Biomaterials for Bone Tissue Engineering Applications
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Week- 03
Lecture- 11

So in the last module we ended at the discussion on the structure and characteristics of a protein molecule. let me remind you that in a typical biological system you have large number of protein molecules and this protein molecules are abundant in any biological cells and therefore some understanding that how this protein structure is built up is necessary. So keeping that in mind I have discussed that two amino acids when they react then they form a peptide bond. So this peptide bond has been shown here.

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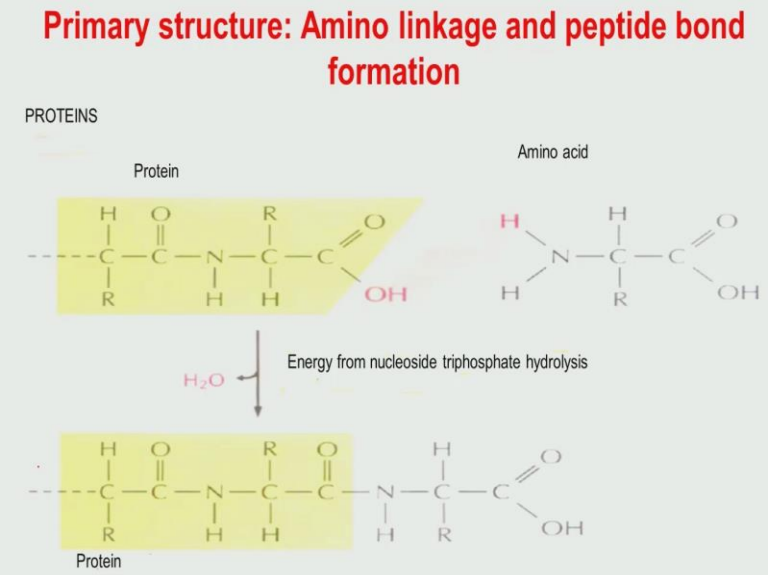

So you have this is C terminal of one amino acid, C terminal of a protein and N terminal of a, so there is a N terminal of a protein here. So here some the N terminal and there is also C terminal of a protein and you have a peptide bond which establish the linkage between 2 amino acid here, and these peptide bonds are formed and then this is your polypeptide chain. And in this polypeptide chain depending on the chemistry of R and R prime, say for example this is R prime, the protein molecule have different conformation.

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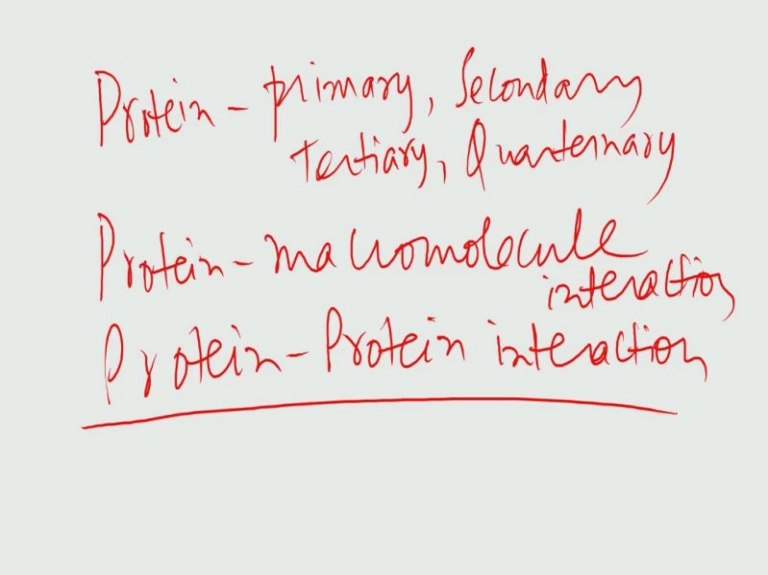

And then other things that I have mentioned in the last lecture, in the last module is that protein has several; protein structure can be described at different level. 1 is the primary structure of a protein, second 1 is the secondary structure of a protein, third 1 is tertiary and fourth 1 is quaternary. So each structure, the higher level structure is essentially being built up based on that lower level structure and as the level increases the complexity of the protein structure also (increase) increases.

