angstroms multiplied by 5 means going to seven angstrom and this angle the turn that you know angle between the 2 conjugative turns it is around 26 degrees so it is a very well made and it is a very kind of well organic kind of a structure and then this distance is kind of 5.1 angstrom.

So you have this 27 angstrom that is the total length of the protein helical structure and this 5.4 angstrom is the length of the each turn and 26 degree is the angle between the 2 conjugative turns so this structure or the protein molecular structure it is important and this kind of basic fundamentals all the students must have so that they can understand that how the protein molecules can be squeeze or protein molecules can be transported from one cell to another cell.

How the protein molecules can be transported from within the cell to the extracellular matrix all those things can be possible the one thing that that I must emphasize here the protein molecules since it is a polymeric macromolecules and it it is composed of carbon hydrogen oxygen nitrogen very light weight the other thing is that between the 2 protein molecules you have the hydrogen bond so this protein this if require this hydrogen bonds can be broken so the 2 protein molecules can be dissociated from each other and if require these 2 protein molecules can come together and make an assembly.

So all these things are possible because of the characteristics structures and the bonds associated with the protein molecules so I repeat here that this is a 2 amino acids which are link together release on water molecules then they form this kind of peptide structure and there is a hydrogen bonds which are present between the 2 protein molecules so these hydrogen bonds can be broken therefore, 2 protein molecules can be separated and they can be transported from the cell to the outside extracellular matrix.

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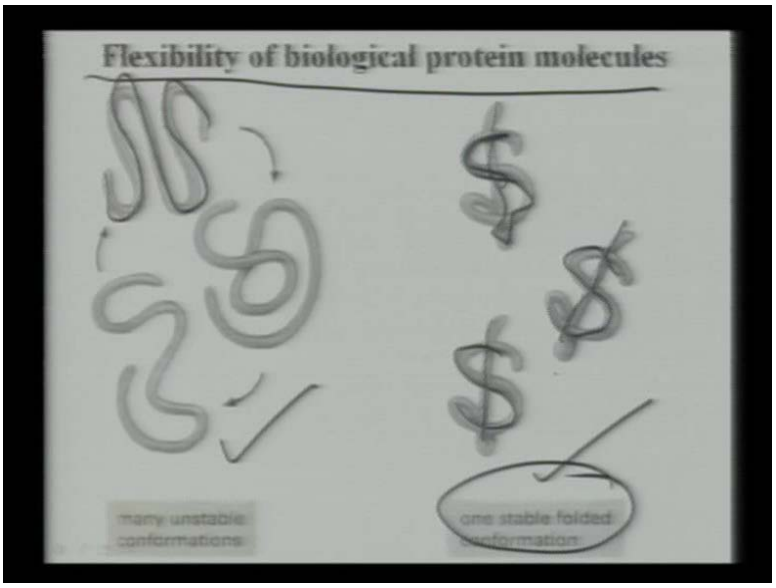

And because of the characteristic bonding structure that you can see that there is a flexibility larger flexibility of the biological protein molecules can you see that it has a typical coil like structures like it can be squeezed and you can see that it has typical very irregular structure and this has a many unstable confirmations that is one thing and that is one stable folded confirmation stable folded confirmation means like how the protein molecules exist in nature it is like a stability means it is biologically stable structure it is like a thermodynamically feasible reactions so similarly, biological stable structure is what when proteins are in the extremely coil form it is not like a simple chain like forms.

So that comes from the natural characteristics natural characteristics of the protein molecule the way this protein molecules are made of like helical pattern and because of the helical pattern protein molecules can be can exist in the coil form and so on.

So now you know that how the protein molecules they look like what is the helical structure of the protein molecule what is the typical dimension of the each strand what is the angle between the 2 conjugative strands and how and why the protein molecules are extremely flexible as a biological molecule right.

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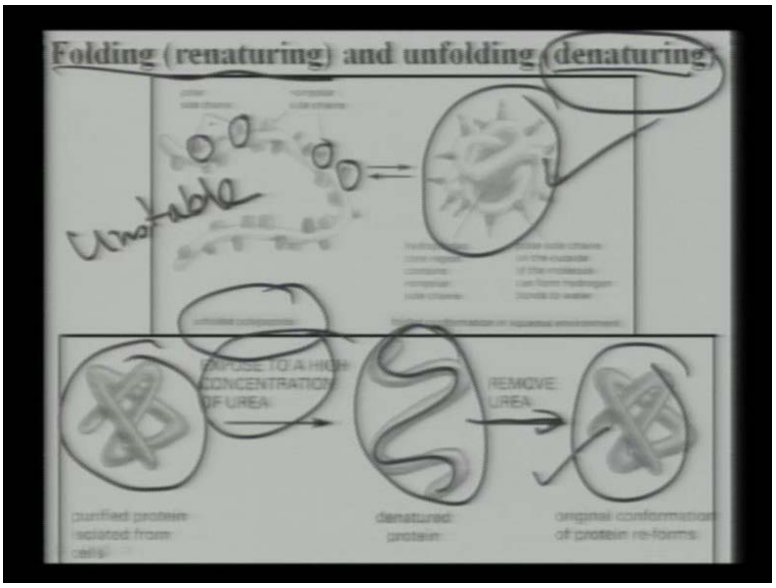

This is like folding renaturing and unfolding denaturing unfolding means like when protein molecules becomes straighten like if it is not in the folded conditions then so then it is called proteins are in the denatured conditions denatured means like in it is losing its natural characteristics of the from the structure point of view renaturing means it is like folding and this folding is more like a stable confirmation if you go back to the earlier slide i've mentioned here that this is the stable confirmation from the biological structure point of view and if you go to this one you can see that this is a more stable folded configuration and this is a unfolded polypeptide so unfolded polypeptide it certainly unstable unstable protein configuration unstable means it is like a protein molecules in the denatured condition.

Denatured means this protein molecules cannot perform certain biological functions which it is supposed to do and because it is not in the natural characteristic shape and size natural characteristic confirmation is that this is like a extremely folded conditions and this is a renatured conditions like here you can see that purified protein isolated from cells from the cells you can extract the protein molecules because cells has a as I mentioned in the last lecture cells are (( )) run ten to the power nine right ten to the nine ten to the twelve like number of protein molecules in each set.

And if you expose the high concentration of urea then it becomes a denature protein denature protein means it is not anymore in the folded configuration it is in the unfolded

configuration and if you remove urea and then it becomes in the more coil formation and then it is more a renature configuration whenever it is a coil configuration or whenever the protein molecules is in like you know extremely squeezed conditions then it is a more natural and stable confirmations natural and stable confirmation means these are like biologically stable conditions this protein molecules come exists.

The other thing in the last lecture I've mentioned that this is the polar group and this is the polar site chain so this is the blue ones now you remember in the original protein molecule composition I have mentioned that it can have r 1 r 2 r 3 three different kind of site chains and these r 1 r 2 r 3 can contain either benzene or can be methyl or ethyl like simple hydrocarbon type of site chain

So different color essentially indicates the different type of site chains with different compositions

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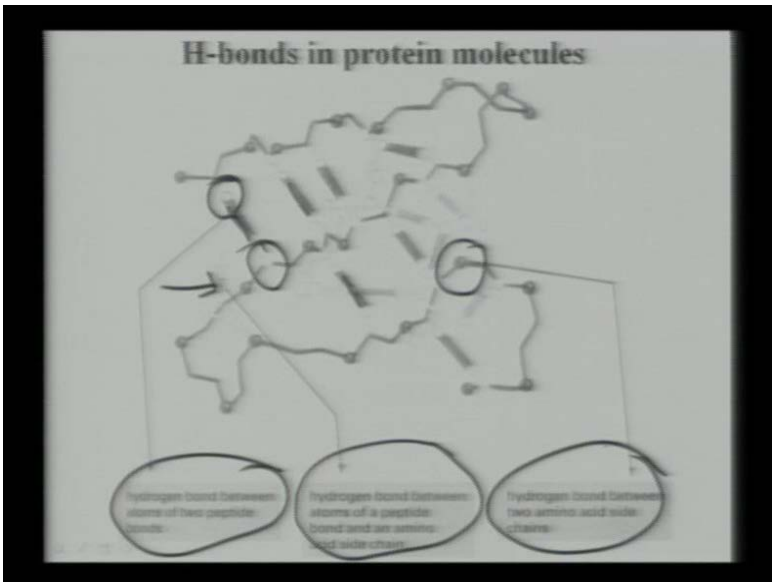

now hydrogen bonds in protein molecules what you can see here this red ones are the hydrogen bonds red ones whereas, this yellow ones also the hydrogen bonds so this red ones hydrogen bonds between atoms of the 2 peptide chain like this is one atoms from one peptide chains this is another atoms from a neighboring peptide chain.

So between the 2 atoms there is a hydrogen bond I am emphasizing on the concept of hydrogen bonds because hydrogen bonds are known to be extremely weak bonds right so

it can be broken very easily and this is the hydrogen bonds yellow hydrogen bonds that is the hydrogen bonds between atoms of a peptide chain and amino acid site chain.

So there are 2 different types of hydrogen bonds that is possible and third hydrogen bond is like hydrogen bond between 2 amino acid site chain so either the atoms from the 2 protein molecules they can form hydrogen bond or 2 site chains can form hydrogen bond or hydrogen bonds can form also between atoms of the peptide chains and peptide bond and an amino acid site chain.

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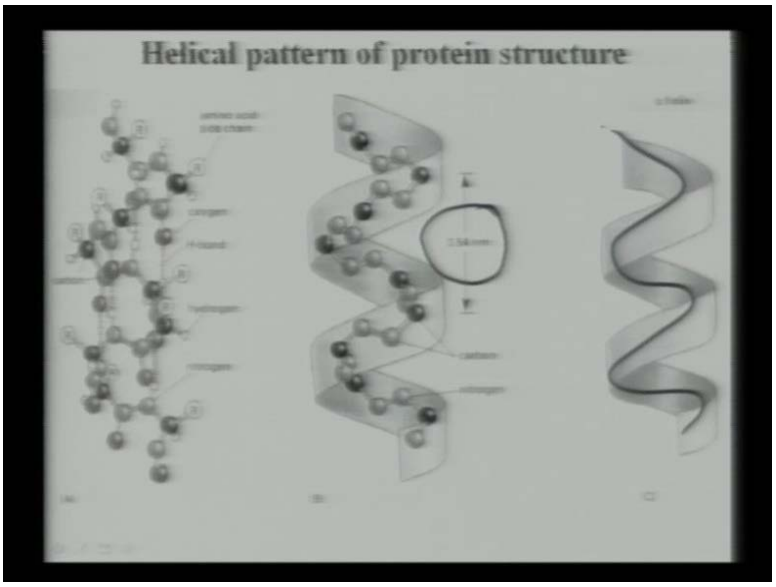

Now this is again I am coming back to this helical pattern like how this protein structure they look like in the biologically stable confirmation and biologically stable confirmation means it has a each turn has a length of 5.4 angstrom 5.4 angstrom means 0.54 nanometer so that means if it has a 5 strands so it total is that what 2.7 nanometer 0.54 multiplied by 5 is 2.4 nanometers so that is the entire length of a protein molecule so 2.7 nanometer is an extremely small molecule right

and this is alpha helix means that is like simple helical pattern the way this proteins structure they appear

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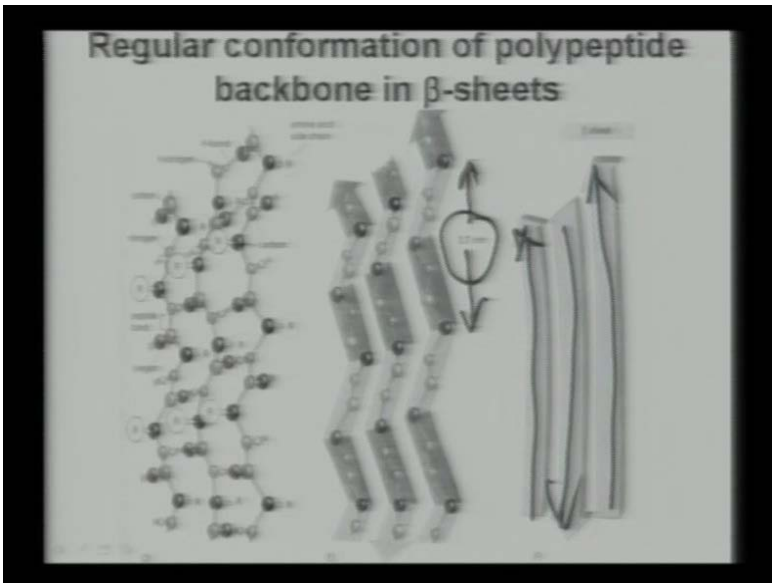

now it can have a beta sheets also that is the regular confirmation of polypeptide backbone in beta sheets now again here this linear length or linear dimension of each turn is like 0.7 nanometer and this point seven nanometer again it has a 5 turns so altogether 0.7 multiplied by 5 is 3.5 nanometers however in both the cases it is even less than 5 nanometer so it is extremely small size that each protein molecules they have.

