Introduction to Biomaterials

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Lecture No. # 04

Structure and properties and cells, protein and cellular adaptation process

In the last few lectures I have first taught you, about that, about the definition of biomaterials, biocompatibility, post-response and as well as, the importance of biomaterials. Then, that was followed by the molecular biological description of biological cell.

So, molecular biological description of biological cell, that time I have focused on that, you know, definition of the cell, what is that, what are the different components of the cellular organelles and how their structure looks like, in terms of the membrane structure like, you know, it has a double membrane structure. Most of the cellular organelles are of the double layer at the membrane and there, includes by the double layer membrane and also on the cytoskeleton, like what are the different components of the cytoskeleton, like active filaments, micro tubules at the intermediate filaments, how their property looks like.

In today's lecture I will continue the description of that further, molecular biological aspect of the biological cells, in terms of the cell cycle and some other relevant information, which are, which should be useful in understanding the cell material interaction as well as the cytocompatability or the biocompatibility behavior of the materials.

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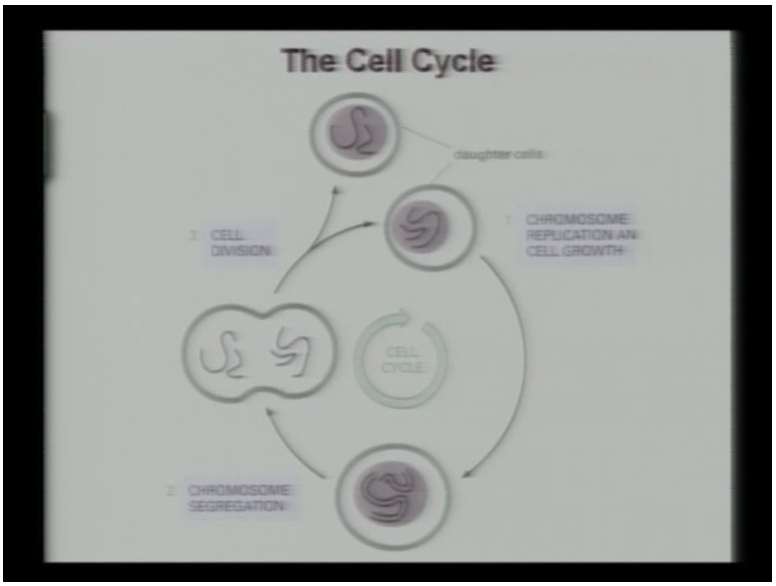

So, cell cycle – now, one biological cell, as a set, in the definition of the biological cell, that is, cell can be defined as a self-duplicating unit capable of duplicating itself by genetic information, which is enclosed in its nucleus. And therefore, that when you start with, that any particular biological cell, that chromosome duplications in cellular growth that occur.

Now, cellular growth is a part of the cell-fate processes; cell-fate processes means, it is a combination of cell proliferation, cell adhesion, cell differentiation, etcetera.

Now, when the cellular growth takes place, then cell also increases in size because I will show, mention it to you later on, that cell has a kind of very much elastic properties and cell can undergo different cellular adaptation process depending on the environment, as well as external stimulus etcetera.

Now, if you see here, that is, a chromosome segregation takes place prior to the cell division process and cell division also composed of two states, in the 1st is the cytokinesis, and cytokinesis, and then, 2nd one is mutation. And cytokinesis, you can see that this chromosome segregation takes place and then sales are also going through a transition in terms of the cell shape.

You can see the cleavage in the cell shape here and you can see this kind of cleavage. So, that is kind of one set of the cell division process and subsequently, the one given cell, it

can be, it can lead to the formation of the two daughter cells and these are the two daughter cells. And these two daughter cells can undergo again, chromosome replication, cell growth processes. So, this is the way cells multiply in a given biological environment.

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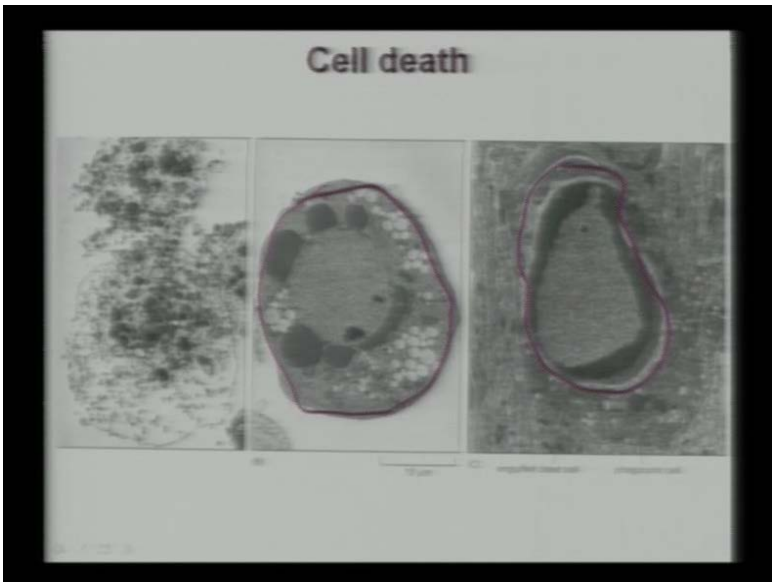

Now, cell death, it is also known as necrosis or also, programmed cell death. Now, when cell death takes place, typically, you can immediately realize on the scanning electro-micrograph or in a, in the flourosis micrograph. If the cells appear like, you know, typical globular shape or typical spherical shape, then you can, you can very well say, that cells or cells undergo apoptotic processes, apoptosis, cell apoptosis means cellular death. Now, you can see that this is the kind of very much spherical type of cells, which are typically observed in the case of mammalian cell lines.

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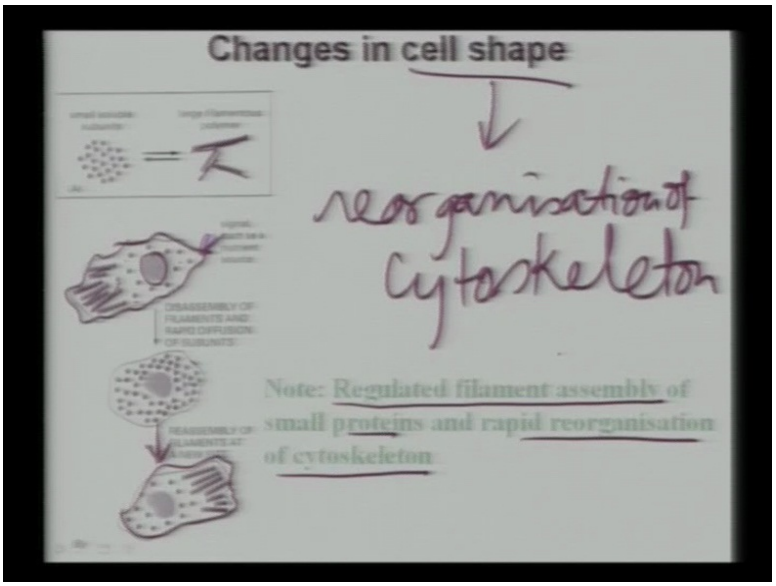

Now, how the changes in cell shape processes take place? As I said, that cells can change its size, cells can change its shape depending on the external environment. Now, if you see the first step, like you know, you have small soluble subunits like protomolecules and then it can form large polymers, filamental polymer in the cytoskeleton. And if they get a signal, such as a nucleon source or something, then that acts of the signal and then you can see, that cells also changes its shape. It is a nucleus, these are like globular soluble subunits and these are like filament forms, which are nothing, but protein aggregates.

Now, what they do, that this large filamentous polymer, they can also break down to smaller globular subunits and subsequently, they can reassemble by taking the proteins or signals taking the nucleon source from the external environment. So, entire process leads to the increase in the cell size, as well as the cell shape. So, that is why, it has been written here, regulated filament assembly of small proteins and rapid reorganization of cytoskeleton.

So, essentially, you have to remember all the time, that cell shape process is nothing, but reorganization of cytoskeleton. Cytoskeleton is kind of a reorganization of cytoskeleton and these cytoskeleton is composed of you know, acting filament, as well as the intermediate filaments and so on, and microtubules. So, if they can, either these assemble

or reorganize itself. When they receive the external stimuli, then cells can change its shape.

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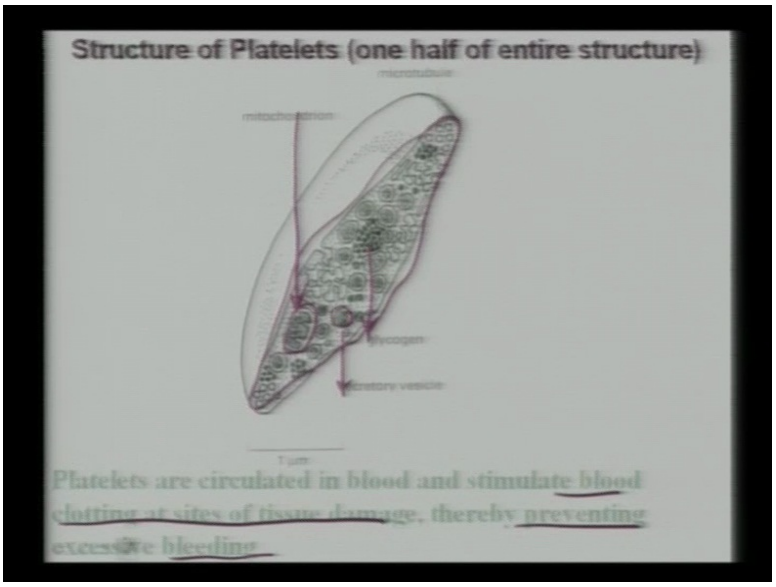

Now, this is the typical structure of the, because till now I have showing to all the different structure of the cellular organelles. Now, this is the typical structure of the platelets, blood platelets, it is like one half of the entire structure; you can see this is like a half of the structure, is actually to show you the internal organelles, which are present there.