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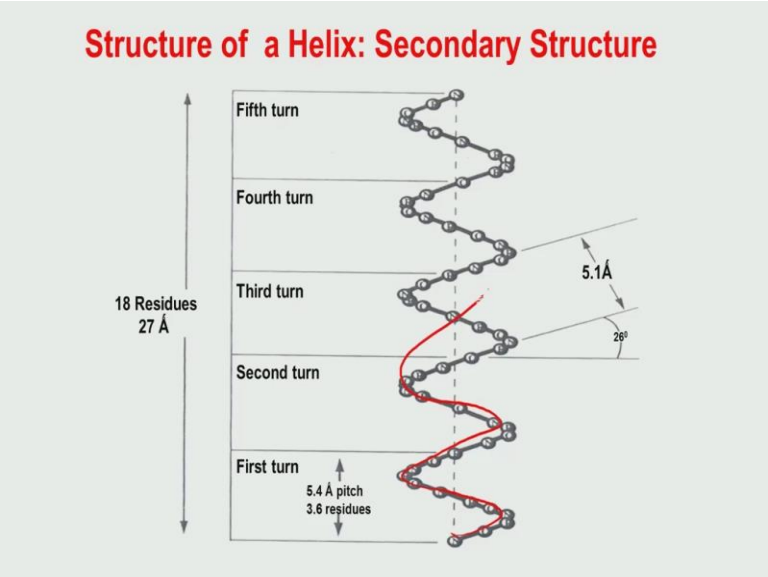

So while discussing the different level of structure I have also mentioned certain length scale that one has to one has to remember. For example this is a typical helical structure of a protein molecule, and it has 5 turns. So total length of this 5 turns is 27 amstrong and with each turn is

measured to be around 5.4 amstrong that is the peach. So each bend that whenever each turn this helical structure is being bend, so that angle of bending is somewhere around 26 degree, and this length is around 5.1 amstrong.

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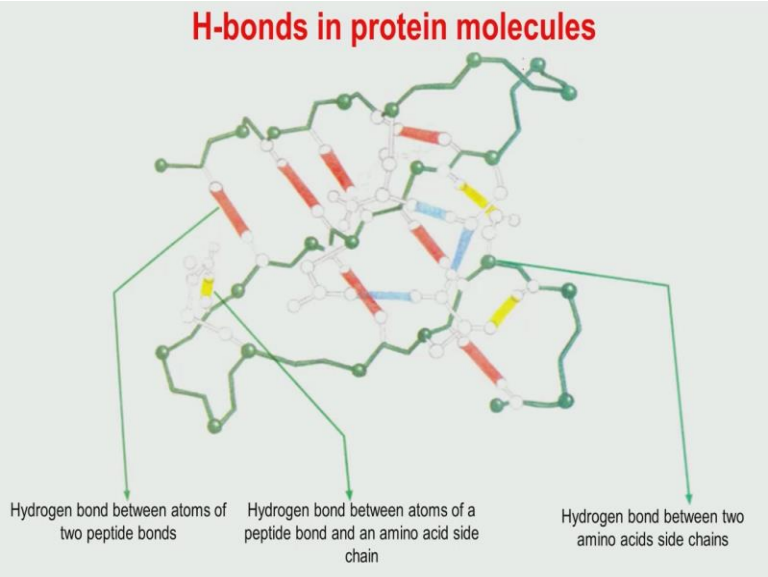

So the interaction between the 2 protein molecules is also established by very weak van der waals bonding or hydrogen bonding which is mentioned in the last module.

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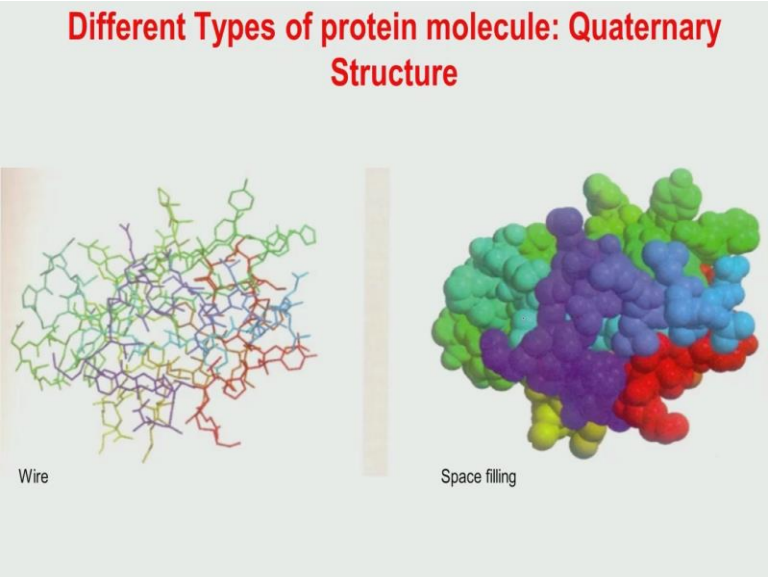

Also mentioned was that, that in the quaternary structure can be described either in the wire model or in the space filling model and this model can be can be understood by computational

modelling of the protein structure which itself belongs to a entire subject of protein mix or protein structure, so that is that goes to the discipline of the biological sciences altogether.