So this kind of dimensions are the students should have a feeling of the number about the size or shape of the biological molecules so that they can correlate with the real time phenomenon the beta sheets essentially you have a parallel sheets and this parallel sheets they are oriented in certain specific orientation and therefore,

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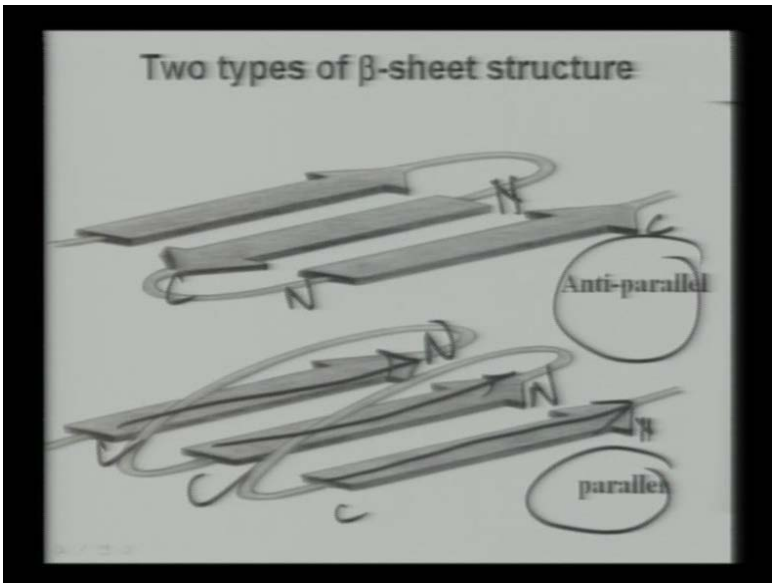

it can have a anti parallel beta sheet structure or it can have parallel beta sheet structure anti parallel means in parallel case both the protein molecules they are carbon and nitrogen ends they are oriented exactly at the same way in the anti parallel configurations means that carbon and nitrogen bonds they in the 2 neighboring protein molecules they just reverse to each other do you understand what I am saying.

So in the one case you have a nitrogen here carbon here and in the next chain it can have a nitrogen here and it can have carbon here the next chain again it is reverse but, in the parallel chain nitrogen and carbon and nitrogen and carbon at they are arranged in the same orientation right so that is what is meant by beta sheets

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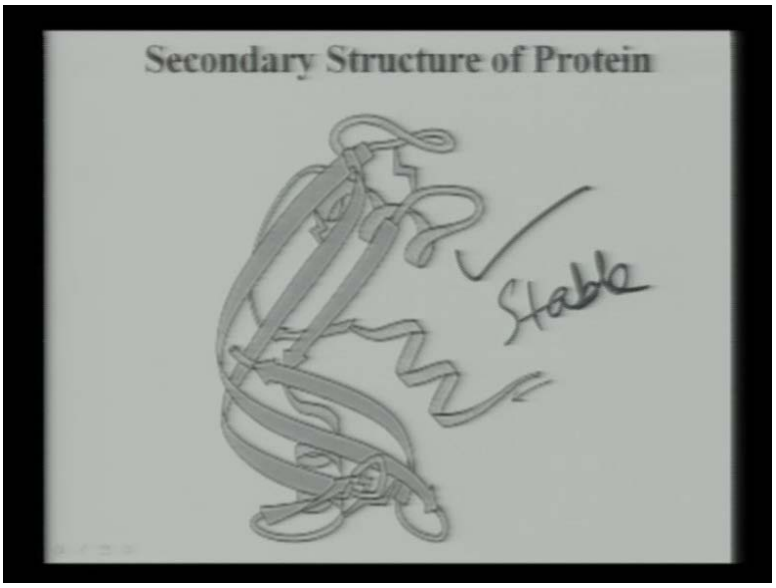

this is again a very biological stable configuration so because it is in the coil form or it is not in a unfolded form

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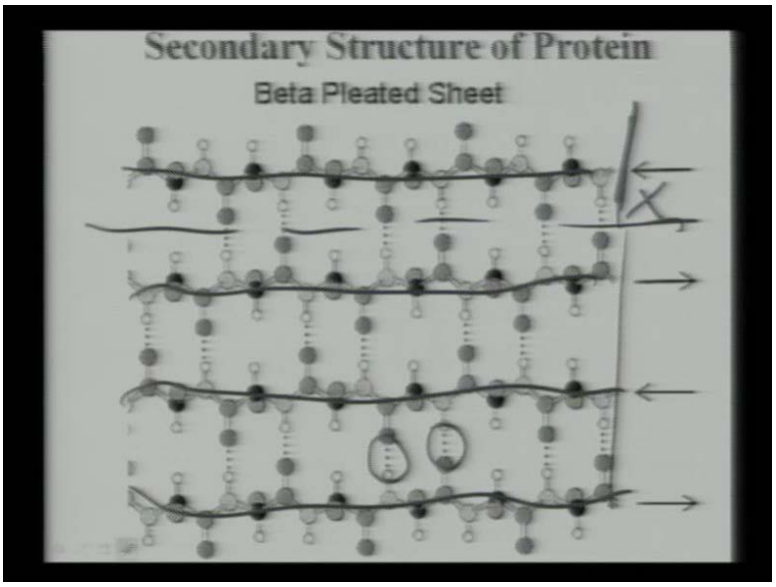

this is like you know different beta plated sheets essentially this contains the parallel polypeptide backbone chains and this between parallel polypeptide backbone chains you can see the weak hydrogen bond they are always dotted bonds.

So that shows that weak hydrogen bonds how they will hold 2 polypeptide backbone chains together and that essentially indicates that any point of time this backbone chain

can be broken right they any (( )) this hydrogen bonds can be broken and this this polypeptide can be separated from the rest of the polypeptide chain do you understand what I am saying so that is possible because the structure has the hydrogen bonds.

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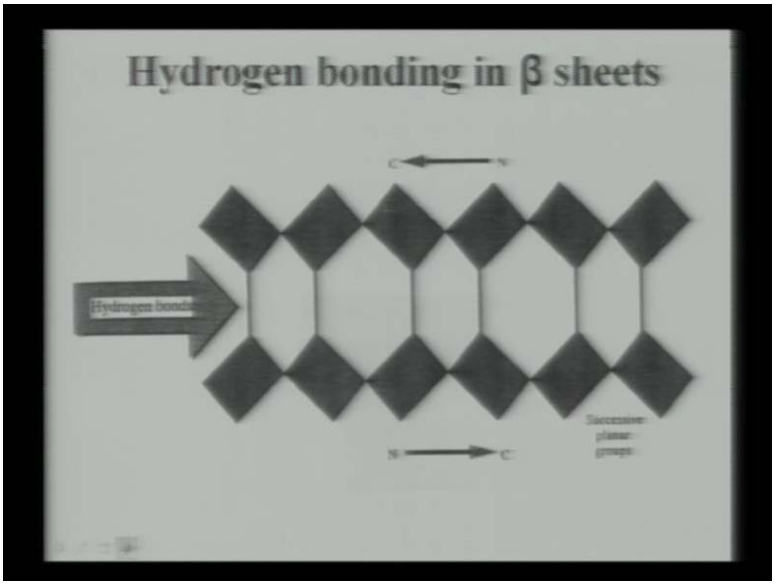

Now this is again showing that you know how this hydrogen bonds they are formed in the beta sheets

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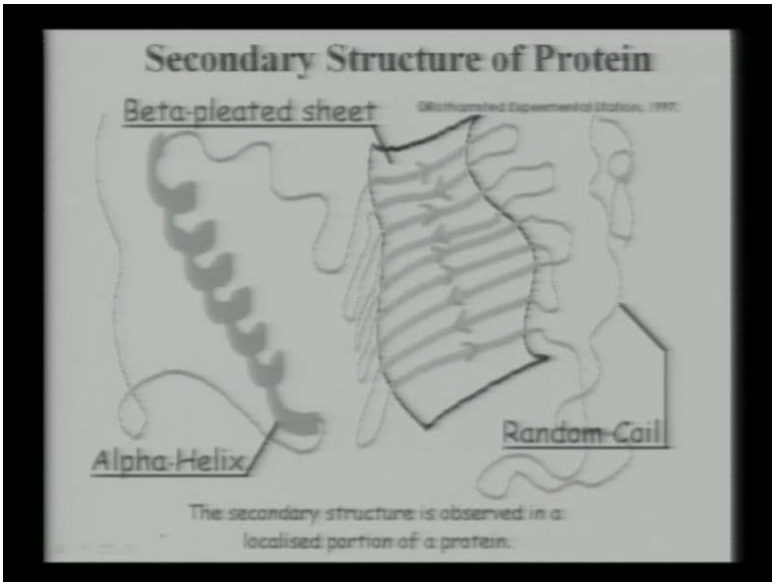

this is the comparison of the alpha helix pattern and the random coil organization in a localized potion of a protein molecules

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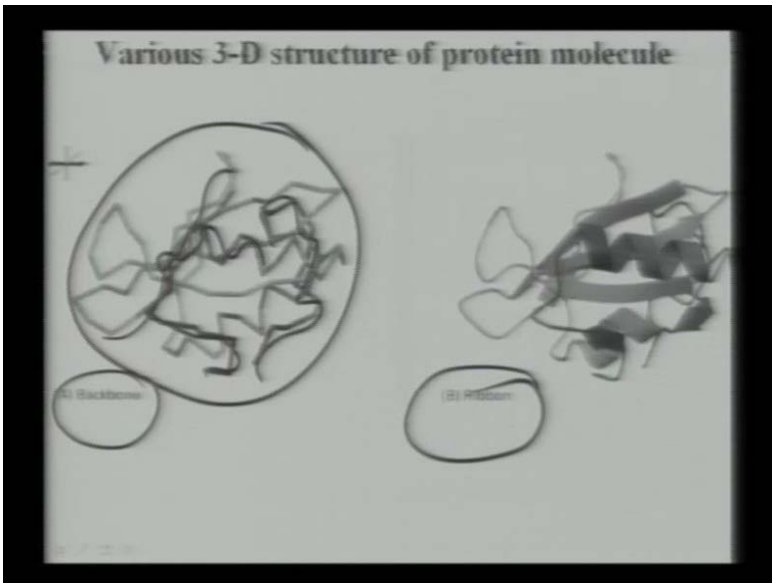

so this is like computer generated protein structures because there are lot of computer stimulation on the protein structure what biology they do in real like stimulated structure it can have a ribbon like structure you know that ribbons how they look like or it can have a typical backbone type of structure and this all this different you know different color this is an indicate that each line each backbone chain has different composition.