Now, you can see these are like globular subunits, which is known as the glycogen. You have also secondary basic cells here and you have the mitochondria. Mitochondria, as you know, it also is enclosed by a double membrane and this platelets are circulated in the blood and they stimulate blood clotting at the sites of tissue damage and thereby, preventing excessive bleeding.

So, once the blood clotting takes place, then bleeding can be stopped and that is essentially enhanced by the presence of the platelets.

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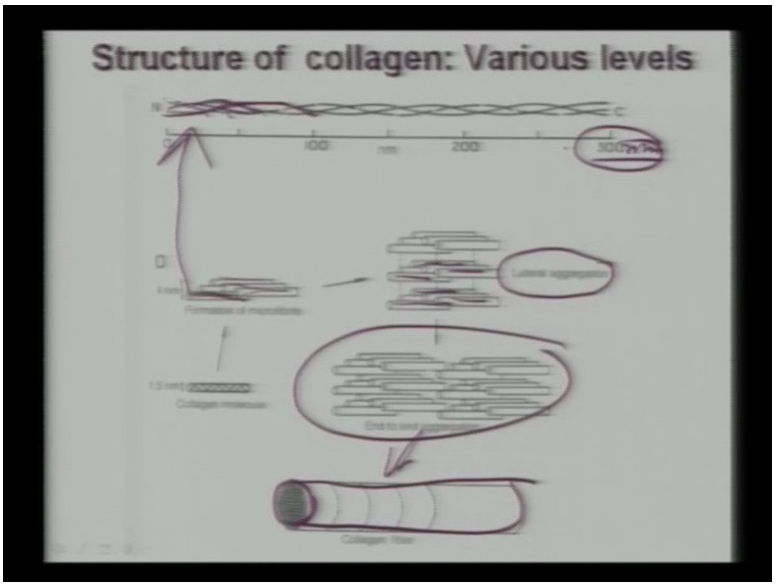

Now couple of slides back I explained to you the structure and composition of the human bone, human natural cortical bone and there, I said, that cortical bone can be described as a polymer ceramic compose, you have the 65 percent collagen and you have the 20, sorry, 65 percent hydroxyapatite particles, nano size, nano size natural hydroxyapatite particles and you have also 25 percent collagen. Now, collagen is essentially polymer, hydroxyapatite is essentially ceramic and therefore, this bone can be very well described as a polymer ceramic composed.

Now, structure of collagen, if you look at the various level, now it also is, it, typically, it has a very micro and ultra micro scale. It has a triple helix type of structure, triple helix means, you can see, that this is the one part of the collagen, this is the dotted, is the another part and then, 3rd one is that typically collagen, composed of triple helix structure and then entire micro fibril, it is somewhere around 300 nano meter.

So, this kind of numbers are important so that you understand, that you know, that collagen, what is the typical length scale and I mean, how collagen also looks like. So, further, you can see, that how this, you know, collagen fiber takes place or the micro scale, I said, the collagen micro fibril and this is that one of the micro-fibril. So, expanded version of the magnified version is this one, which has a typical length of up to 300 nano meter.

Now, this micro fibrils, essentially the aggregate first and then, this cluster of micro-fibrils, they are bonded by the hydrogen bonds, by lateral hydrogen bonds and that leads to the lateral aggregation. And this end to end aggregation that leads to kind of a collagen fiber, you can see, it is like a fiber bundle.

You could remember, in the material science many times people teach you about the fiber reinforce composites, so how the fiber looks like? So, that is the how the collagen, also collagen fiber also will look like and, but this collagen fiber basic building unit is the triple helix structure, which has a length scale of around 300 nano meter.

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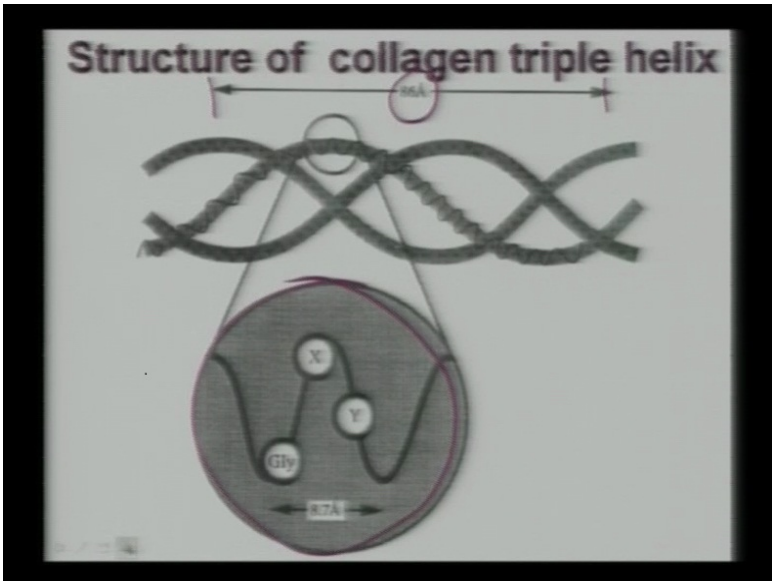

And on an individual triple helix, it has a length scale of 86 Angstrom, which is extremely fine and this is the glycoprotein, and then other types of proteins. And collagen, there are several types of collagen, like collagen type 1 and so on. That depends on the, how the structure of the collagen, individual collagen micro-fibril, that changes in terms of composition as well as to orientations.

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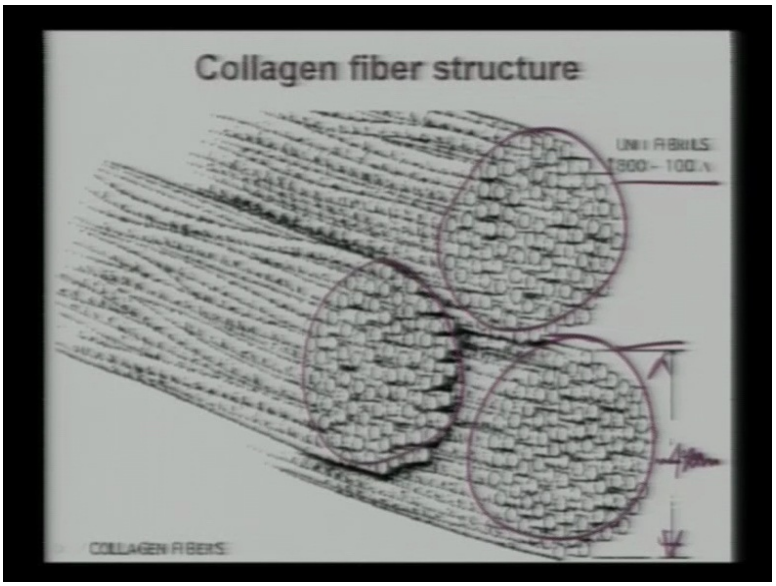

This is like more details about the collagen fiber structure. You can see, that this is one collagen micro-fibril aggregate, this is another collagen micro-fibril aggregate, this is the 3rd aggregate.

So, this is the spot of the collagen, large collagen fiber and each micro-fibril aggregate if you notice, they have a kind of dimension of around 4 micrometer, 4 micrometer, that is the kind of a diameter and unit fibrils, they have a radius of 800-1000 angstroms. So, this is the typical dimension of the collagen fibers structure.

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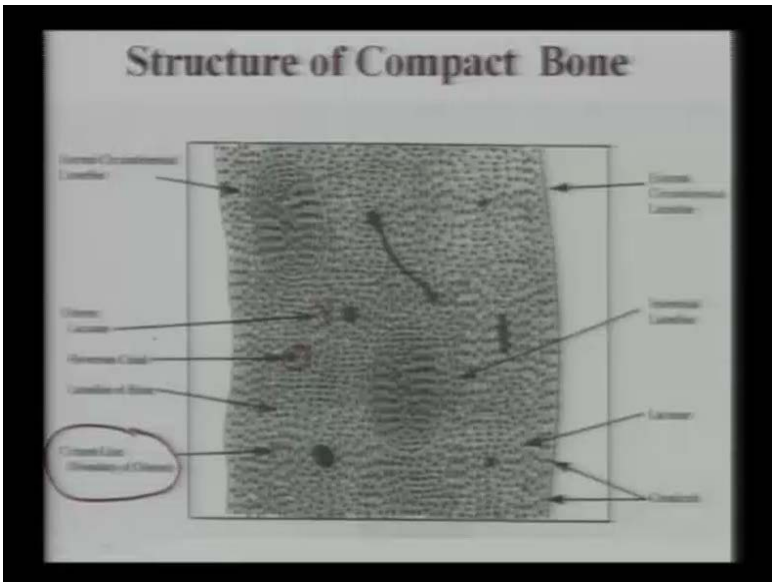

Now, how this compact bone looks like? Now, you can very well realize, that how does this compact bone, they look like in real life. Now, in the compact bone, you have this collagen fiber matrix and then you have these very nano sized hydroxyapatite particles, which are dispersed uniformly in the matrix and if you look at these little bit in details of this, how this compact bone looks like.