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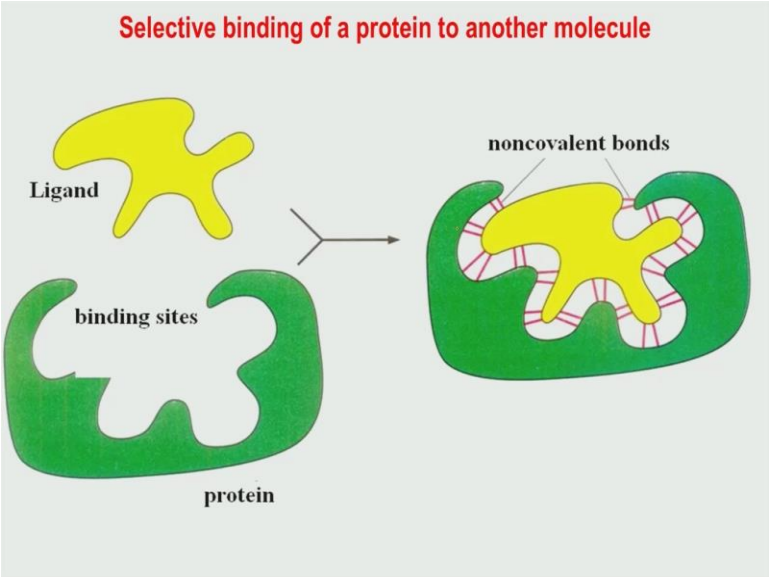

The selective binding of a protein to another biological macro molecule is important and then protein, protein interaction both are important. So what I mentioned in the last, in the, in the last module is that protein versus biological macro molecule interaction, and, and also this protein, protein interaction both are important in the context of particularly biomaterial science; simply because that as I will be dealing in today's module that cell material interaction essentially mediated by the protein, protein interaction.

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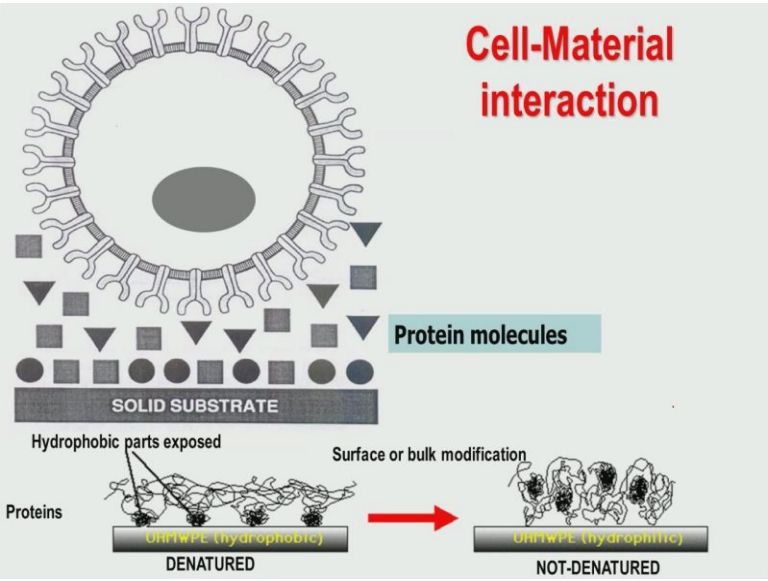

So now coming to this cell material interaction, let me explain this central concept, and which is very important in the overall development of this biomaterials. Because one has to understand this cell material interaction to the finest level as much as possible. So let me begin with the typical description of an eukaryotic cell; in the last lecture I have mentioned that eukaryotic cell represents a much more mature development of a cellular architecture, with double layer membrane structure. This double layer membrane has a typical thickness of few nanometre. We have a very well defined nucleus structure. In this nucleus you have a DNA that is a triple, DNA has a typical helical structure.

Now there are various organelles which are dispersed in the cytoplasm which include mitochondria and so on. I have also mentioned in the last module that cell membrane has certain characteristics, and these characteristics include presence of certain trans membrane proteins. Trans membrane proteins means I repeat, proteins which extend from cytoplasm of the cell to the extra cellular space. Now these proteins, this integrates essentially take place, (take) take an active part in the cell material interaction.

The other things that I had mentioned in the last lecture; just to refresh your mind, that cell membrane also has some well defined pore channels. Now this pore channels have certain specificity towards the transport of the some of the ions. For example, some of the pore channels can allow either sodium potassium, exchange to take place between the cell and the, cytoplasm and the intercellular extra cellular region.

Whereas some of the channels are specific and they have, and they can only allow the transport of the calcium channel; and that too particularly in the, in response of certain external, I mean the, these are like volta sensitive so the response to a volta sensitivity so they allow this calcium transport out or inside the cytoplasm.