And that is what is indicated the each backbone chain has different composition means if you have r 1 r 2 r 3 different site chains immediately you have the backbone chain of the same.

So essentially what I am trying to say here this constitutes the total protein molecules assembly but, individual fraction length of this individual of this backbone chain can have different composition

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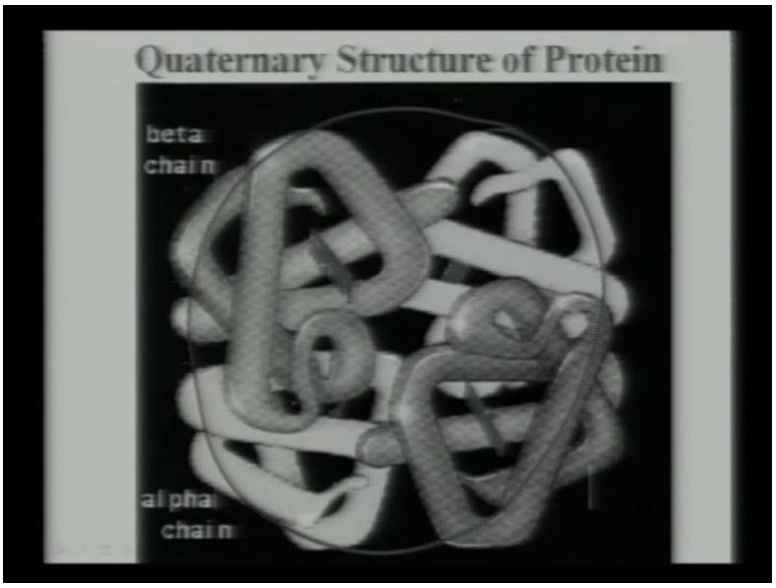

so this is like quaternary structure of a protein you can see that it is more like a how a snake they looks like they they look like in the real life right so it is more like snake like pattern and then you know typical snake how they can squeeze them to fill up the space if it is a very coil like pattern then it can feel the space more optimally but, if this coil you can just straighten it then it will be a very long molecular structure like a backbone type of structure but, then it will it does not occupy the 3 dimensional space as effectively like the way this coil like configuration they occupy 3 dimensional space do you understand do you realize what I am trying to say so that is important is that how this you know different protein molecules they take the shape in the different conditions biological conditions.

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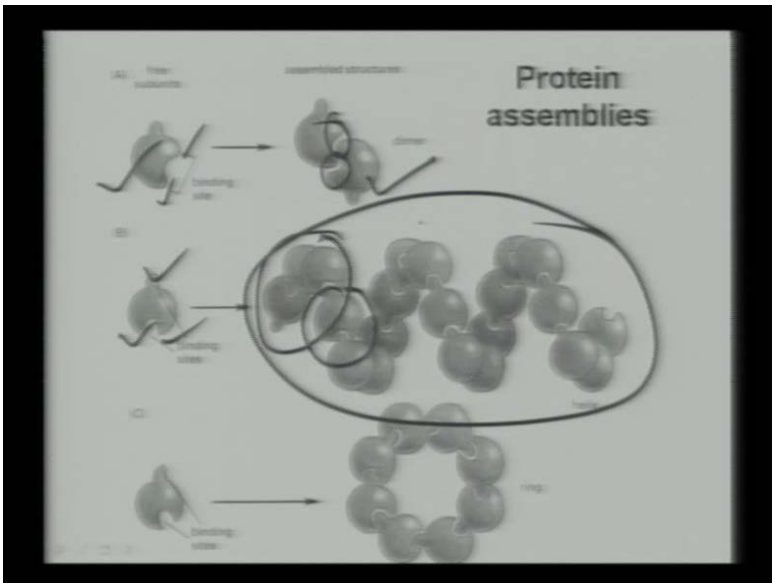

Now protein assemblies how the protein assemblies step one like you have a binding sites now this binding sites can be you have the site chains like r 1 r 2 r 3 or you have the potential formation of hydrogen bonds here at potential formation of hydrogen bonds here so if you have one protein molecule is this one another protein molecule they come together in this joints they can form the hydrogen bond then they can form a assemble at the same time a protein molecules can have a 3 binding sites and 3 binding sites can bind 3 protein molecules similarly, next protein molecules also equally will have 3 binding sites.

So accordingly that entire globular assemblies that can form by just by very weak hydrogen bonds interacts do you understand what I am trying to say here so all these things are possible ones you know how the structure of a protein molecule they form in real life

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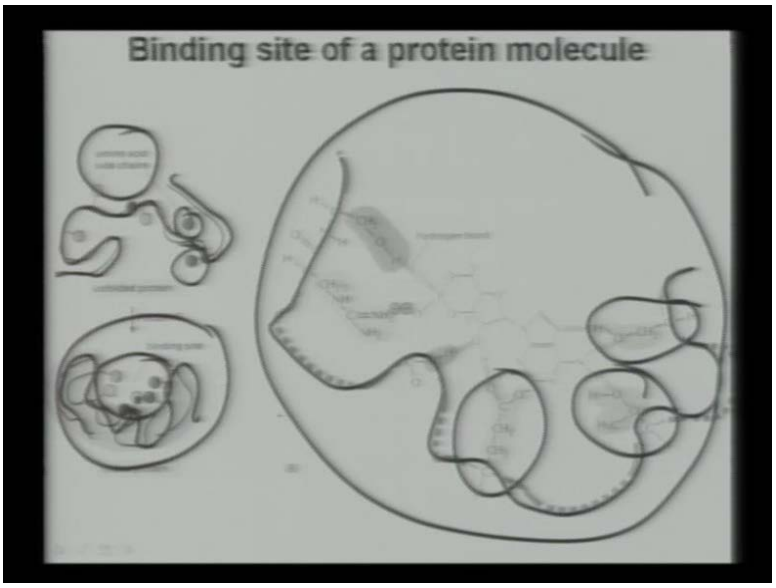

now binding site of a protein molecules now what you can see here it is an unfolded protein and this unfolded protein it is a it has a different kind of a amino acids site chains and the different color essentially means that amino acids site chains can be different chemistry like it can be methyl group it can be ethyl group it can be benzene ring whatever it is

And in a folding chains also you can see that it contains when it is in folding condition now it has a more binding sites which are coming together now essentially if you have a more binding sites that means it can attract more number of protein molecules to come together and it can form a very easy protein assembly you understand what I am saying and that is what is presented here when you have a large structure large backbone polypeptide backbone chain and this large polypeptide backbone chain you can add different protein molecules and then 5 weak hydrogen bonds then you can form a large protein molecules

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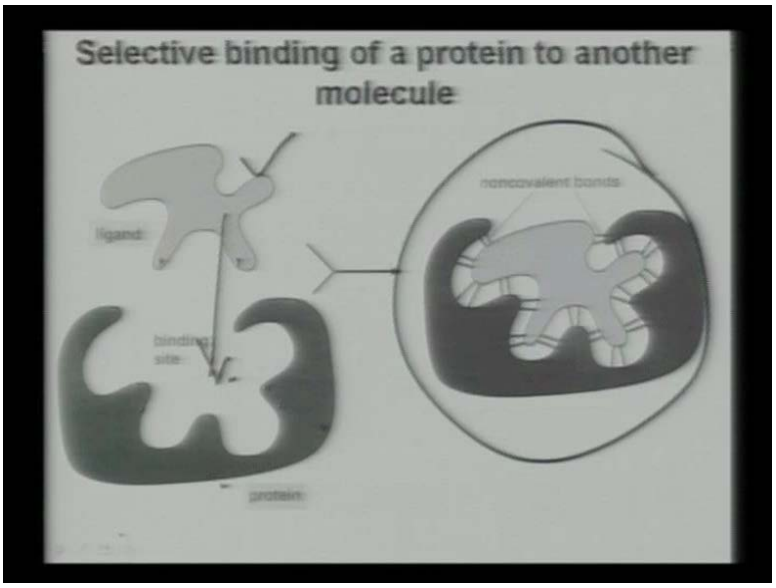

this is how this non coherent bonds they are formed so essentially it has a ligand and then this is a binding sites of a protein molecule now this ligands can come they can bind together or the different binding sites and they can form this kind of a structure therefore, protein molecules can bind another organic ligand if necessary or if require.

So this kind of binding is possible just because the protein molecules have some available binding sites

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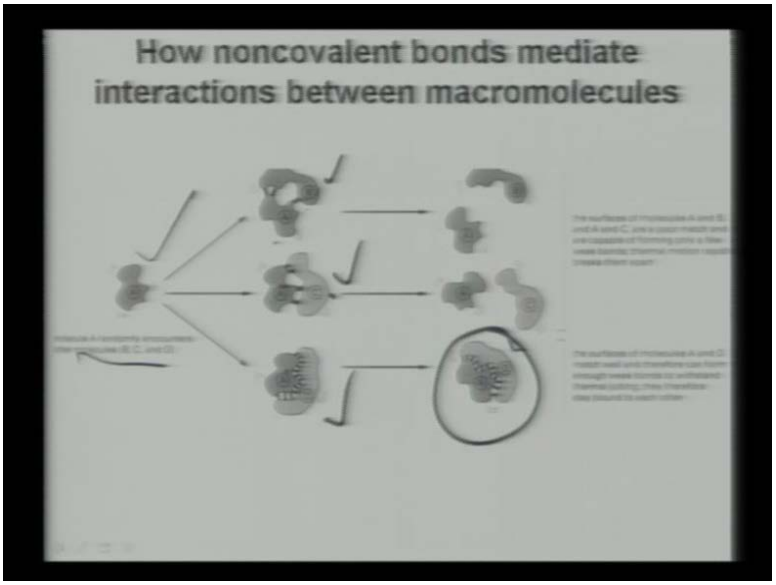

this is the how the coherent bonds immediately interactions between macromolecules so essentially protein is a polymer macromolecular structure and suppose it is an example if a molecule a which randomly encounter other molecules b c or d now how see different cases like how the a is getting connected or getting bound to b or how is getting connected to c or how a getting connected to d now there is certain comments which are mentioned here in this slide that is surfaces of molecules a and d match well and therefore, can form enough (( )) which can form (( )) they therefore, stay bound to each other what it means that here if there are binding sites which are more number of binding sites are available that means it is like this so If we have a more binding sites or more finger tips then you can grab certain object more firmly but, if you lose your (( )) finger and if you have only 2 fingers then what will happen you cannot grab that object that firmly what you can what you can do very easily with 5 fingers you understand.

So similarly, if the protein molecules have the more binding sites then they can firmly grip although the same hydrogen bonds will again be available for the binding to this particular molecule but, since you have more binding sites available so that binding would be more firm or that binding will be more strong compare to the protein molecules which has less number of binding sites so that is what I am we are trying to mention here.

And when the binding is formed or binding is strong then what will happen whatever thermal activation or if you have some changes in the physiological environment inside the body this assembly will never be destroyed so they can remain stable because the binding is quite strong.

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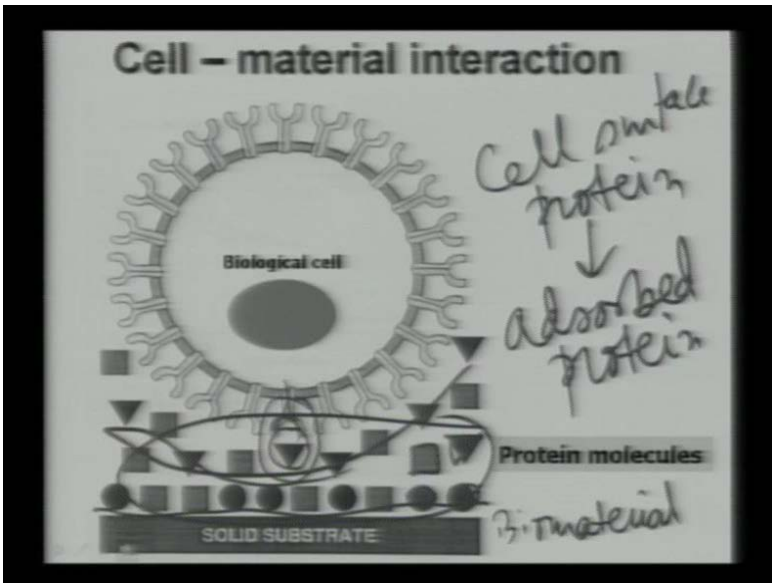

Ok next few slides I will discuss with the cell material interaction essentially now you have got enough understanding of the how the collagen molecules they look like how the protein structure they look like and how you can characterized these protein structures both compositionally size wise morphologically shape wise.