Now, first one, you can see that Haversian canal and what is Haversian canal? It is the kind of a channel, which supplies the nucleons in the blood to the bone cell. Then, 2nd one is the cement line, these cement line is the kind of boundary osteon and the osteon is nothing, but this is like individual osteon here, which is shown and this is like osteon lacunae.

Now, cement is required to build a house because what it does… So, it essentially makes the bond between the two bricks. So, similarly, here the cement line means, it is biological cement, which actually acts as a glue to put all the structure, collagen hydroxyapatite structure together.

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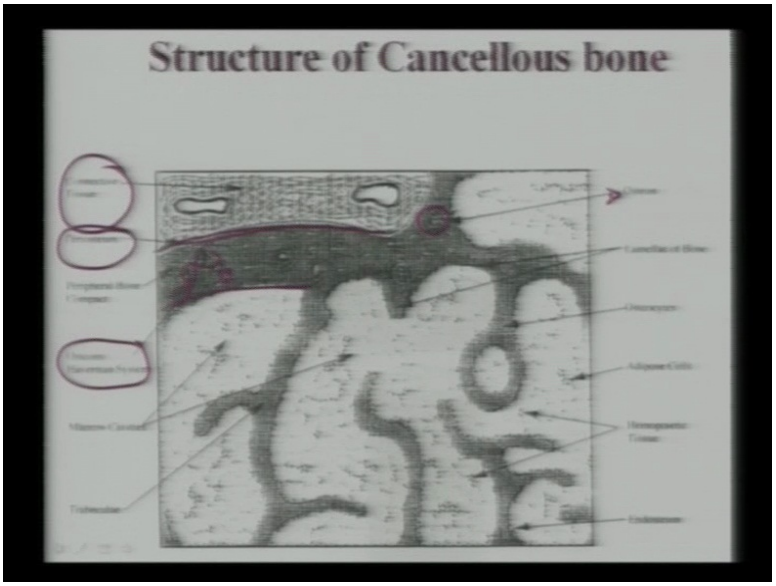

And this is the structure of the cancellous bone. So, there are two types of bones, as I mentioned, one is the compact bone, one is the cancellous bone. Cancellous bone is more porous type of structure; compact bone is more strong type of structure because it is a nonporous type of structure.

And in the compact, cancellous bone you can see, you can see the connective tissue and that cell lines of the connective tissue, as I said, fiber blast type of cell lines. You, still you can see, that osteons, which is the very small subunit of the typical contact bone. You can see, that osteons and Haversian systems, that is, the Haversian canals and so on and you can also see the periostium. Periostium means, like you have, like shirt in a, shirt has this kind of cover. So, like similar things, it is also present in the bone and this periostium region is that outer part of the bone structure. So, that is what is known as the periostium.

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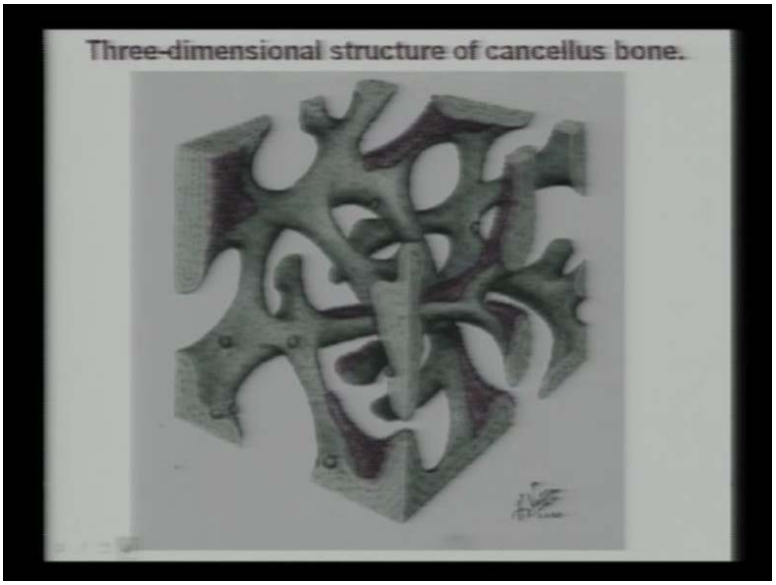

This is the unit three-dimensional structure of the cancellous bone. What you can see, that it has a highly porous structure and this porous structure, they essentially lead to very weaker mechanical properties of the cancellous bone.

Whenever a structure is porous, then it has a poor mechanical property, whereas compact bone, which is a very non porous type of structure, very strong structure; it has also very good mechanical properties.

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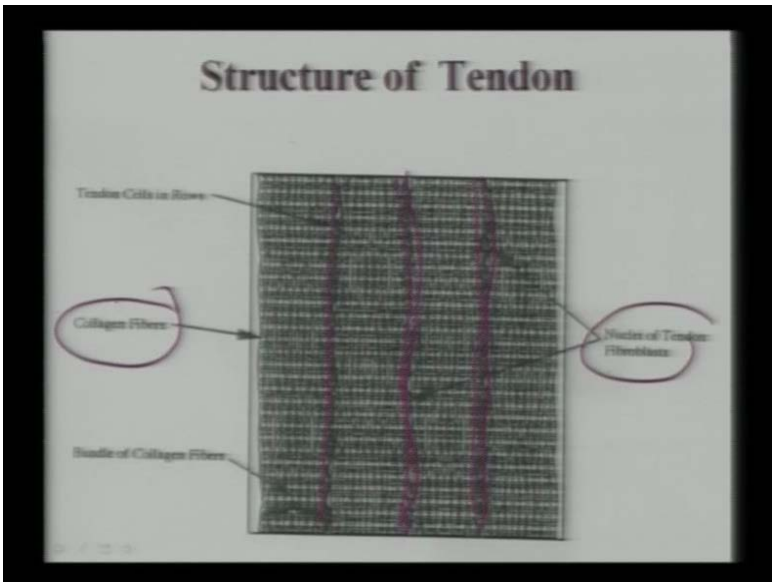

This is the typical structure of the tendon and this tendon cells typically present in a kind of a row and mostly it is a fibroblast or you know, connective tissue cell lines, which are present as an aggregate and then number of cells, which are kind of organize themselves in particular fashion and you also have the collagen fibers in these tendon cells.

Now, from the typical cell size and number of molecules you can find out, that what is the typical clinically relevant number of cell lines?

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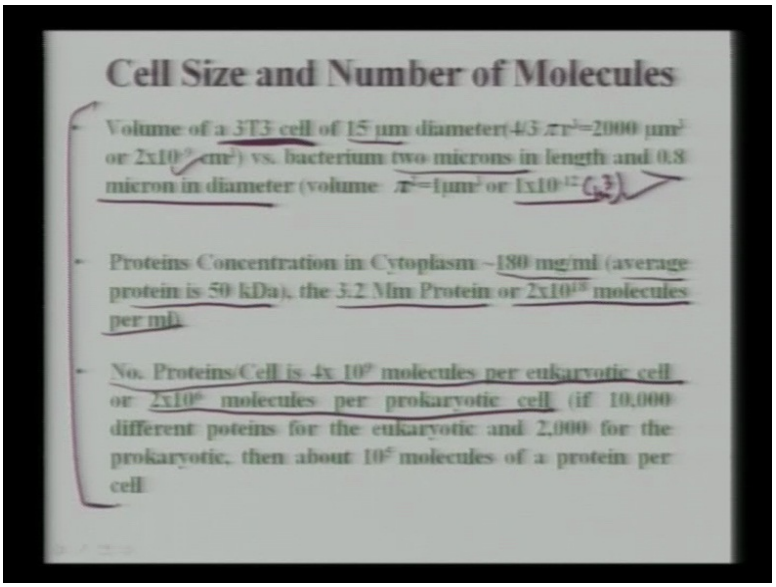

Let us say, this 3T3 cells, this is also fibroblast type of cells, like you can use the real 9 to 9, that is, small fibroblast cells. You can also, they use this 3T3 cells, this is also again fibroblast type of cells and it has a typical diameter of 15 micron. So, 15 micron diameter means, you can calculate the polymers 2 into 10 to the minus 9 centimeter cube and if you consider the bacteria, which is 2 micron in length and 0.8 micron in diameter. Now, it has a volume of typically 1 into 10 to the power minus 12, this is like centimeter.

Now, what you can see here? The typical bacteria like Escherichia Coli, which is a long shape and this long shape means it has a particular aspect ratio, like it has a particular length and particular diameter. Now, if you typically take this length and diameter is 2 micron and 0.8 micron respectively, then you can calculate, the volume is 1 into 10 to the minus 12 centimeter cube.

If you consider that versus that volume of a cell, now volume of a cell is nearly 3 orders of magnitude larger compared to that of the bacterial cell lines, then protein concentration is, cytoplasm is typically 180 milligram per milliliter and average protein, average protein mass is 50 kilo-Dalton and therefore, 3.2 million proteins or 2 into 10 to the power of 8 molecules per milliliter of proteins is available in the cytoplasm and therefore, number of proteins per cell is 4 into 10 to the minus 4 into 10 to the power 9 molecules per eukaryotic cells, or 2 into 10 to the power of 6 molecules per prokaryotic cells.

So, eukaryotic cells means, cells with nucleus; prokaryotic cells means, cell without nucleus; that is the example of the bacteria cells. Now, what you can see from this simple calculations, that you can have 10 to the power 9 molecules for eukaryotic cells, like mammalian cells or biological cells, whereas you can have 10 to the power 6 molecules because there is a 3 out of the magnitude difference in terms of the volume, so 10 to the power 6 molecules in the case of the bacterial cells. So, it is a huge number of protein molecules.