So having said this, this, this kind of ion transport, particularly calcium as well as sodium, potassium are important as far as the cellular homey structures is concerned. Particularly calcium is also important when we talk about this functionality of the bone cells, bone mineralisation and so on. Now this is all about cells.

Now coming to the proteins you see the different kind of shapes of this geometric objects. I have mentioned here. Now each geometric object essentially represents different protein conformation or different type of proteins. Now this protein molecules you see this is a solid substrate here,

this solid substrate can be either dense, that means non porous or solid substrate can be porous also. Porous means it has certain micro porosity or macro porosity.

The distinction of the micro porosity and (micro) macro porosity is based on the size of the pores, the size of the pore itself. If it is somewhere between 1 to 10 micron we call typically micro porous structure, if it is more than 40 to 100 micron, so on, then we call it macro porous structure. Okay so whatever is this porosity or dense structure it is the solid substrate, proteins will get adsorbed to this solid substrate immediately after the solid substrate is placed in the culture medium. Or in solid substrate is implanted into the into the animal tissue then this solid substrate will have immediate interaction with the protein molecules, simply because you have such an abundant pool of the protein molecules.

Okay now once this proteins gets adsorbed, which takes place within just few minutes after the after after after placing the solid substrate in the culture medium; then what is the next stage, next level of this one. Then next level is essentially is that how this protein molecule their cell surface in different proteins, or cell surface receptors interact with these proteins which are adsorbed in the material substance.

So this interaction essentially takes place like a tong. You (have) you remember a tong and this tong can be used to hold some (sample) hold some objects; similarly if you consider the transferable protein as the tong, then these adsorbed protein has one of the objects that they tend to get hooked to and therefore this will be hooked to that individual cell surface receptors. So this is essentially a physical mechanism, which takes place in the early stage of the cell material interaction.

Now other things that I must mention here, ideally a cell is a cell has more in a typical freely cultured medium (or or or) or in a culture medium, cell has typically a spherical shape. So the stability of a spherical object on an otherwise flat substrate is also involved, or it also determines that how a cell would interact with a, how a cell itself would interact with a material substrate.

Now before going further, or before going into more depth on the cell material interaction, let me also tell you that this proteins depending on the surface wettability characteristics or the surface properties. Then protein can get either denatured, so denaturing means essentially that protein molecules, (protein) proteins would not express its desired functionality, or it would not express its desired function, and then it will not be in a simple coil form or it is trying to straighten up and so on.

So this example is shown here with the help of this ultra molecular polyethylene which has typically a hydrophobic surface. Now you can do certain surface treatment like oxy plasma treatment, or UV treatment or some other, some of the surface treatment, to make this ultra molecular polyethylene has a hydrophilic surface. Now once the surface becomes hydrophilic either by surface or bulk modification then (same) same protein molecules get, (same) same protein molecules are not denatured anymore.

So this denatured and not denatured state are important because if the protein molecules proteins are denatured then it cannot effectively contribute to the interaction with the cell surface receptors. So it is important to for the protein molecules to retain its original conformation so that it express its desired function.

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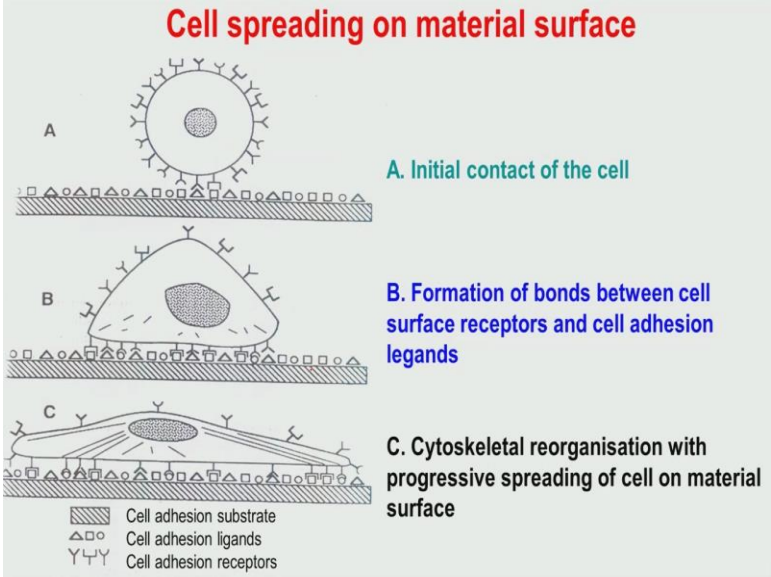

Okay now, I mean having explained this one, now let me tell you that what is the next stage or what is the next level of interaction of a biological cell on a material substrate. At the next level what happens; as I said that a spherical object, the stability of a spherical object is much more stronger or would be much more stronger if the spherical object now changes its shape. So that it becomes more flat towards the material end, or towards the, towards the side which gets exposed to the biomaterial substrate. And that is what exactly happens in the next stage of the cell material interaction.