Now protein molecules as you know that you know it is like abandoned in number in kind of in the body and these protein molecules what happens when a solid substrate when a biomaterial so this is a biomaterial now when a biomaterial is placed inside the body and environment in vivo environment or in a cell culture solution then what will happen this immediately within a few seconds now you can see here this protein molecules of different shape here so one is (( )) triangle one is square one is (( )).

So essentially what I try to elasted here this different type of protein molecules in large number they come and adhere on the poly surface biomaterial surface and these protein molecules is also supplied in the cell culture solution right each cell culture solution you have a large number of protein molecules and this protein molecules once they adhere on the on the material surface then they can easily interact with the cell surface proteins remember the membrane structure of the cell that I have described you earlier this membrane has a double layer if it bilayer structure and this membrane also has a protein molecule so this protein molecules then they can attract they can adhere or they can make physical contact with the protein molecules or different shape and size it is like a

tongue you want to capture object with the tongue and this tongue is the cell surface protein and you have large number of protein molecules they are available in the material surface so within this tongue this protein molecules can immediately get hook or can giving to get attack so this hooking the physical hooking or physical attachment that is more possible because of the cells surface protein with adsorb protein molecules so essentially cell will not directly interact with the biomaterial per say but, cell surface proteins so it it is essentially cell surface protein which will interact with the adsorbed protein on the surface and this is what is meant by cell surface interaction so this is what happens at the initial stage of the cell material interacts right

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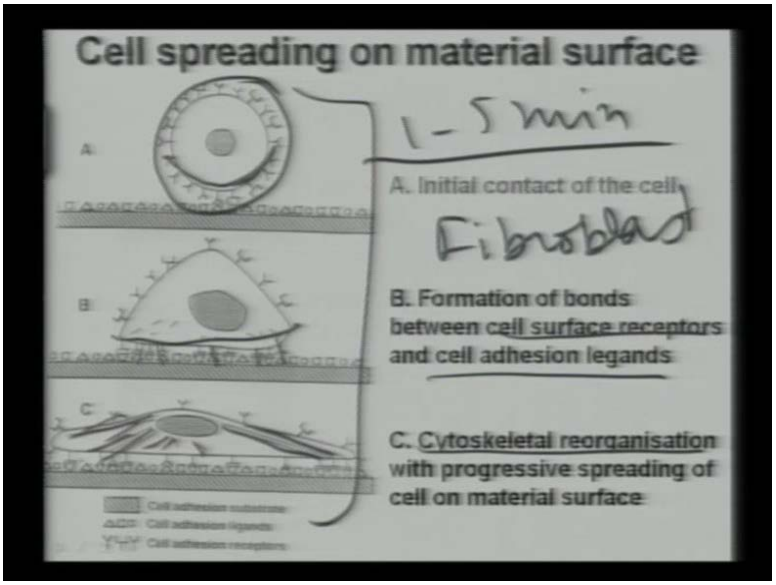

now if you go and describing that how it happens that entire cell adhesion so initially cells have a very spherical like shape right now ones this proteins are getting adsorbed now you can understand this from the basic physical concept now a cell or a molecule they can adhere to the surface more if there is more surface is in contact with the another surface so in order to have the more form interaction between a cell and material what it happens this spherical surface now becomes more (( )) so that more number of cells surface proteins can now get physically hook to more number of surface adsorb proteins on the biomaterial surface.

Now once in taken place then more cells spreading can take place and as I have mention to earlier that cell this lines essentially indicates cytoskeleton so this is the part of the

cytoskeleton like microtubules of the acting filaments or the intermediate filaments and so on of the cytoskeleton now this cytoskeletons are also essentially polymeric macro molecule so they can be also easily stretched the way you want so essentially to have more enhance interaction between the cells surface proteins and the adsorbed proteins the cell there will be more cells surface receptors and more cell adhesion legands they will interacts and cytoskeletal reorganization will take place reorganization means like the cytoskeletal proteins that acting filaments and microtubules they can also reorganize themselves as the cells will create on the material surface so this entire physical process can take place very easily within few 1 to 5 minutes of the implantation or within one to 5 minutes of the putting the (( )) putting material on the cell surface.

Now this is lecture one type of cells let say this is a fibroblast or osteoblast type of cells now the theory says that one single cell they are getting (( )) material then that information or that communication is sent to other cells also in the solution and therefore, that adhesion of one cell will essentially facilitate the adhesion of more number of cells from the solution to getting attracted or to get attract to the material surface and therefore, slowly more number of cells will get a adhere to the material surface so this is what as meant by the cells (( )) material surface so to summarize first protein molecules they get adsorbed because they are much larger in number compare to the number of cells in the solution and this protein molecules they will adsorbed on the material surface and remember this protein molecules have a size of very small right 2 point seven nanometer so 2.7 nanometers and if your samples are 10 millimeter in diameter so large number of protein molecules can immediately adhere now one this nanometer size protein molecules they are adsorbed from the material surface then they interact directly with the cell surface proteins they are getting attracted they are getting physically attach to each other and In order to have more strong interaction between cell surface protein adsorbed proteins now cell surface itself will undergo shape chain and this shape change and this shape is possible because as I mentioned in the last lecture cells have the unique properties that cells can adopt himself and this (( )) cells can adopt themselves and this cellular adaptation can caused change in size change in shape right change in numbers and change in type so all this things is possible so one single cell type first they are adhere then that will recruit the multiple cell types and as a result a tissue will form on the material surface so this is the basic theory of the cell materials interaction.

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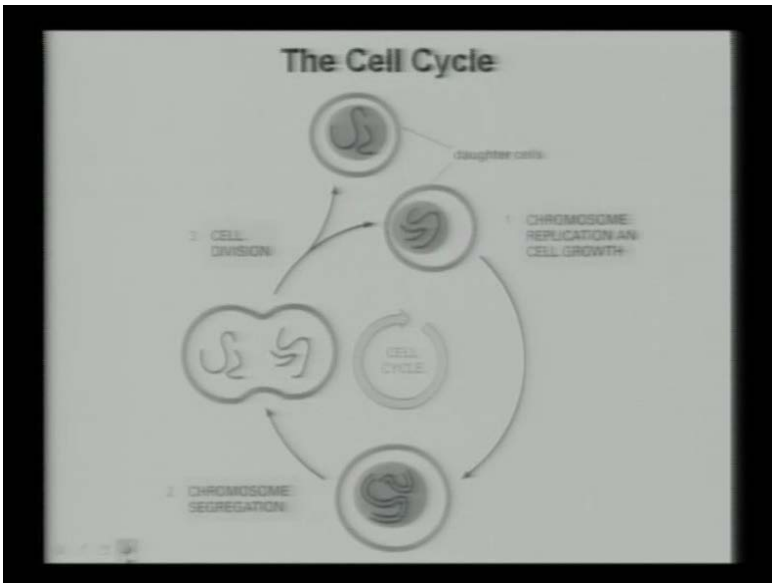

Now this is the cell cycle that I have already mentioned to you earlier

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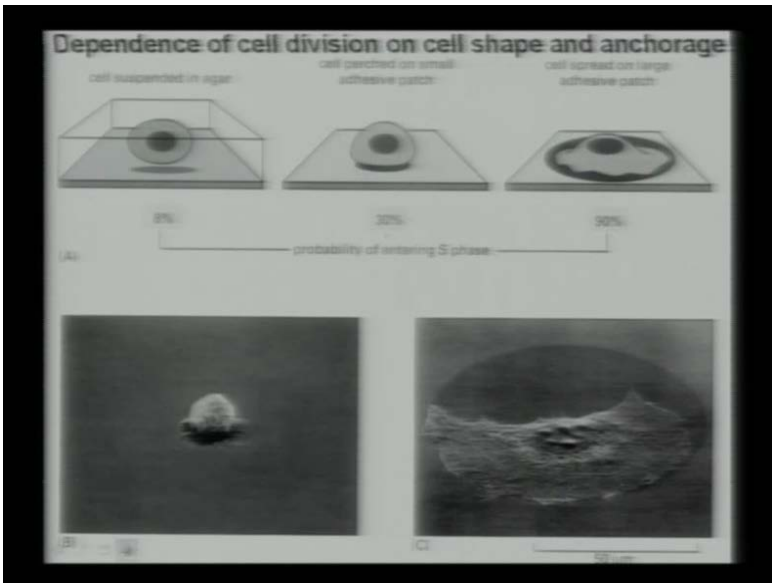

now dependence of the cell division and the cell shape and anchorage now this is the cell suspended in agar and this is the possibility of these are like it is entering into different phase of the cell cycles s phase is one of the one of the phase of the cell cycle now as you can see that is an anchorage dependent cell like fibroblast for example, they can spread very easily on the material surface

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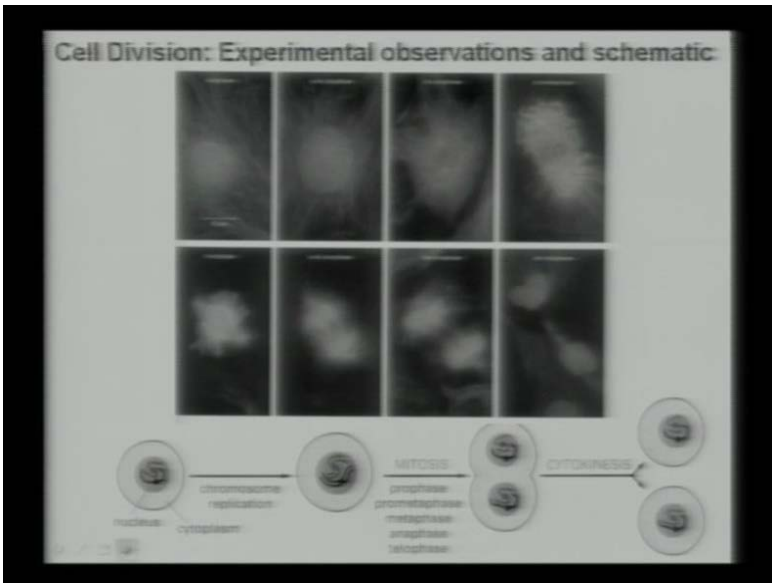

and this is the fluorescent images so you can see that it is (( )) and this (( )) is in different color it is expressed so after this (( )) you can see that how this cell division take place now you can see this is a mother cell now this mother cell is essentially is divided into 2 daughter cells and 2 daughter cells are now separated and you can see very wagely the cytoskeleton which are kind of expanded to form the e c m that is extracellular matrix so this is at mitosis you have the cytokinesis all this processes

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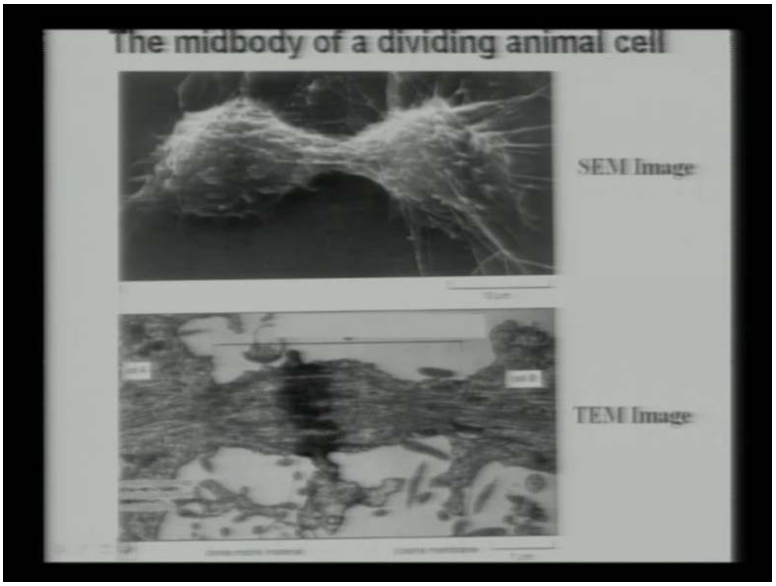

this shows that how a midbody of a dividing animal cell so this is one daughter cell this is another daughter cell so this is the connection with this cell which cell exists.