They are enclosed within a single unit cell because from the engineering aspect, it is important for you to have an idea of the numbers, like when you say, that large number of protein molecules, people would immediately ask you, what is the typical number? Typical number is 10 to the power 9 order of magnitude. Protein molecules, they are

contained in a single biological cell, 10 to the power 6 number of molecules; they are contained in a single bacterial cell.

Now, bone, composition-wise bone has an organic framework. Organic framework means, it has a kind of a, it is mostly like, like, either cells or collagens or other organic molecules.

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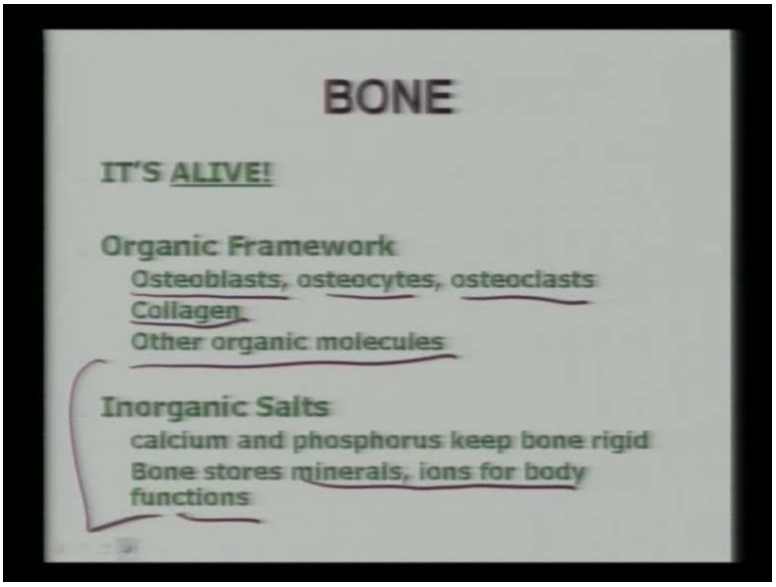

Now, cells, it can have bone cells, osteoblasts, that is, the bone formation cells. It can have also osteocytes and you can have osteoclasts, osteoclasts are the bone resorption cells. Bone resorption cells means, if any bone, they have a large number of osteoclasts, then this bone will dissolve in the (( )), so bone cannot survive. But if you have large number of osteoblasts, that means, bone growth can take place, that bones can survive and bones can grow.

Now, we can have collagen also in the bone, as I said, and other organic molecules. Now, inorganic salts is that calcium and phosphorous, so calcium phosphate type of free stress, they are present in the major fractions and therefore, bone stores minerals and ions for body functions, this is one of the important factor. Why we always test as osteoclasts (( )) bone mineralization test as part of the (( )) study? For any synthetic materials you would like to check, whether it has a bone mineralization ability or bone mineralization property for that particular material and osteocalcine actually tells you, that whether this bone mineralization is capability of a particular material.

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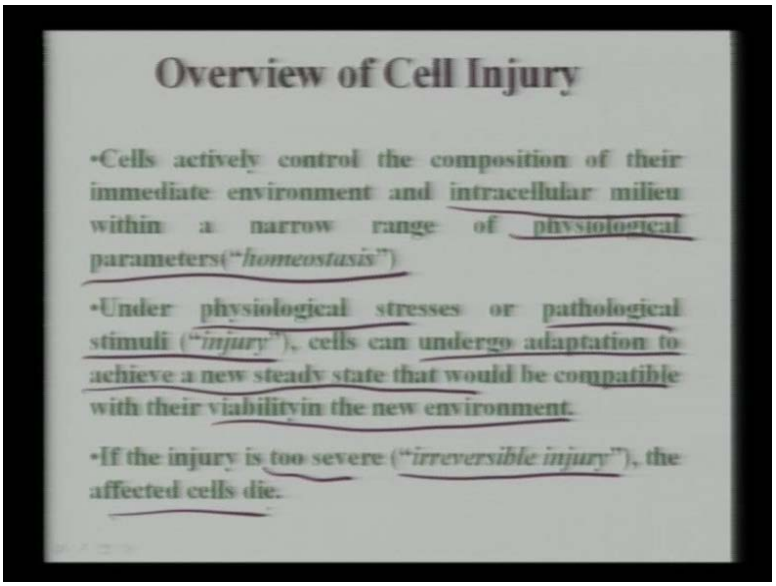

Now, cell injury - cells actively control the composition of their immediate environmental and intracellular milieu. Intracellular milieu means, within a cell membrane the area, which is enclosed by the cell membrane within a narrow range of physiological parameters, like homeostasis, so physiological parameters means, like you know, in pH, like temperature, etcetera. So, essentially, what is the meaning of the first statement? That, cells will actively control the composition of their intermediate environment all the time and they also try to maintain the environment of the intercellular space, then under physiological stresses or pathological stimuli injury.

So, physiological stresses or pathological stimuli, like you know, you are getting some wound or you are having an accident or fracture, then under these kind of conditions, cells can undergo adaptation to achieve a new steady state, that would be compatible with their viability in the new environment. What does it mean? This means, when you are getting some damage, physical damage or physical injury, then cells will quickly adopt, so that cells can survive under the changed environment, under the changed scenario. So, that is the unique capability of the biological cells, that you know, cells can change its size, shape or whatever physiological conditions, the way external environment also, I mean, with the change in the external environment.

So, like in the normal conditions, normal healthy conditions, cells will have one set of physiological condition or physiological situations. When the external environment

changes, cells can change itself, so that they can survive well. So, that is the unique capability of biological cell.

If the injury is too severe, irreversible injury, then affected cells die now. However, this adaptation, cellular adaptation to the new environment also can take place to a maximum extent. Now, if the injury is too much, then cells will die and that is what takes place in that case of paralysis or any other kind of disease. Then what happens, that tissue or organs, that completely fail to do its original function, for example, paralysis means hand paralysis, so that hands cannot, you cannot really move to any moment or leg pain or leg side pain, that means, legs cannot move itself. So, all the tissues and the cells, they cannot perform their normal function because the injury is too severe. So, that is the meaning of this particular statement in the slide.

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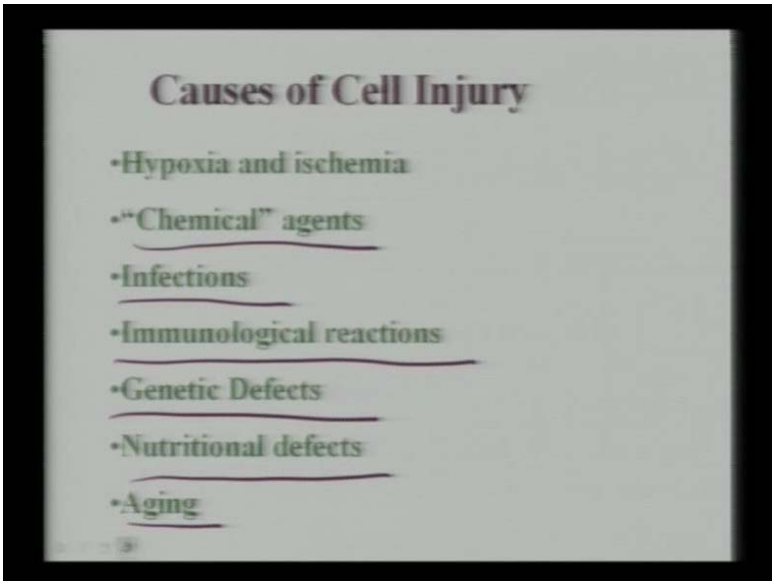

Now, causes of cell injury – now, there are different kinds of causes. So, 1st one is the hypoxia and ischemia. Hypoxia means lack of oxygen, so, whether, when there is lack of oxygen at a particular implant side, then what will happen? It will have kind of Hypoxia situations, as like it will have, these cells will essentially lose their normal function.

Then, chemical agents; chemical agents means, like you know, some particular chemical agents, they enter the body immune system. Infections, several kinds of infections is possible; immunological reactions. Genetic defect, genetic defect means inside the nucleus at the DNA, RNA level. If you have any genetic defects, nutritional defects and

aging, aging means, like when people become old, they cannot walk that fast, why? Because the normal function of the cells, that also deteriorate with aging, like the more you become older, the less you become, more, and less you become active. So, that is the reason, that aging also causes the cell injury.

This is not a cell injury; it is like you know that functioning of the normal cells will not be there that many times, that is why people stress upon biological experiments, that it is the healthy cell line. Healthy cell line means, cells are at their optimum stage of functioning. So, that is call healthy cell line.

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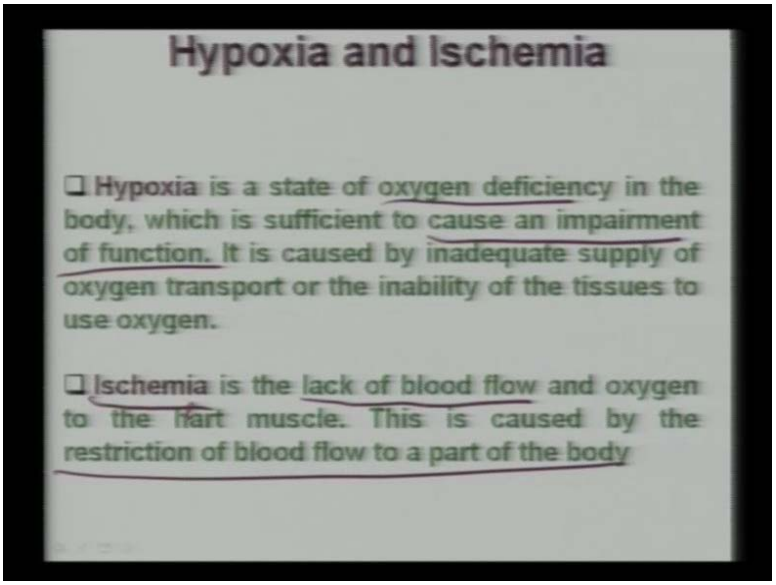

Hypoxia and ischemia - hypoxia is a state of oxygen deficiency in the body, which is sufficient to cause an impairment of function. So, it is oxygen deficiency, that causes an impairment of function and it is caused by inadequate supply of oxygen transport or the inability of the tissues to use oxygen.