You can see that this shape is like off spherical shape. So this is your off spherical shape and this off spherical shape essentially allows larger number of cell surface receptors, the way I am drawing in or I am trying to sketch it; large number of cell surface receptors to interact with more

adsorbed protein molecules on the biomaterial substrate. So from this simple description or from the simple schematic illustration, you must be in a point to realise that; larger this cell surface receptor protein interaction; more would be the stability of a, of this particular , of this particular spherical, spherical shaped eukaryotic cell.

Now the way I am describing these things it does not take place so easily or so quickly on a material substrate. Simply because any change in the cells, any any change in the cells shape has to synchronise with that of the cytoskeleton reorganisation process, which I have emphasised to a greater extent in the last and last 2 modules. That is cytoskeleton reorganisation, essentially means that this acting filamental structure has to be depolymerised and repolymerised.

So this depolymerisation which I have mentioned in the last module also, so depolymerisation means that acting filament, (go) acting filament is forming lot of monomers and then repolymerisation means these monomers get assembled together to form again acting filaments. And then the third stage you can see that the cytoskeletal reorganisation is also time dependant phenomena, this depolymerisation and repolymerisation, therefore the more the time the cells spend in contact with the biomaterial substrates, more is this dynamical cytoskeletal reorganisation process takes place. As a result a single biological cell can be stretched to a maximum extent.

And let me also tell you the more, more the stretching of the cytoskeleton; or the more the shape change that the cell would experience; more would be the cell adhesion on a biomaterial substrate. Now the cell adhesion; another term that also is important here is the focal adhesion complexes. Now this focal adhesion complexes are essentially a cluster of a cluster of cell surface receptor and adsorbed protein clusters. Now the more the focal adhesion complexes that form on a biomaterial substrate, more stronger would be the cell adhesion on that particular substrate. Now these points you should keep it in mind and then (we will) we may recall at certain point of time later on during the cell fed processes, okay.

Now, to sarise it that you have the eukaryotic cells truly nucleated cells; you also have the, the cytoskeletal reorganisation process, dynamic cell shape changes, focal adhesion complexes formation and cell adhesion takes place.

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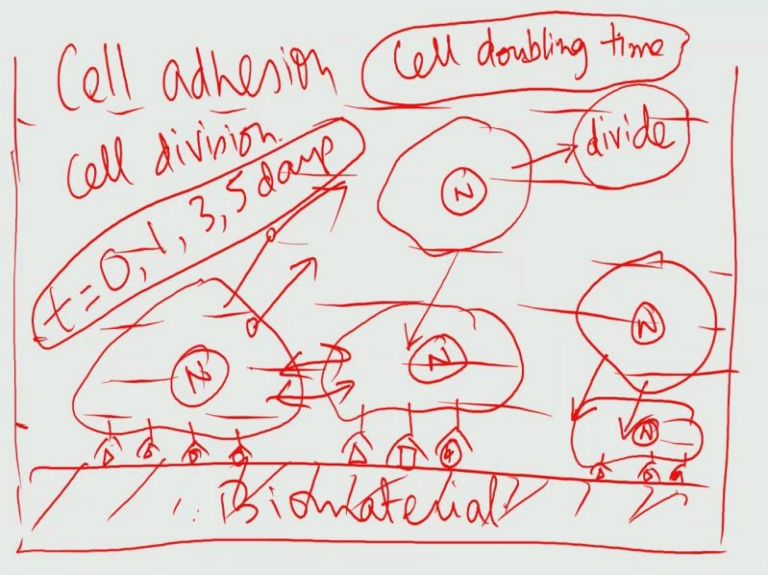

Now the moment the cell adhesion takes place on a material substrate; so the cell adhesion here you have seen that protein protein interaction, protein protein interaction takes place a major role, right? Which I have mentioned here that protein protein interaction takes a major role in the way cell material interaction takes place.

So the moment cell adhesion takes place, next thing is that; suppose this is your biomaterial substrate, this entire biomaterial substrate is placed in a culture medium. So culture medium typically has 10 to the power 4; 10 to the power 5 number of cells. So this is a large number of cells and here your eukaryotic cell; here you have this cells surface receptors and adsorbed protein molecule interaction and so and so forth.