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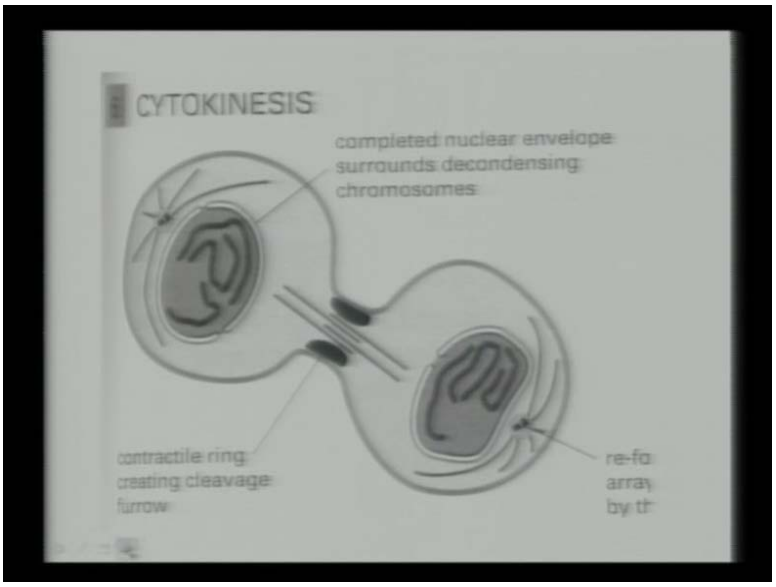

So this is the process of the cytokinesis cytokinesis means like this is a nucleus with a chromosome with a containing chromosome and this is the this cells now will is going to divide and it can form 2 cells 2 daughter cells so this is the contractile ring creating cleavage furrow so that is what is important

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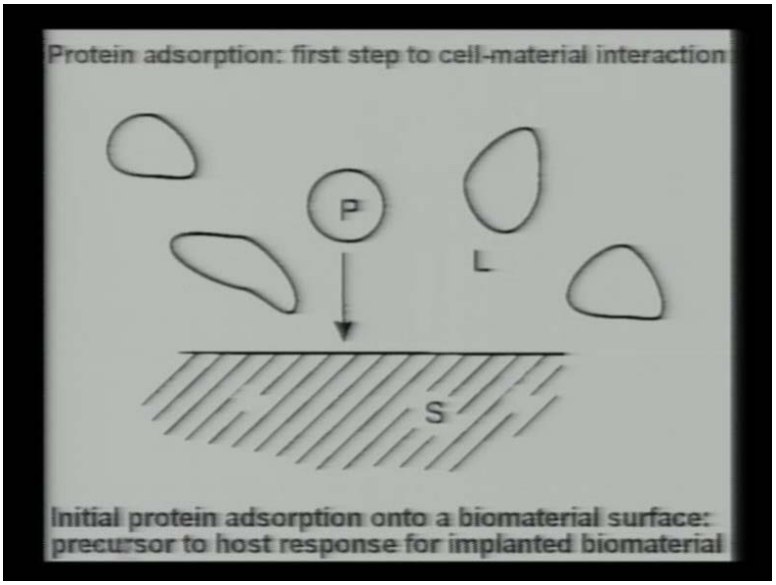

now as I said that protein adsorption is the first step to the cell material interaction therefore, you need to know that how much how far the proteins will get adsorb in the material surface you can see that this as the different shapes of the protein molecules and this is called it substrate so this is exactly written (( )) initial protein adsorption in a biomaterial surface is the precursor of the host response of the implanted materials

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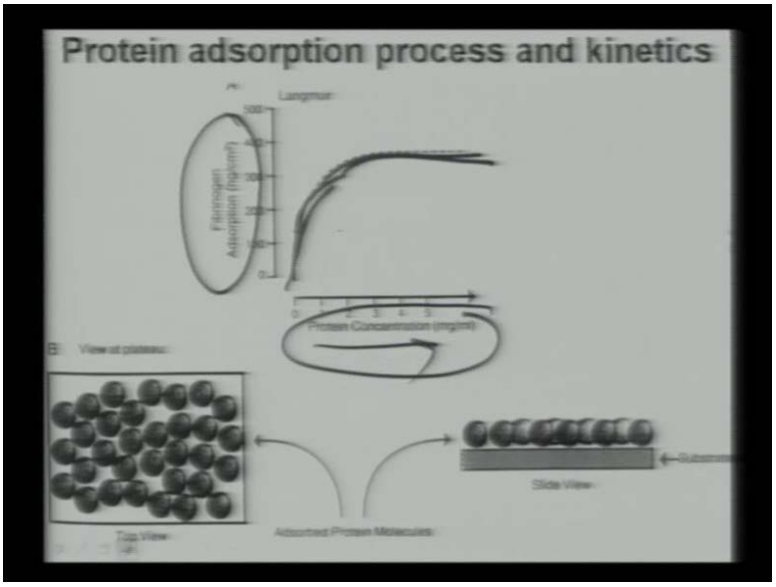

now this is the protein adsorption isotherm what you see here this is the fibrinogen this is one type of protein and this is a protein concentration milligram per milliliter And this is the langmuir isotherm so if you add more and more concentration of the protein and then you have this more and more protein fibrinogen absorption and then you can see that this undergoes initial increase and then it goes through a steady state and therefore, more protein absorption cannot take place because a entire software is now cohered with the protein molecules.

So that is what is meant by so even if we add more protein concentrate even if you add more protein to the solution these adsorption isotherm will not change.

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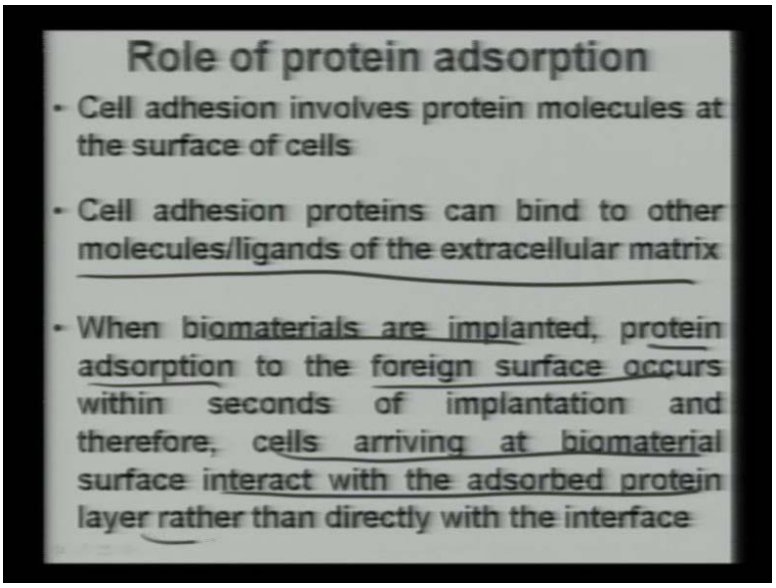

Now this is the role of protein adsorption it essentially tells you some theoretical aspects.

A Cell adhesion involves protein molecules at the surface of the protein cells now cell adhesion proteins can bind to other molecules or ligands and to form the extracellular matrix and this is the when biomaterials are implanted as I mention already protein adsorption to the foreign surface occurs within seconds of the implantation and cells arriving at the biomaterial surface interact with the adsorbed protein layer rather than directly with the interface.

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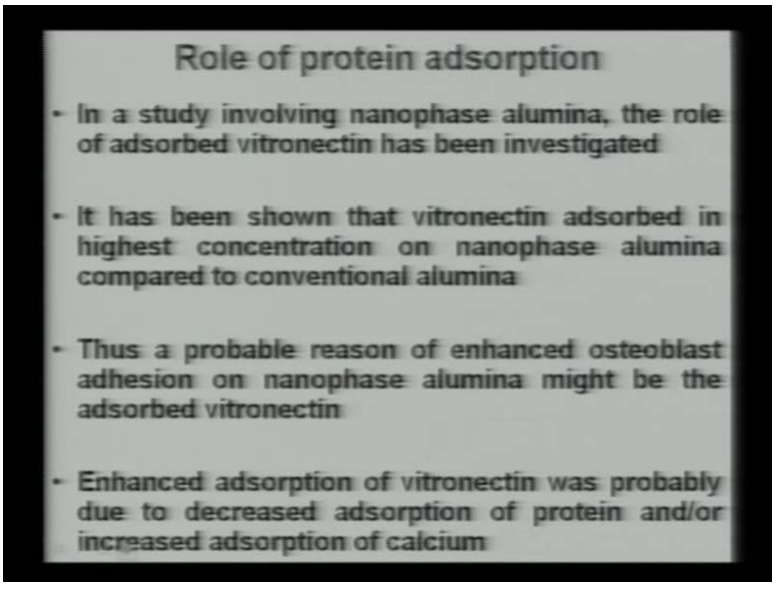

So these things are clear to you already.

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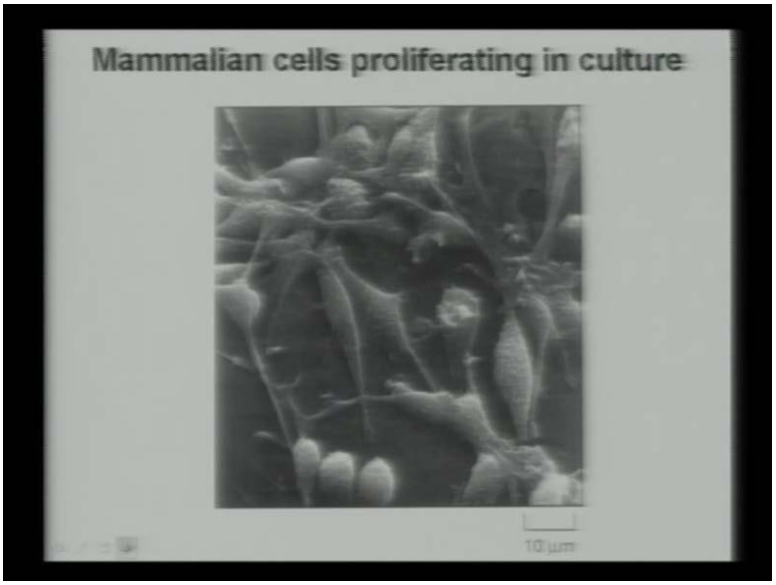

Now I'll show you couple of examples before I finish this lecture essentially so that you are comfortable or you have a clear idea that how cells paired how cells proliferate on the material surface this is the mammalian cells like any animals animal animal cell lines proliferating in the culture.

Now this is one cells this is another cells this is the third cell (( )).

Now we can see that is a lamilopodia or philopodia extension that gets connected to the are debarring of neighboring cell surface and then it forms a cellular network.

This is a preferential attachment of the cells on the hydroxapatite particles but, not in the polyethylene matrix.