So, there are two things it can happen, one is, that there is an inadequate supply of oxygen, that means, you do not have sufficient amount of oxygen available or access around the cell. Or, the other thing is, that tissue, although oxygen is available, the tissue cannot actually use or tissues are not able to use that oxygen to do its normal function.

Now, ischemia, ischemia is another kind of cell injury, it is the lack of blood flow and oxygen to the heart muscle. So, this is a small spelling mistake, heart muscle. So, what it

means? It means that it is lack of blood flow and through blood your oxygen also is circulated. So, if there is lack of blood flow to a particular location, then what will happen? Your tissues or cells, they will not be able to get sufficient amount of oxygen and that is caused by the restriction, restriction of the blood flow to a part of the body.

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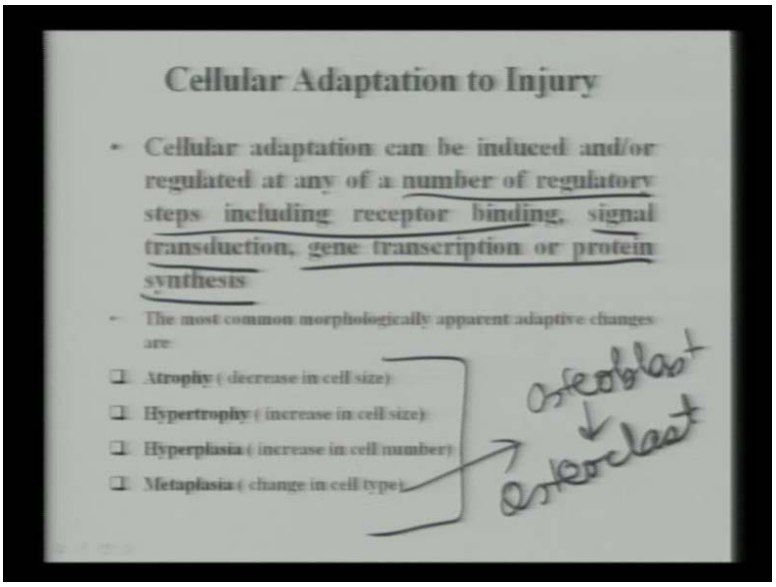

Now, cellular adaptation, as I said, can be induced or regulated at any, at any of a number of regulatory steps and these steps includes receptor binding, signal transduction, gene transcription or protein synthesis. So, the cellular adaptation process can be regulated at any of a number of regulatory steps.

And what are the four different adaptive changes that cells can undergo? First one is atrophy; atrophy means, decrease in the cell size, like cells will get squeezed. Then, hypertrophy, that is, the increase in the cell size; hyper means more, hypertrophy means it is an increase in the cell size; atrophy means it is less in cell size, that is, decrease in cell size.

Hyperplasia, that means, increase in the cell number and metaplasia, that is, change in the cell type. Now, let me give you some examples. For example, metaplasia, if osteoblast cells, they transform to or they go transition to osteoclast, then it is an example of the metaplasia. You remember osteoblast is the bone forming cells; osteoclast is the bone resorption cells. So, if the bone forming cells, by certain biological process, if they undergo transition to osteoclast cell lines, then it is, this process is called metaplasia.

Hyperplasia means, it is an increase in the cell number, that means, under certain changes in the external environment, cells are undergoing more number of cell division process, so that, you know, that number of particular cell types actually increases. Now, those kind of situation or cellular adaptation process is known as hyperplasia.

Remember, this four cellular adaptation process, that is, cells undergo when there is a change in the external environment of the cell.

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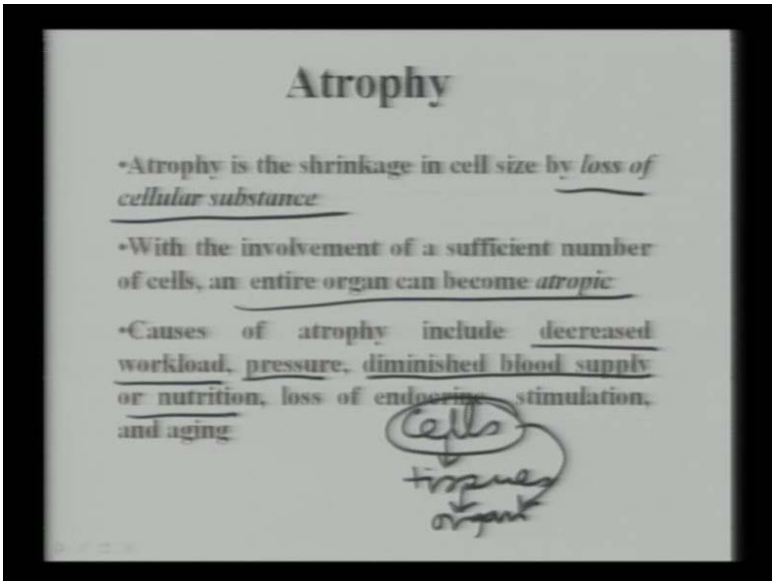

Little bit more description of this differential adaptation process, now atrophy.

So, atrophy is the shrinkage of the cells and how this shrinkage takes place? Now, physically you can see that if there is any change in the size, that size change or decrease in the size can only be accomplished when the cellular content is somehow squeezed out and that is a loss of the cellular substance, and with the involvement of the sufficient number of cells, an entire organ can become atrophic.

Now, what is the genesis of these things? Now, as I explained to, earlier cells they will form tissues. Tissues are like what? Tissues are defined as the self organized structure of a number of cells, which are organized in a particular fashion to form a tissue. Now, a number of tissues, they form a organ.

Now, what is explained here, that if a number of cell lines or sufficient number of cells are becoming atrophic, means (( )) really decrease in size, so the entire organ, which is

composed of that particular cell line can become also atrophic in nature. And this causes of atrophy include decreased workload, like you know, you are very lazy, do not work, you always sleep and sit, and then you have a atrophy, then decreased pressure, diminished blood supply or nutrition. So, these are like atrophic nature and also aging.

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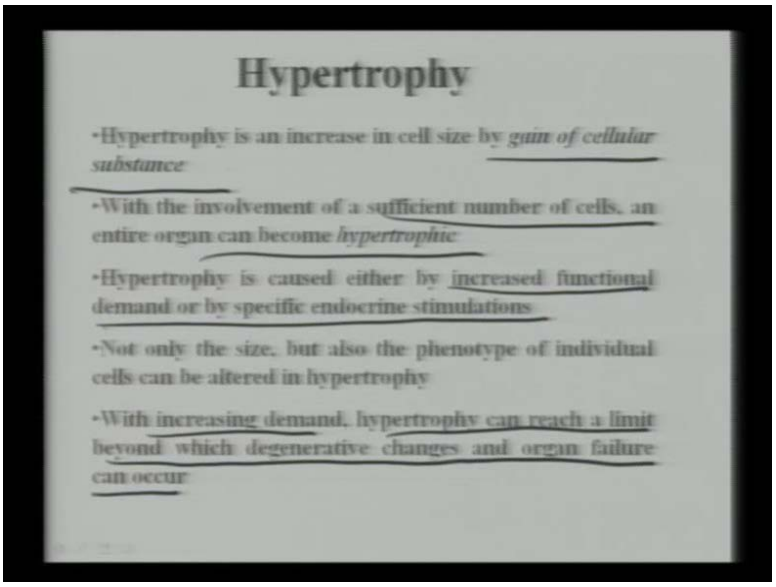

Hypertrophy, hypertrophy is, again is increase in the cell size. So, that is possible by gain of the cellular substance. Now, with the involvement of a sufficient number of cells, an entire organ can be hypertrophic, just like atrophic, an organ, entire organ can be atrophic.

Hypertrophy is caused by increased functional demand or by specific endocrionic stimulation, not only the size, but also the phenotype of individual cells can be altered in hypertrophy and with increase in demand, hypertrophy can reach a limit beyond which degenerative changes and organ failure, they can occur. Now, what is the last statement, that tells, that tells, that hypertrophy, that if the cells increase their size and therefore, a number of cells, they content in organ, that also changes its size, then it undergoes degenerative changes; degenerative changes means, normal biological function cannot take place and then, organ failure can essentially be, be essentially occur.

So, organ failure means, there are people still can have, that particular organ, but that organ cannot work out, that organ cannot function in a normal sequence.