So once this particular cell establishes a rather firm interaction with this biomaterial substrate; what it will do? It will send out the cellular signals, or cell signals to the extra cellular region. Now if some of the cells are floating here or some cell is floating here, what it does? They will send the signalling, (s s) they will send the signal to the neighbouring cells, so that this neighbouring cells also would come and also would try to interact with the biomaterial substrate because this biomaterial substrate does not cause any toxicity to the cell whatsoever.

Now this, when this guy also comes; this guy also kind of interacts with the same substrates. Now when we have multiple cells that are lined up on a same substrate then that is something called cellular crosstalk also takes place. Crosstalk means just like 2 persons, 2 human beings; if they stand at a bus stop so they can talk to each other. So similarly that when the, when these 2 cells they are sitting on the same biomaterial substrate, they can also establish the crosstalk.

Crosstalk essentially means there is a continuous signalling processes that is going on between these 2 neighbouring cells.

So cell adhesion, then cell signalling and then that more number of cells will come into, come in contact with the biomaterial substrate, then what? Now once this cell signalling processes enables that more number of cells to be attached to the biomaterial substrate; the next step would be that, we have to understand that whether this, so let us say if it is a stem cell or if it is some other type of cells like osteoblast cells and some. So whether this cell; this particular cell when being attached to the biomaterial substrate; whether it would be able to divide the way this particular cell would divide here.

So as I said the fundamental definition of cell, is that cell is a self contained unit with a capability to propagate itself given the nutrient and proper environment. That means when you are dispersing the 10 to the power 4, 10 to power 5 number of cells in a culture medium, which contains nutrients, which contains antibiotic, which also contains several protein molecules, the point is that; that in a phyllic suspension condition the cells can grow, cells can divide one to 2, 2 to four and so on.

Now the point is that whether same cell division process can take place when a biological cell is now adhered to a, or now adheres to a biomaterial substrate. So in other words, so one can see that whether cell division takes place. And that typically people, that typically, that typically people investigate by culturing them over a (la) over a different time point in culture, in a in a culture medium.

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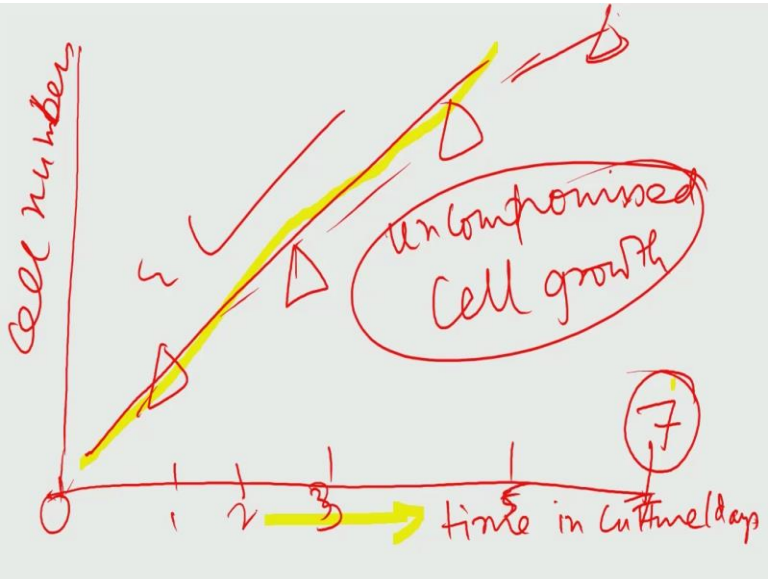

Let me explain this particular point little bit more, and this what I am saying is that suppose you are culturing this cells, any (ce) any particular cell at a given time point; suppose you are culturing cells at a given time point, let us say this is the point that you, you are essentially you are you start the culturing, cell, you are start the cell culture process and this is your cell numbers.

So suppose this cell numbers if you plot as a function of time of culture here; so along x axis if you have a time in culture, so this. So now if you plot along the y axis cell numbers. Okay so what I showing here is that suppose you start the time, cell culture at a time equal to 0, T is equal to 0 time point then you allow the cell growth to take place for 0, 1 day, 3 day, 5 days.

Let us consider the hypothetical culture system where you are growing a cell of cell type X for different time period in culture. Now after the experiments are over; now if you are counting or quantifying the cell numbers and along x axis you are plotting time in culture, let us say in terms of days. Then here you started with T is equal to 0 then you were seeing that how cell growth is taking place, so they said it is day 1, this is 1, this is 3, this is 5 and then you have a data point which is falling closely more or less like a linear linear variation.

That means if the cell numbers increases more or less linearly with time that means cell growth is also linear in nature, and that is a testimony to the fact that cell cells while being attached to a biomaterial substrate their cell growth or (share) cell division is not being compromised. Or it is a like a testimony to the fact this is a kind of a uncompromised cell growth take place.