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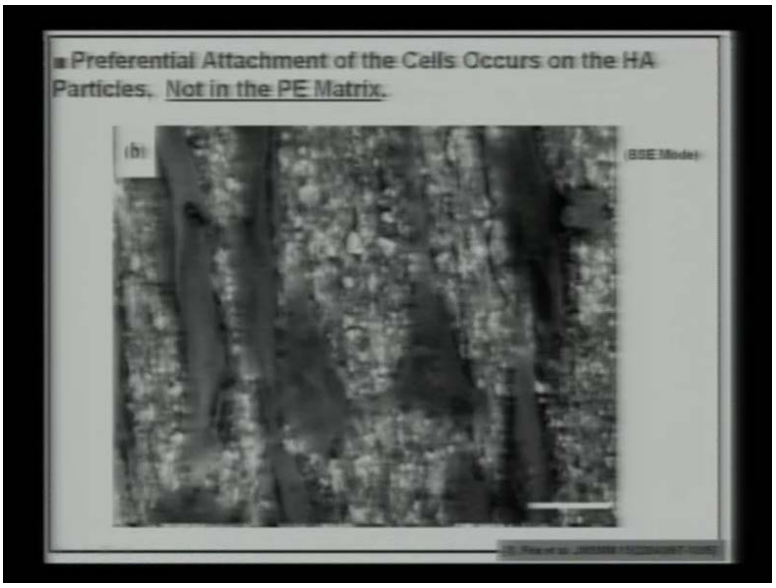

So you have this polyethylene particles there and you have the hydroxapatite particles here and this is the cells which are getting at a head on the polyethylene hydroxapatite particles because it is very much (( )).

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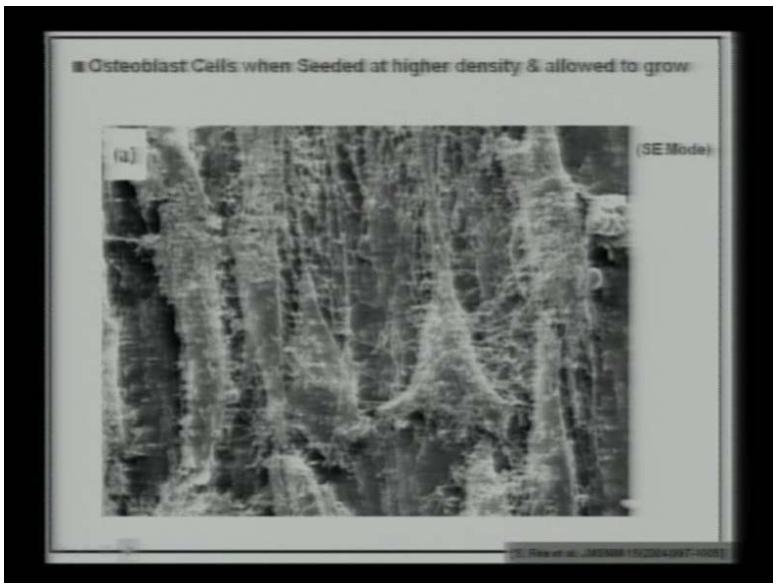

This is the osteoblast cells when seeded at higher density and when they are allowed to grow.

Now here this you can see very clearly the human osteoblast cells lines and this is the form of the E C M that is the extracellular matrix this is this collagen protein which extracellular matrix that can be also formed.

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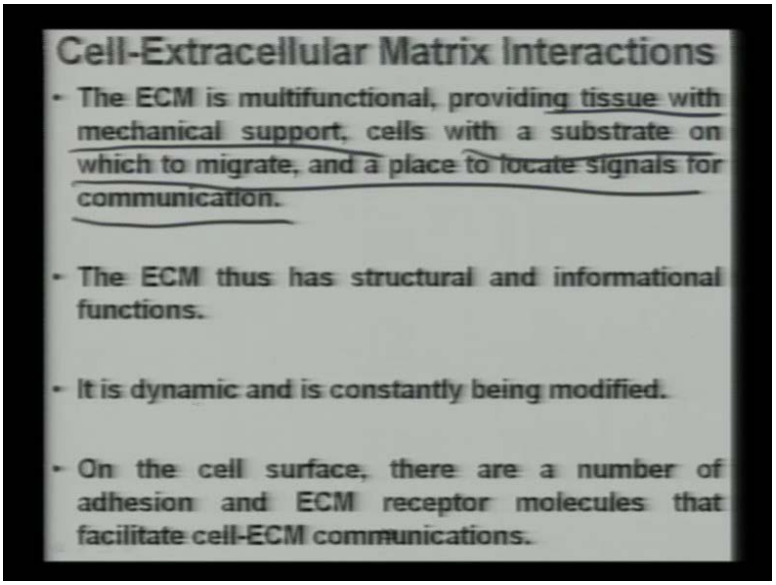

Now E C M E C M is as you already know that it is a multifunctional it provides tissue with mechanical support I told you in the earlier lecture that E C M count is not a collagen fibre and because of the fibres whenever any material has lot of fibres those fibres actually will we are mechanical load.

So that is what E C M does so it does provide tissue with a mechanical support and cells with a substrate in which to migrate a place to place to locate signals for communication and E C M also has structural informational functions and it is a dynamic and constantly being modified.

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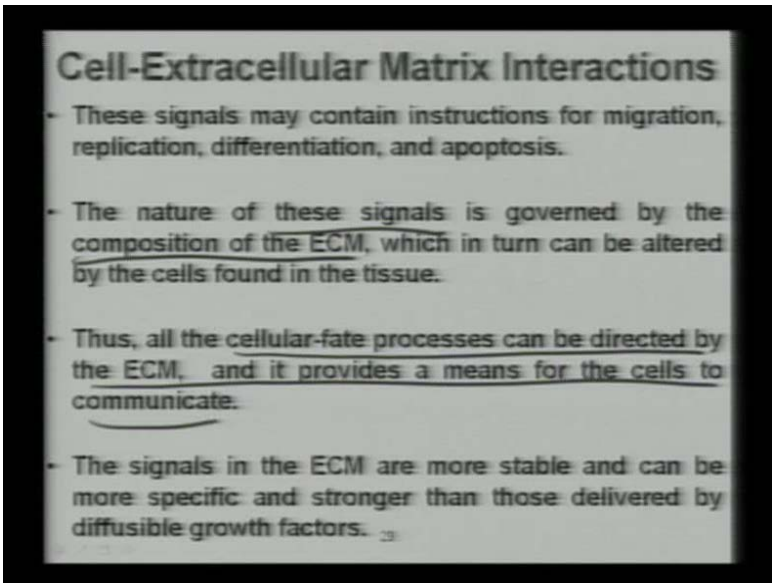

now cell-extracellular matrix interactions are be essentially mediated by the signals which may contain instructions for migration replication and differentiation and apoptosis.

Now migration means when the cells are adhered on the surface now cells can move just like a human being cells can move on the surface.

Now this migration is not possible unless cells gets some kind of signal so it is like a chain modes on the track railway track right.

But at the certain points there are signals if the signals are red that cells that trains will move if the signals are red if the signals are green then train will move if the signal is red then same train will stop it will not move on the railway track.

Similarly cells also requires from signals before it can migrate and it can replicate means it can replicates itself it can differentiate means cells can differentiate from (( )) cells to the all the other cell lines or cells can can undergo necrosis or apoptosis apoptosis means cells will die.

So all these are (( )) essentially signal dependent and this nature of this signals is perform by the composition of the e c m.

So if the e c m composition is change so the signals also will be change and therefore, all the cellular processes can be directed by the e c m and it provides a means for the cells to communicate.

So essentially if you go here you what you can see that this is the extracellular protein extracellular matrix and these are the cells this is cells one this is cells 2 this is 3 4 5 6 7 something like that but, that extracellular matrix is the entire matrix which is outside the cell so all this signals are essentially transfer to the cells by the extracellular matrix proteins only.

So therefore, extracellular matrix are the much larger role to play one is the mechanical support and second one is the transfer the communication or transfer the signals to the adhered cells so that cells can perform either migration or diffusion or differentiation or apoptosis.

So that is what is meant here some theory which is mentioned here that cellular-fate processes that means replication differentiation on migration these are all directed by the e c m and therefore, it provides to means for the cells to communicate and the signals in the e c m are more stable and can be more specific and stronger than those delivered by diffusible growth processes.

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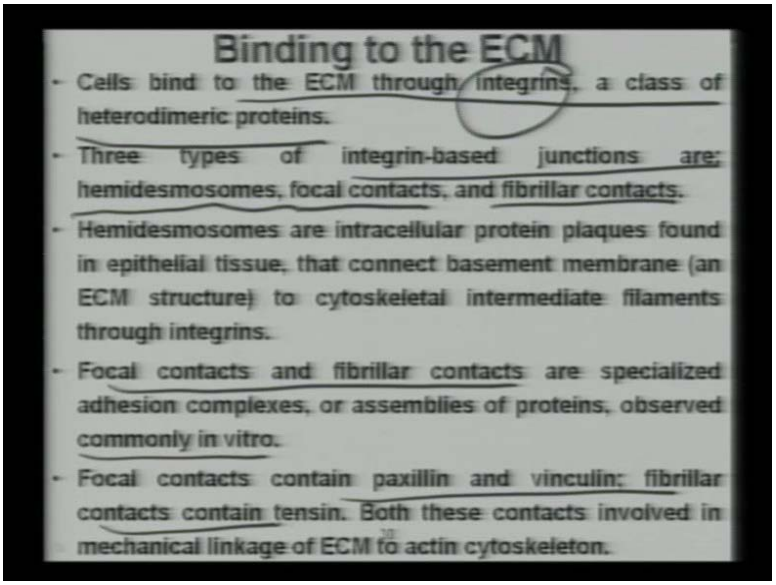

Now binding to the e c m like in a how cells are bound to the extracellular matrix now cells bind to the extracellular matrix to integrins a that is a class of heterodimeric protein.

So this integrin protein is essentially the protein which helps in the binding of the cells to the extracellular matrix and 3 types of integrant base functions are possible that is that focal contacts fibrillar contacts and hemidesmosomes that is the 3 integrant based junctions which also facilitate the binding of the cells to extracellular matrix.

Now focal contacts and fibrillar contacts are specialized adhesion complexes or assembles of proteins observed commonly in vitro.

So when you do this laboratory simulator (( )) normally you can see the focal contacts are fibrillar contacts focal contacts means it is a like a focal contact proteins which helps just to bind the extracellular matrix to the protein at the specific location and that is the responsible a focal contact and focal contacts also contain they have different type of proteins and both these contacts involved a mechanical linkage of e c m to the actin cytoskeleton.

Now if you go to this diagram then you can understand more clearly now you can see that cytoskeletons here also expanded under e c m also contains lots of molecules or integrin proteins.

Now these e c m proteins they directly connect to the cytoskeleton protein and therefore, these protein molecules they connect to each other and they form a strong adhesion bonds now do you or if if this over all biological picture getting clear that how the cells are getting bound to the e c m.

So first if you go back to the slide because this is very important concept I will summarize it and before I'll go ahead.

So essentially your surface protein they are absorb it forms a monolayer then the surface (( )) proteins they interact with the cell surface proteins then cells will also change their shape you have more adhesion then they will be cytoskeleton reorganization that takes place right.

Now then cells also will secrete protein molecules now this protein molecules can come out from the cellular membrane and this protein molecules and with they will form the extracellular matrix.

Now the question is at how this cells will be directly getting connected to the extracellular matrix the answer is that all the cytoskeleton proteins they will directly connected to the integrin proteins which are present in the extracellular matrix and as a result they can get directly getting hook to the extracellular matrix and that will give you direct biological bonds as well as the mechanical bonds also.