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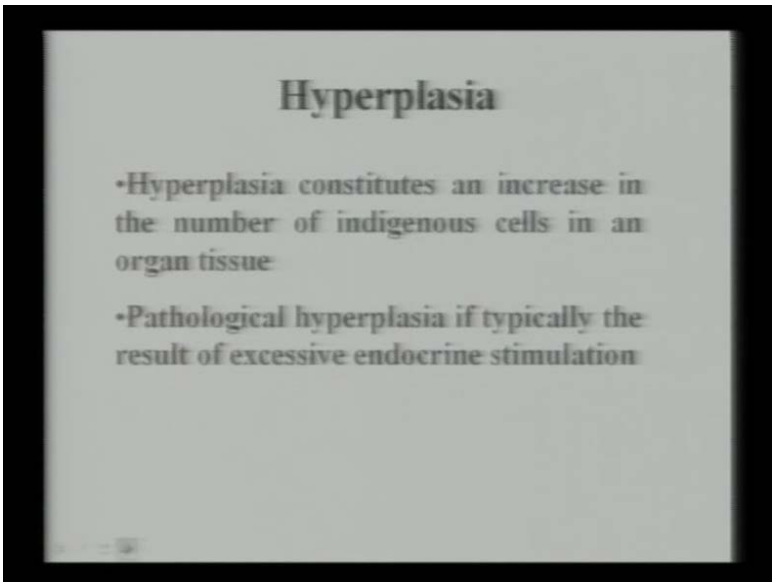

Hyperplasia, that is, constitutes an increase in the number of indigenous cells in an organ tissue and pathological hyperplasia occurs, if, and if typically the result of the excessive endocrine stimulation.

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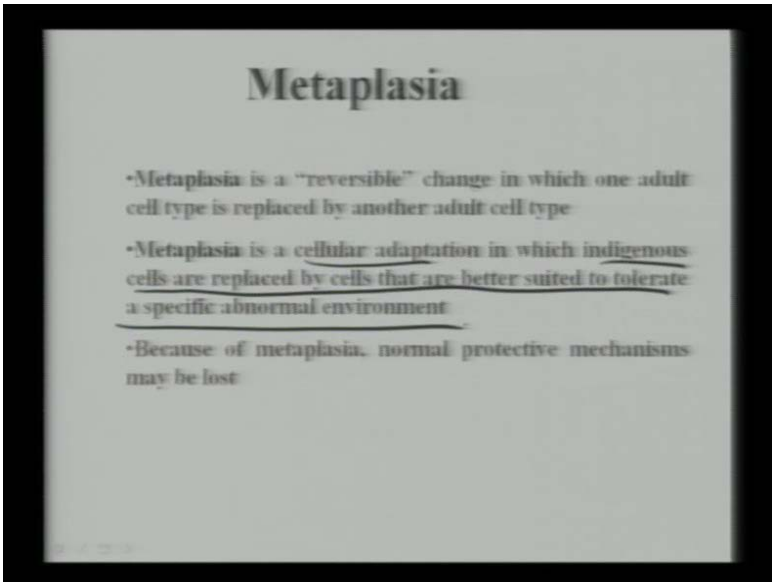

Now, metaplasia is a reversible change in which one adult cell type is replaced by another adult cell type that is the example, that I have given to you, that is, the osteoblast cell line, they undergo or they change it to osteoclast. And metaplasia is a cellular adaptation in which the indigenous cells are replaced by cells that are better suited to

tolerate a specific abnormal environment. Abnormal environment means, when the external environment is not suitable for the survival of that particular cell lines, then they quickly change to another cell type and the example I have given, for the bone cells.

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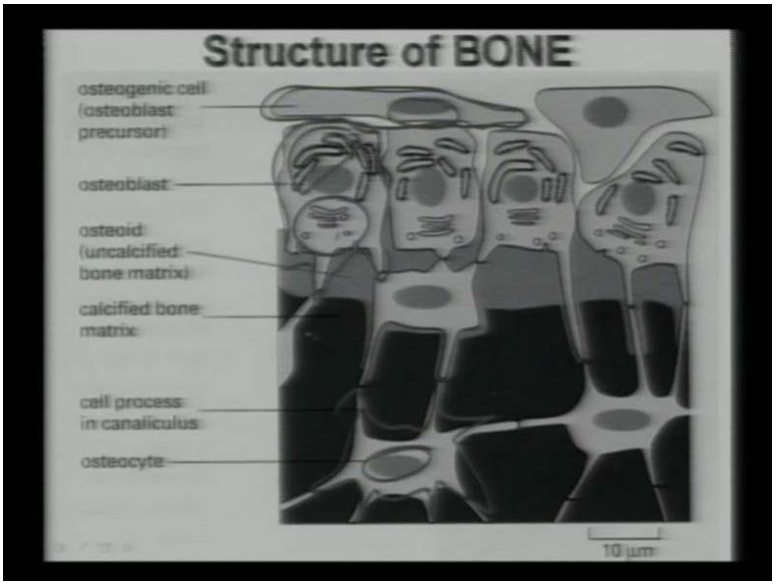

So, you, here you can see, that a typical bone structure, in terms of the different cells, they are, they are present in this bone. So, osteoblast cells, they have a typically irregular type of cells and with the nucleus. You also have osteocytes, which have also typical star type of cells and this star type of cells, star shaped cells, it has also central nucleus. So, these are examples of eukaryotic cells.

Now, these are, also this osteoblast cells are of different sizes here and you can see, golgi apparatus, mitochondria, all this important cellular organelles. You have the calcified bone matrix; calcified means, it has the hydroxyapatite, that is why, it is called calcified. So, whenever you see, that calcified bone matrix, that is right. Many times, you know when you visit the doctor, doctor will tell you to take, you take calcium tablet; why calcium tablets? Because your bone has loss of calcium, so to compensate with the loss of calcium you need to take the calcium tablet, so that certain calcium level is maintained in your body. So, that is the scientific meaning of taking this calcium drugs.

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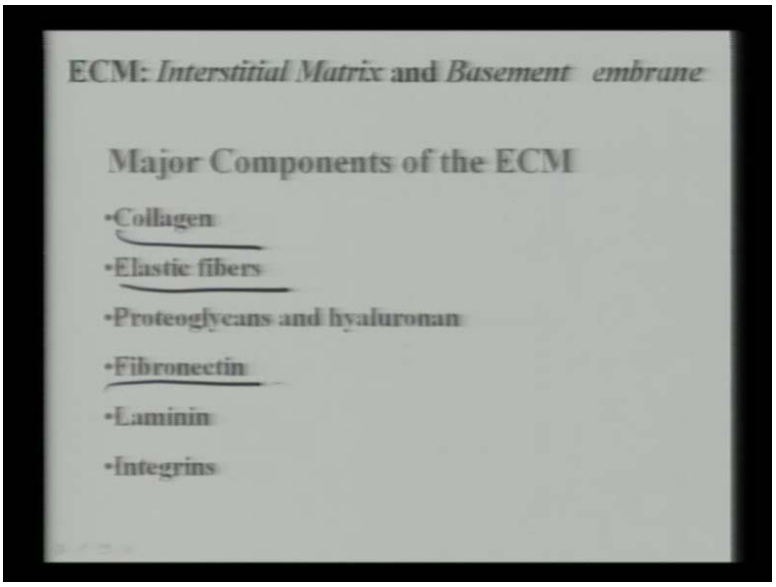

Now, ECM, as I said earlier, ECM stands for extracellular matrix and extra cellular matrix means, matrix contains typically, collagen, it has also fibers like, elastic fibers. Remember, I have mentioned, that you know, this extracellular matrix contains different fibers and that fibers give the ECM mechanical support. So, ECM you can constitute as a matrix, where the different cells are present, so that ECM has to support the, support the, you know, support the all the cells, that it contains. So, if it has fibers like structure, then it is mechanically also suitable.

We have the different proteins, which is called fiberonectin and also it has integrins.

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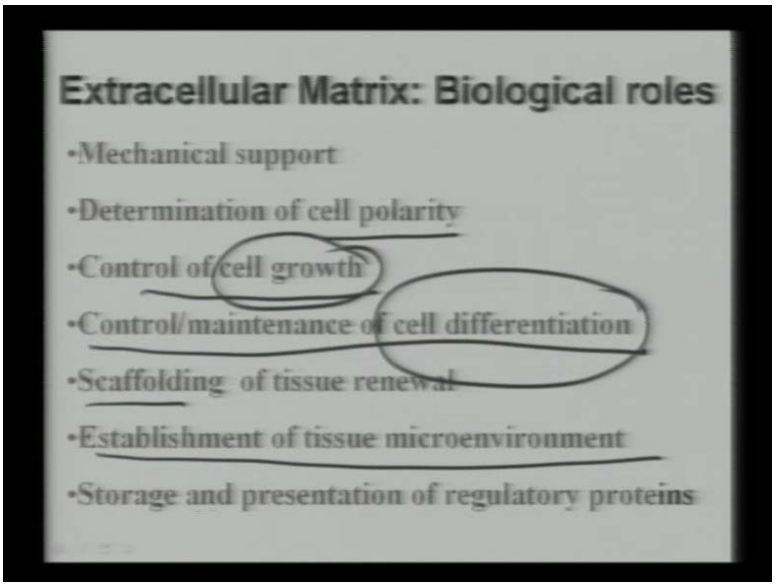

Now, what is the biological role of the extracellular matrix? Like as I, as I just mentioned to you, that it, it, it acts like a mechanical support, it also determine the cellular polarity. Many times, you know, cells have a electrical charge and it has positive and negative polarity, that is also controlled by ECM. It also control the cellular growth or cell (( )) processes, control or maintenance of cell differentiation.

Now, cell growth, cell proliferation, cell differentiation, these are like part of the cell fate processes. And scaffolding for tissue renewal, establishment of tissue microenvironment, storage and preservation of regulatory proteins, so these are, like, all the different biological roles of the extracellular matrix.