And these things is important for one is to establish, simply because that typically biologists; they grow cells in a culture medium without anything, without any substrate on it. Or in a culture, or, or in a tissue culture plastic just as a control sample, but not a stiff biomaterial stiff biomaterial substrate. So therefore if you want to describe this particular scientific process to any person who does not know much about the biomaterials; first question they would like to ask you, that what about the process or how the cell functionality would be changed which I will explain to you more in detail later on.

But at this time, at this stage you must (re) understand and realise that first thing 1 should check when you are culturing cells in a biomaterial substrate; is to see that when you are growing the cells at, at least at 3 different time points and quantify the cell numbers and see whether the cell growth has taken place linearly or there is no change in the cell number.

If there is no change in the cell number which is little bit unrealistic to consider, then you cannot say that this biomaterial substrate, this hatched separate biomaterial substrate can be used as a cell growth substrate. You can only confirm that point only when this particular kind of behaviour you can establish by culturing the cells on their biomaterial substrate.

Other points that I must mention at this juncture is that; that how one can select that what would be the different time points at which you will culture or what are the different time intervals that you should check that how the cell numbers are increasing or something. So this depends on what is the cell doubling time. Cell doubling time means what is the typical time frame or what is the typical time line at which cell is a, an isolated cell is expected to, an isolated cell in a culture medium is expected to divide.

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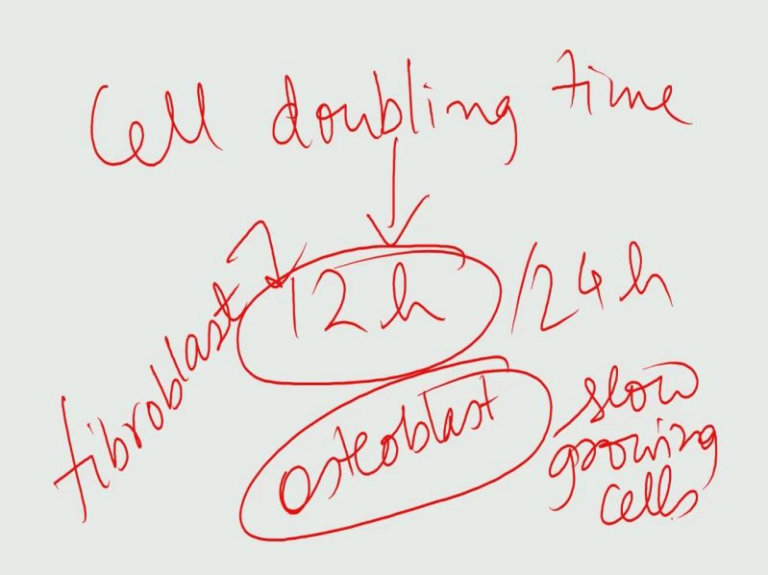

Now cell doubling time is somehow varies somewhere between 12 hours, 24 hours or 36 hours. This is a typical human cell, human cell I am talking about; so, so 12 hours or 24 hours and so on. So some, for example fibroblast cells they are fast growing cells, fibroblast means connective tissue cells. But osteoblast cells they are relatively slow growing cells that is the bone forming cells, so this is the slow growing cells.

So osteoblast cells they take typically much longer time to divide and therefore you may need to extend this just to see that what is the cell growth pattern on a biomaterial substrate particularly when you use osteoblast cells; you may need to grow them for upto 7 days, because if you grow them upto one day you do not see (major m) many of the, many of the cells on the substrate. But if you start growing them at day 3, day 5 and day 7, you should be able to see, you should be able to see more and more number of cells, simply because osteoblast cells are slow growing cells.

However for fibroblast cells which is also used in many cell culture experiments involving biomaterials so fibroblast cells since it is fast growing cells then you can culture them upto 1 day, 2 day and 3 days also.

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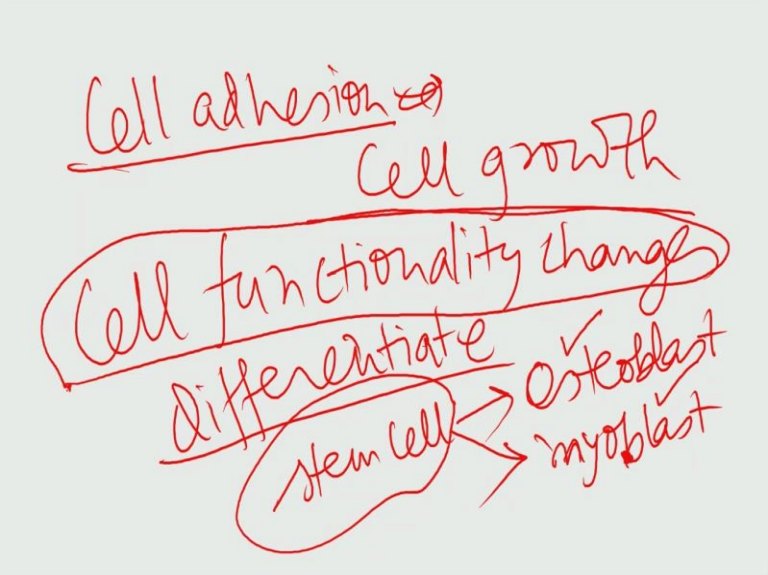