So that cells can immediately bound to the extracellular matrix

So this is the kind of total biological picture about the how these cell material interaction and the cell formation and then cell e c m interaction and that takes place

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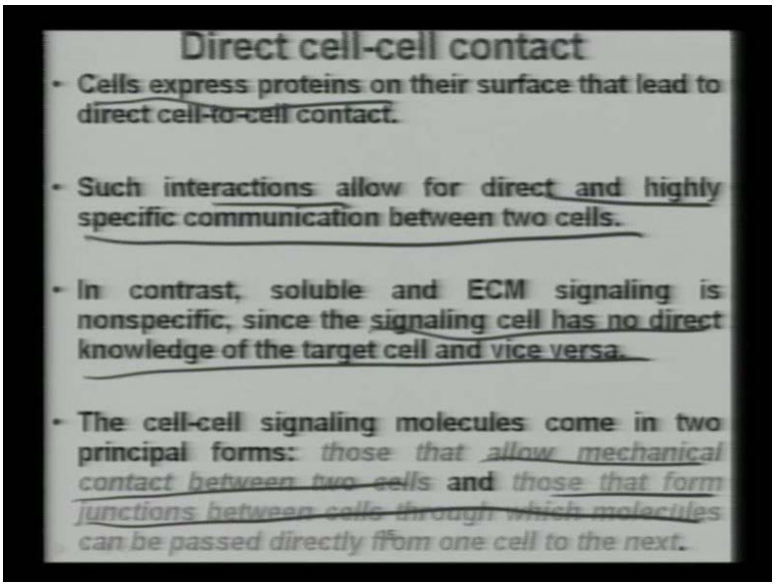

now direct cell to cell contact of cellular bridge formation that is possible when cells express proteins on the surface that lead to direct so cells express protein means cells will secrete certain protein molecules which come out of the cells and going to the intercellular matrix and these interactions allow for the direct and highly specific communication between 2 cells.

Now when 2 cells are getting connected to each other than through this cellular network or cellular bridge formations one cell can directly transfer that communicate bridge to the other cell.

It is just like 2 human beings communicate to each other similarly, one cell living cell can directly communicate to another living cell when their getting connected to each other.

Now in contrast soluble and e c m signaling is nonspecific e c m signaling means whatever signals that is transfer from the extracellular matrix to the cells that is nonspecific nonspecific means that interaction is not direct the way one cell gets transfer the communication to another cell since the signaling cell has no direct knowledge of the target cell and vice versa because here like 2 human beings when they directly communicate to each other both of them they know each other

But when you change the signal to the extracellular matrix you do not know whether the the where the signal will reach what is the your target cell right so that therefore, it is a nonspecific interaction when a cell to cell communication is a direct specific interaction the other things is that cell to cell signaling molecules come in 2 principle form those that allow mechanical contact between 2 cells and those that form junctions between cells through which molecules can be pass directly from one cell to the another.

Now these junctions when there is a direct link from one cell to another then there is very easy for the molecules or protein molecules to get transfer from one cell to another cell otherwise protein molecules have to form some fascicles they have to get out of the cell cell surface going to the (( )) cellular matrix they will be transported to another cell.

So that process can be that long process can be cut shot if the one cell is directly bound to another cell

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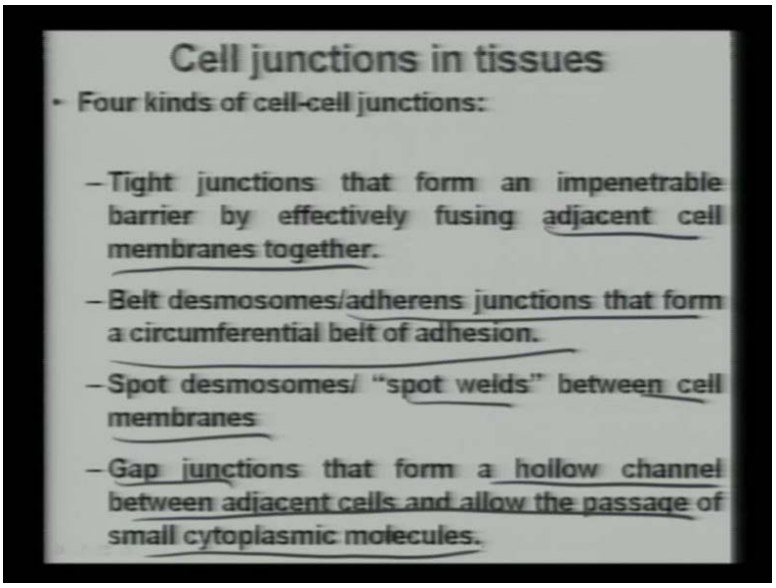

Now there are 2 4 kinds of cell to cell junctions one is the tight junctions that form an in penetrable barrier by effectively fusing adjacent cell membranes together then there is a belt desmosomes are adherens junction that form a circumferential belt or adhesion then there is spot well between the cell membranes is just like a spot welding process then there is a gap junctions that form a hollow channel between adjacent cells and allow the passage of small cytoplasmic molecules.

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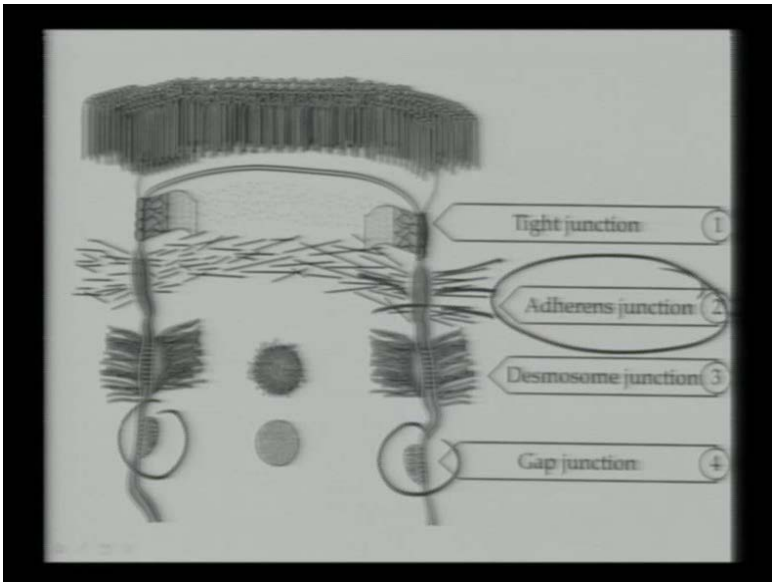

Let us see a how they look like.

Now tight junctions is the number one so it is like a very tight mechanically and both biologically it has more like a cytoskeleton type of molecules which form the adherent junctions then gap junctions these this is the type of gap junctions here

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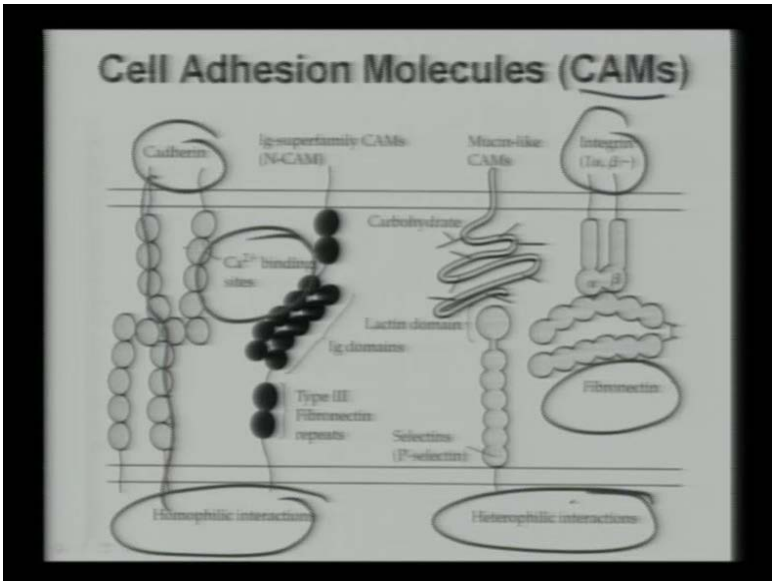

and there are certain molecules which are express from the cells and these are called cell adhesion molecules and these cell adhesion molecules in short they are known as cams like cadherin.

Now cadherin is the cell adhesion molecules then you have also hemophilic interactions and you have also heterophilic interactions like fibronectin is a kind of proteins which are connected to integrins and also there are other type of proteins which has a calcium ca plus to binding sites.

So ca plus to binding size that is the calcium and binding sites and you have the more calcium and binding sites means that helps in the bimineralisation process also.

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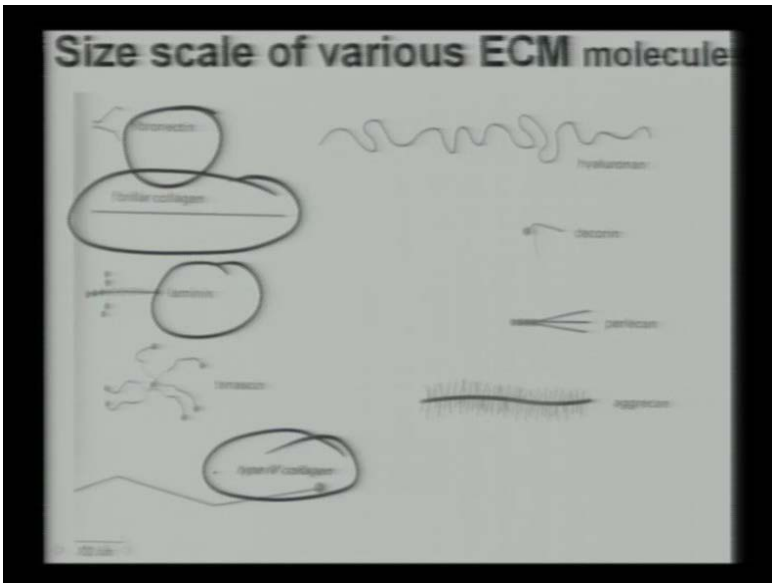

Now a few minutes back I was mentioning that about that extracellular matrix composition is also important in terms of transferring the signals or communication to establish communication between 2 cells if the matrix if the (( )) matrix molecules they are composition they change then the transfer of signals process also change.

Now what about different types of e c m molecules one is the fibronectin that is a protein and these proteins also there is a fibrillar collagen there is laminin type of proteins and there is a type 4 collagen.

So all these different types of collagen essentially means you have different size groups and these size groups is a change in different types of collagen

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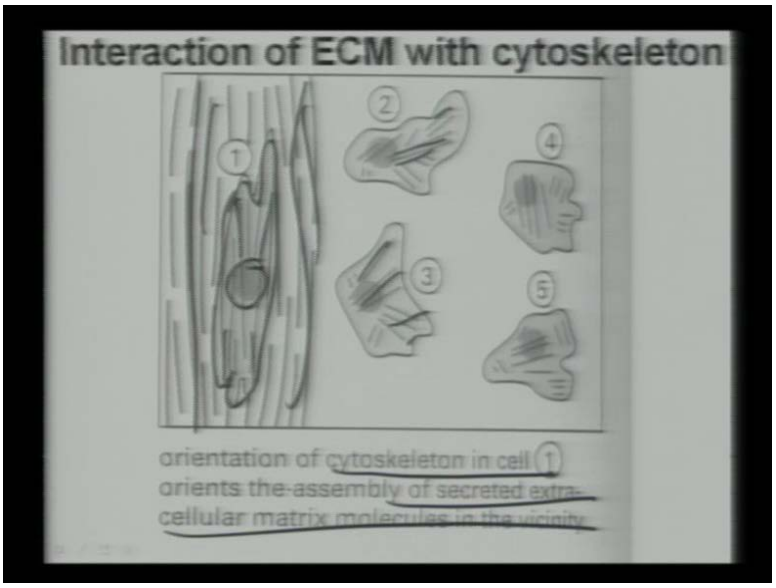

this is like different types of extracellular matrix molecules.

Now what happens and how this extracellular matrix they interact with the cell this is what is been shown here.

Now orientation of the cell your cytoskeleton in cell in 1 for example.

Now orients the assembly of second secreted extracellular matrix molecules in the vicinity.