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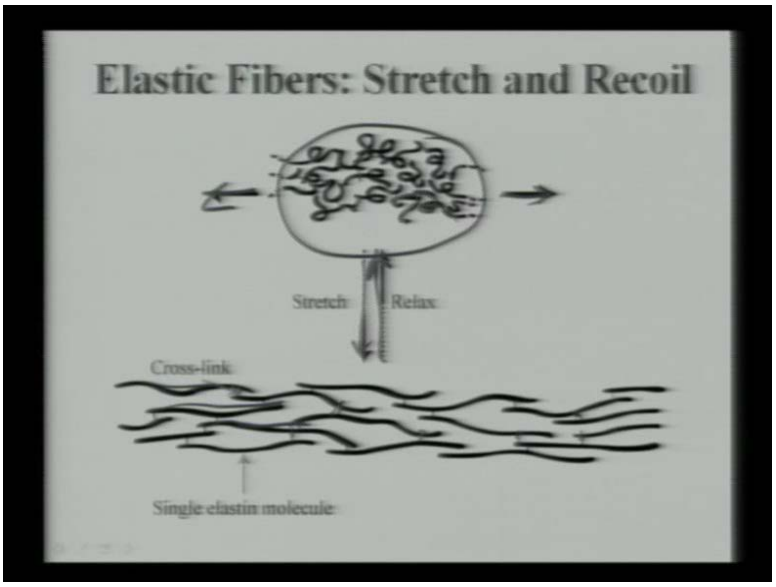

Now, elastic fibers, as I mentioned to you earlier, that extracellular matrix, they have, of this collagen fiber, they have the elastin fibers, they have fibronectin protein, they have laminin. Now, the question is that how the elastin fibers they look like? Now, elastin fibers if you see, that it has, it is, it, the shape is more like a snake, like you know, you have, if you squeeze that something and this, these are like proteinous, proteinous, protein base fibers.

However, there is a link between these two fibers here and this link is weakly and these weak bonds are mostly hydrogen bonds. Now, when you stretch it, what, what is shown here? That when you stretch it, like you know, tension, then what will happen? This elastin, that it is, there is a cross-linking between these two fibers. So, it is like, you can say, polymer macromolecular structure. Each chain, two, two neighboring chains, they have a cross-link and typically, in polymers, this cross-linking, it is taking place by adding sulphur to the polymer, like. So, due to the presence of certain elements, this cross-link is established.

Now, when this squeeze kind of shape, it is stretched, then you can see, that all these fibers, they are all equally stretched in a particular orientation, that is, the orientation along the tensile axis and two fibers, they are each bonds of their cross-linking bonds, these are clearly feasible, but it has a highly elastic nature. If you release the load, then it can go back to the original coil kind of configuration. So, this is like, what we call, coil

type of configuration and this is more, like a more expanded version and this expanded version is more like a, you know, that individual elastin fibers, they are getting stretched. And therefore, you can see like, more like, you know, when the fibers are getting stretched, how they look like. The similar conditions you can find it here.

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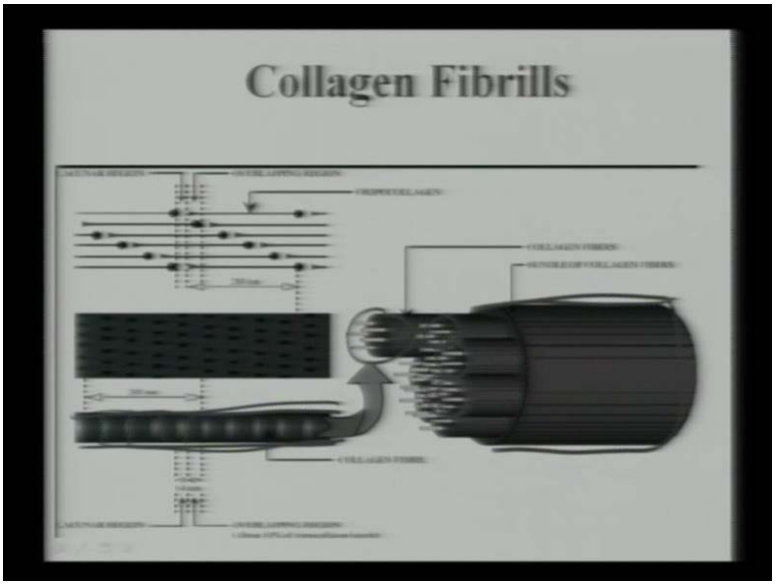

This is more kind of vivid, more detailed description, how the collagen fibrils, they look like? Now, if you see this collagen fibril, they have, like different numbers are there and this numbers are, like you have a 280 nanometers, so this entire length is, kind of, 560 nanometers, this entire length, and they have a triple helix type of structure. And individual collagen bundle, they contain, that number of collagen microfibril aggregate, that I have shown you earlier and this, each microfibril, in each of the microfibril aggregate, individual microfibril, they are attached to each other by the weak hydrogen bond.

Coming back to the description of the collagen fibrils, so what you can see here, that it has this long microfibril type of structure, which has attached to each of the hydrogen bonding, they form this microfibril aggregate, which are contained in a large collagen bundle of collagen fiber.

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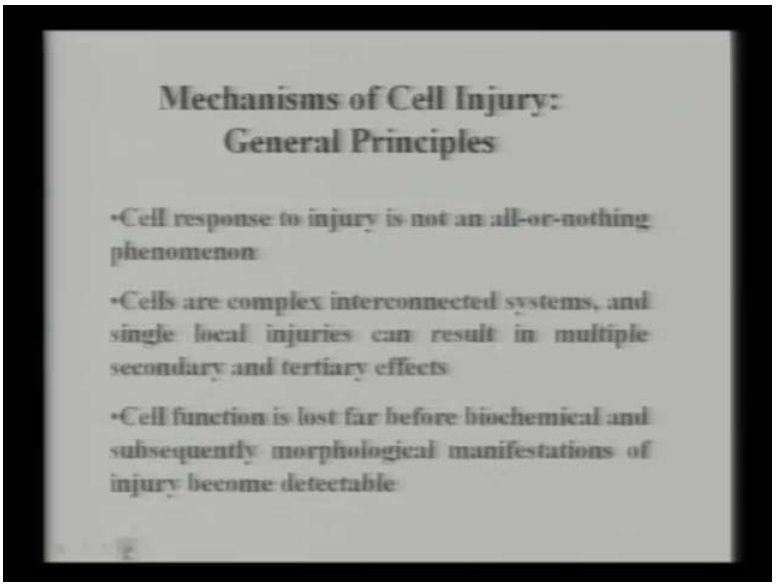

Now, mechanisms of cell injury, like the cellular adaptation or cell response to injury is not an all or nothing kind of phenomenon. And cells are complex interconnected systems and single local injuries can result in multiple secondary or tertiary effects. So, secondary and tertiary effects means, cells can change size, shape or type. So, shape change is called atrophy or hypertrophy, number change is called hyperplasia, type change is called metaplasia and cell function is loss, be, far before biochemical and subsequently morphological manifestations of injury become detectable.

What it says, that cellular, that organ failure, like when a human patient has a failure of the organ, that takes place much before, that this is detectable because all this biochemical and morphological manifestations, that take place fast leading to the final organ failure, so that needs to be understood.

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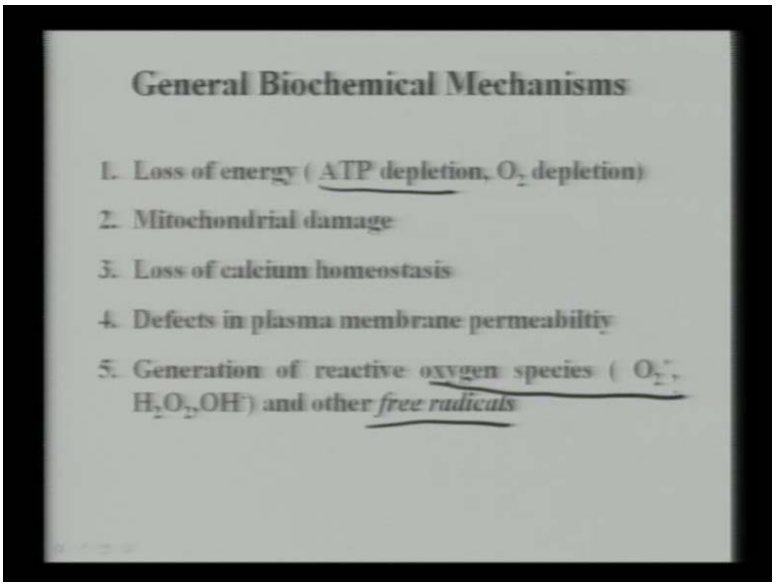

And what are the different biochemical mechanisms, that is, number one is the loss of energy, like ATP depletion or oxygen depletion; ATP is the adenosine triphosphate.

Mitochondrial damage, mitochondrial damage, if any damage takes place in the mitochondria, mitochondria is known as the energy store of the cell, like it is the place where the energy is produced by the reaction of the ADP to ATP conversion, right, and that conversion adds the energy. Now, if the mitochondria itself is damaged, that means, cells will not be able to produce energy for its own survival, and if the cells do not have sufficient amount of energy, cells cannot survive by itself. Because remember, the basic definition of cells, it is self content unit, like whatever is required for its own survival and duplication, that is already contained in the, inside the cell, like mitochondria is energy storage, you have the protein synthesis unit, that is itself is required for its own survival as extracellular matrix, that is, ribosomes. You have, that genetic transformation or this genetic translation, like you know, cell diffusion, that is contained in its nucleus. So, all those units are contained in the cell.