Okay so now that cell adhesion takes place and cell growth subsequently takes place, so cell adhesion, then cell growth. The other functionality of the cell, so essentially if the cell growth takes place on a biomaterial substrate that is a reflection of the fact that cells while being attached to a biomaterial substrate is able to divide.

Now what about the cell functionality changes? Cell functionality changes means, suppose you are growing a stem cell and this stem cells can can differentiate; differentiate means that is a differential cell (ex) gene expression; so can differentiate to other cell types. So stem cells, let us they can differentiate to let us take the example of human mesenchymal stem cell or bone marrow derived stem cells, then it can differentiate to osteoblast cells for example, bone cells. Or it can differentiate to myoblast cells; that is the muscle cells.

So, all this cell differentiation process is possible from the stem cells. Now this cell differentiation means your genes, specific genes related to the osteoblast, specific genes related to the myoblast are to be up regulated or to be over expressed compared to the compared to some housekeeping genes. Now this cell functionality changes suddenly takes little longer time than the time frame typically you grow cells just to check for the cell growth process.

So this cell differentiation is one of the important aspects because when you are growing a cell on a biomaterial X or growing a cell in biomaterial Y, one should be curious enough to see that whether that growth of the cell in a (particular) on a particular biomaterial substrate would also influence the cell functionality changes or not.

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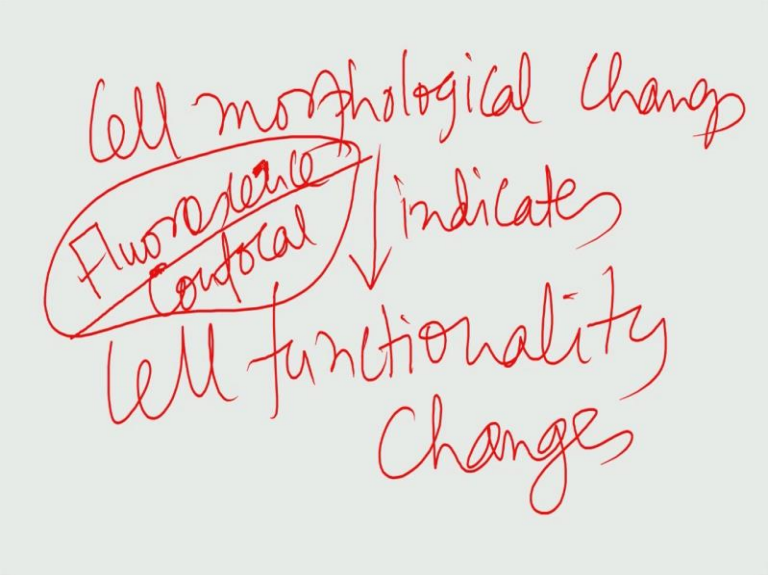

Now to answer the questions; you need to grow the cells much for a much longer time point, let us say a few weeks, let us say 2 to 3 weeks, 2 to 3 weeks just to understand or just to confirm whether there is any indication of the cell functionality changes. Now as I said that indication many times comes from the way the cell morphological changes. As I said that morphology of the cell is important, so cell morphological changes essentially indicates any cell functionality changes, okay.

So the cell, so cell morphological changes essentially can be investigated using some of the microscopy techniques, this microscopy techniques are something which is very special to biological sciences discipline and not that much used in traditional material science research. And this microscopy techniques are; one is called fluorescence microscope and another one is confocal microscope. So if time permits, I will explain to these 2 microscopy techniques with little bit more details.

But at this stage you should know that this fluorescence microscope and confocal microscope; since they are specially biological research oriented microscopes, you need to prepare the samples little bit differently than one normally do in conventional material science research. So (you) you cannot use the samples just like after culturing you dry and put it on the, put it below the fluorescence microscope put it under the fluorescence microscope, that is simply not not possible. You need to do certain sample preparation, you (used) you need to use certain fluorescent dyes to excite certain proteins on the; certain proteins in the cellular structure before one can record certain fluorescence (Micro) fluorescence images.

So therefore not only quantification but also that how this morphology is changing with time in culture; that also provides lot of important information whether the cell is undergoing any cell functionality changes or no. I think I will stop here and then I will continue this in the next module.