Now how it is done like you know this this is the cell living cell and you have a nucleus you have extracellular matrix molecule sorry this as cytoskeleton and we have the extracellular matrix proteins they are oriented in the neighborhood.

Now what will happen when these other cells this is the 2 3 4 5 differ 4 different cells and these different cells as you can see the cytoskeleton there oriented not parallel to the extracellular matrix proteins right they are oriented in different directions.

Now what will happen how this extracellular matrix will will actually interact with these proteins unless this bones are formed between the cytoskeleton proteins with the extracellular matrix molecules that cells will not be bound to the e c m.

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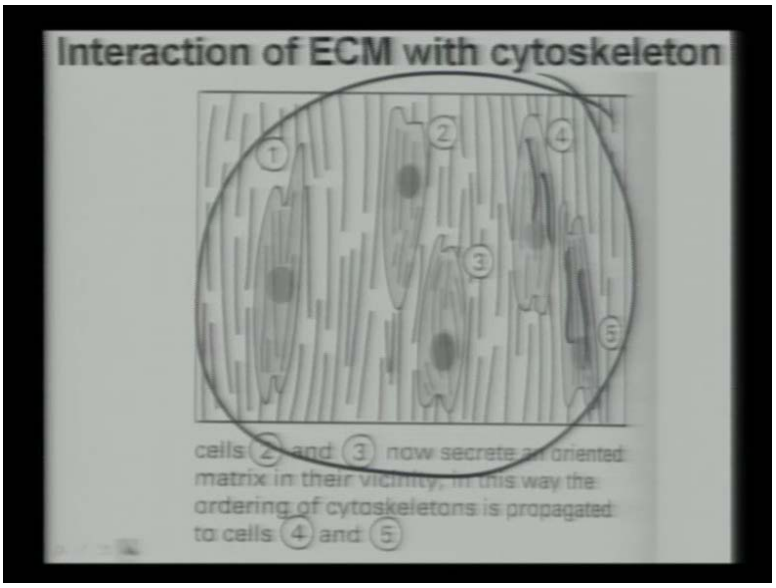

Now how it happens you can see here that as this extracellular matrix more forms more and more they slowly in cell number 2 and cell number 3 the cytoskeletons also they reorganize or they orient themselves parallel to the e c m and therefore, these orientation actually here in more interaction that is what is meant here the oriented extracellular matrix reaches cells 2 and 3 and orients the cytoskeleton of those cell.

Now slowly the cells number 4 and cells number 5 they are also cytoskeletons they are oriented parallel to the extracellular matrix.

Now all this 1 to 5 cells all the cytoskeletons they are oriented parallel to the e c m molecules and therefore, now all the 5 cells are now bound to the a extracellular matrix ‘

So this is how a extracellular matrix they interact with that cells

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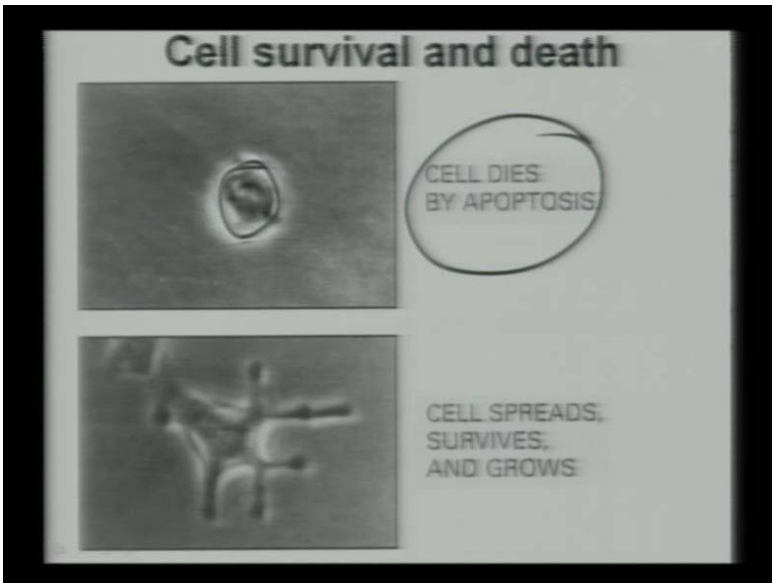

other things that I have mention to you earlier going when the cell dies by apoptosis then cells form a globular shape and from this globular shape you can immediately see that that is the sign of the cell death

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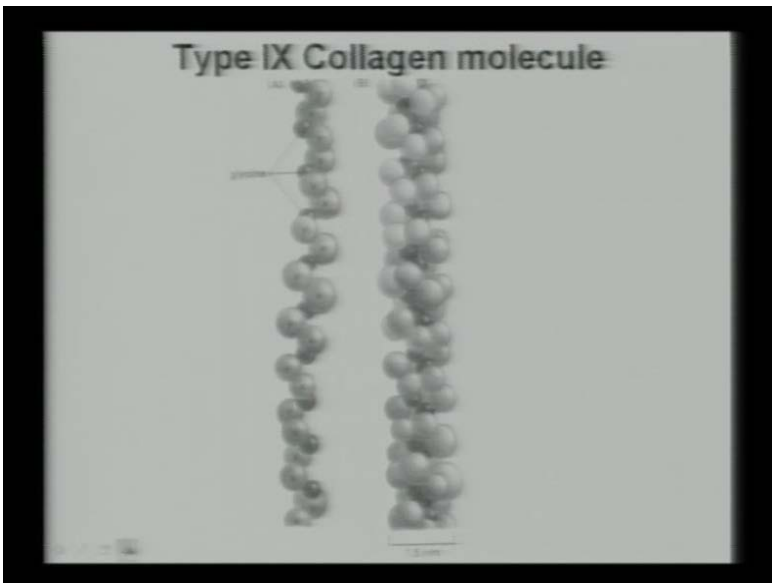

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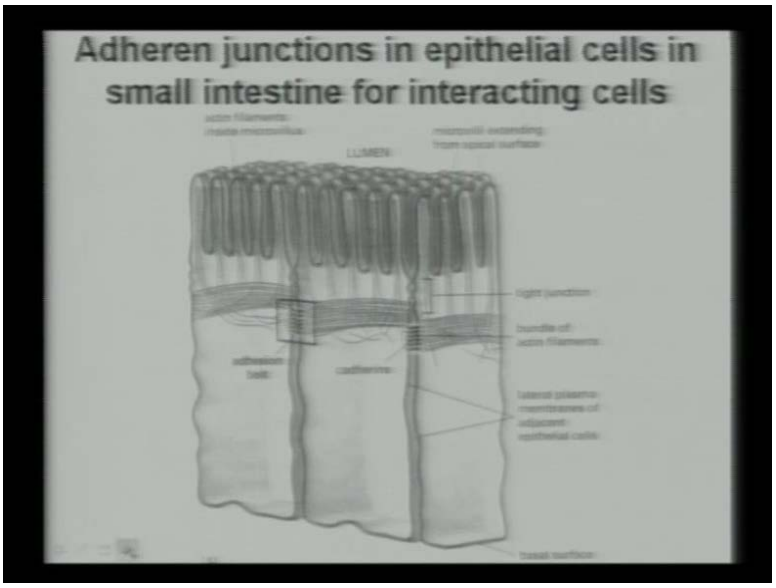

cell death on necrosis

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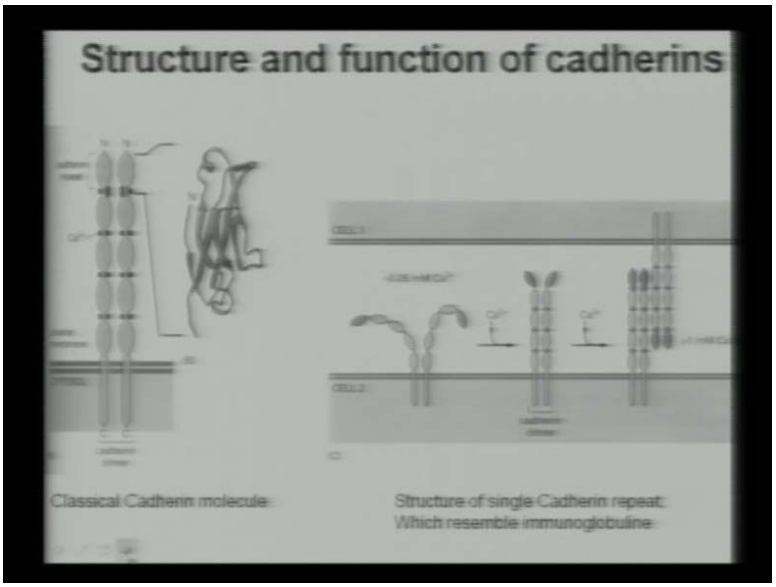

are so on.

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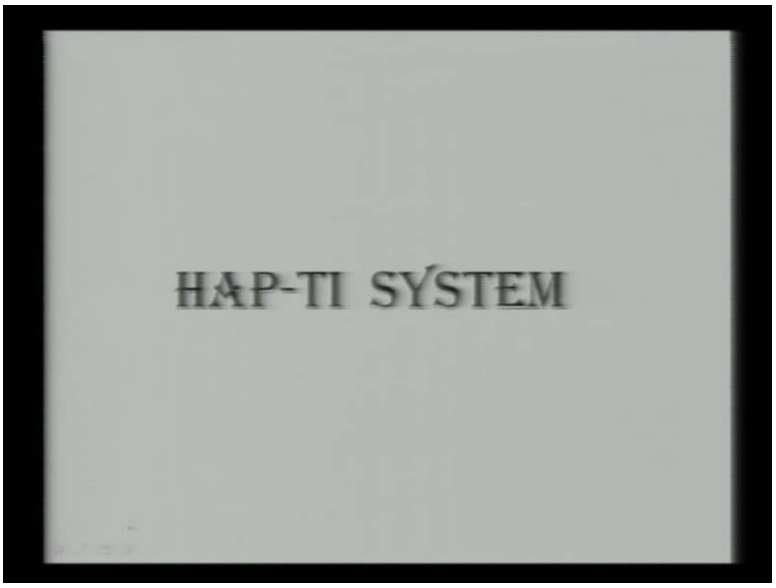

Now we have perform

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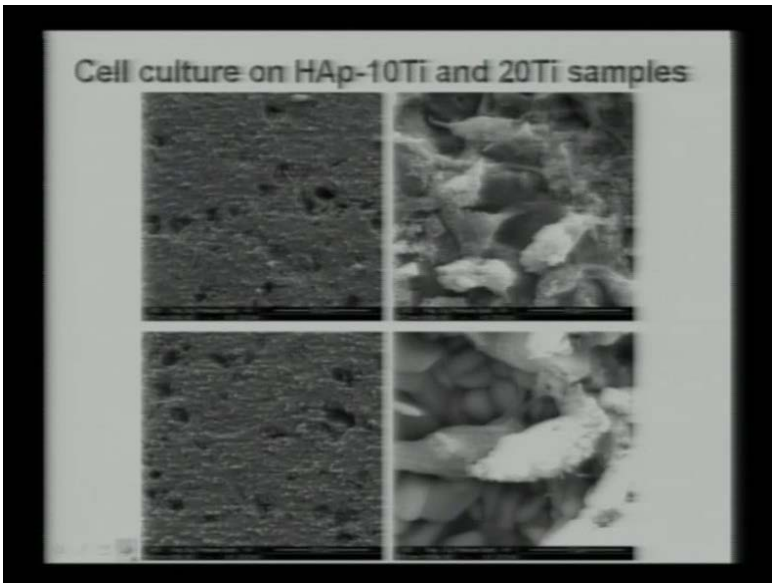

different experiments of these hydroxapatite (( )) those things I will mention when i'll discuss individual systems which are based on the hydroxapatite.

So I think with these I'll finish the finish the lecture of the cell adhesion and how the cell material interaction they take place.