Then, 3rd one is the loss of calcium homeostasis. So, as I said, that bone contains the collagen as well as the synthetic natural hydroxyapatite and if the calcium deficiency is there, then also there will be bone loss.

Then, defects in the plasma membrane permeability and 5th is the generation of reactive oxygen species, O 2 minus H 2 O 2 or O H minus and other free radicals. Now, if there are other free radicals and oxygen species are present, then also cells can die because it can directly attack the cellular membrane and if the cellular membrane is damaged, then that all the internal intercellular milieu, whatever is there, that can come out. So, cells cannot survive like.

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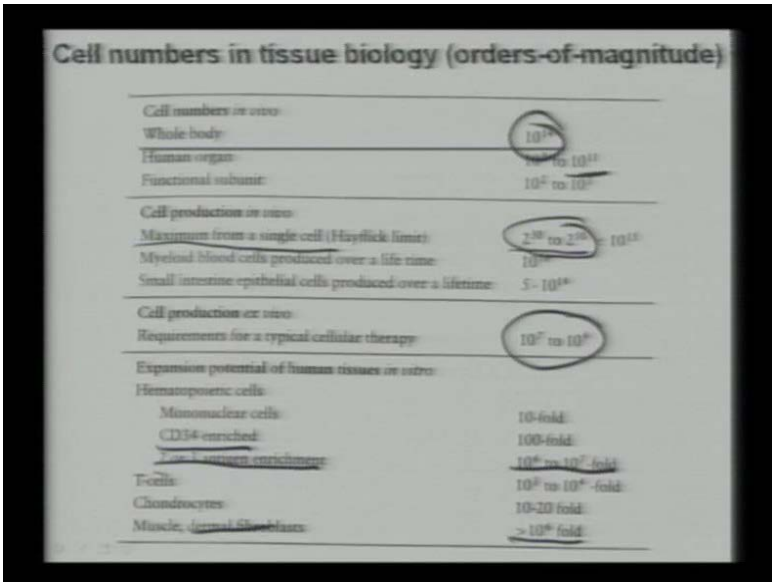

Now, what is the cell numbers in tissue biology kind orders of magnitude? Now, in the in vivo conditions of whole body, it has a, in vivo condition is that 10 to the power of 40 and human organ is 10 to the power 8 to 10 to the power of 11. Now, what is the cell production in vivo? Maximum from a single cell, that is the 2 to the power 30 or 2 to the power 50, that is the huge number of cells can be produced, which is, roughly you have, you know, 2 to the 30, 2 to the 50, and huge number of cells there can be produced from the individual single cells in the vivo condition. Remember, in vivo environment and in vitro environment is different and accordingly, these number of, huge number of cells may not be generated in the in vitro conditions. So, this is under in vivo.

Now, cell production ex vivo, like requirements, typical cellular therapy… Ex vivo means, outside the patient or outside the animal, so that is 10 to the power 7 to 10 to the power 9 number of cells, that is large number of cells there can be produced in ex vivo conditions. Now, expansion potential of human tissues in vitro, like there are different

series, 34 enriched or 2 to 3 antigens enrichment, like they can be expanded, like you know, many orders of magnitude, like dermal fibroblasts, that can be more than 10 to the power 6 fold increased or expansion, that is possible in the in vitro conditions.

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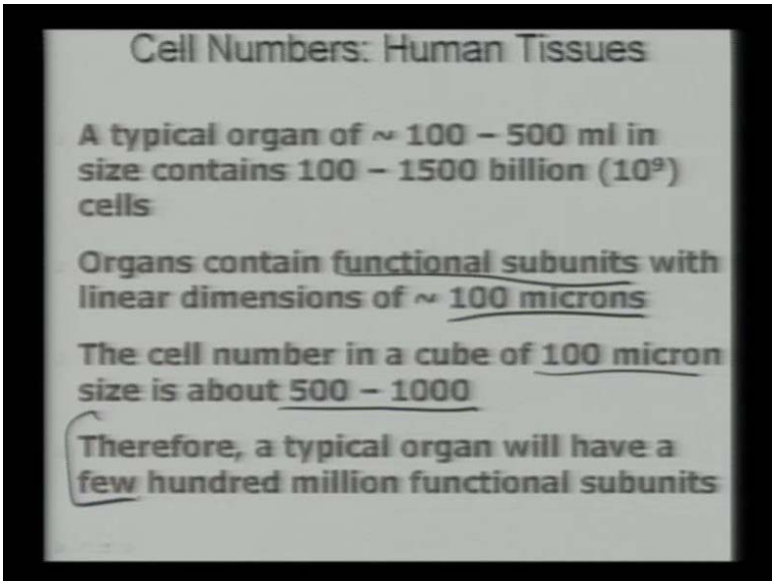

Now, a typical organ, which contains 100-500 milliliter in size, contains 100-500 billions, that is, 10 to the power 9 numbers of cells. So, a typical organ contains huge number of cells, that is, 10 to the power 9 and this organ contains functional subunits, which is like each of the linear dimensions of 100 microns and the cell number in a cube of 100 micron size is roughly 500 to 1000. So, each cube, individual cube there, that itself contains 500-1000 cells and therefore, a typical organ will have a few 100 million functional subunits. Functional subunits mean what? Like in individual organ this subunit, they are supposed to perform different, different functions for that you know, for that overall, towards overall functioning of the organ and individual functional subunit contains that, 500-1000 number cell.

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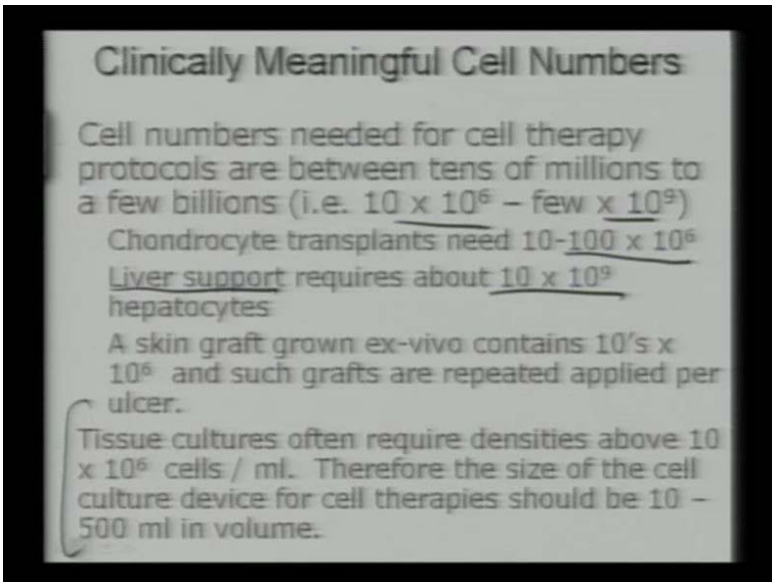

Now, cell numbers needed for cell therapy protocols, that is, between tens of millions to a few billions, like 10 to the 10, 10 to, 10 to the power of 6 to few into 10 to the power 9 and chondrocytes transplants need 100 into 10 to the power of 6, that means, 10 to the power of 8 and others, of other examples is a liver support, that requires about 10 to the power 10 hepatocytes, that is the another cell lines.

And tissue culture often requires cells of the 10 to the power 6 cells per milliliters, therefore the size of the cell culture device for cell therapies should be somewhat 10 to 500 milliliter in volume.

So, typically, in your cell culture studies, you often suspend how many number of cells, 10 to the 5 number of cells, 10 to the 4 number of cells, that is typically you require and that is the why they are required, that, that is explained in this particular slide, the typical unit to disperse this much number of cycles, so that cells will have a good physiological milieu.

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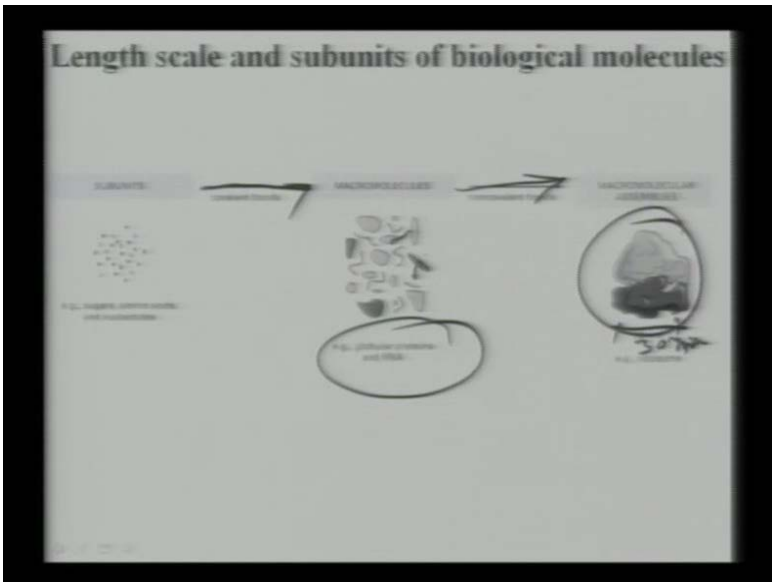

Now, next few minutes I will just explain to you the typical structure and composition of the biological molecules are and the subunits. Now, sugar amino acids, they are like globular as spherical separate subunits and then they are, they are covalently bonded, then they form the macromolecular type of structure, like globular proteins and RNA, ribonucleic acid. And then, if it is non-covalent bonds, that between these two molecules, non-covalent bond between these two non-covalent bonds, then it is called ribosomes.

So, ribosomes are the typically length scale of 30 nanometers. So, 30 nanometer is extremely small and you can see, therefore, ribosomes as small organelles in the cell structure.

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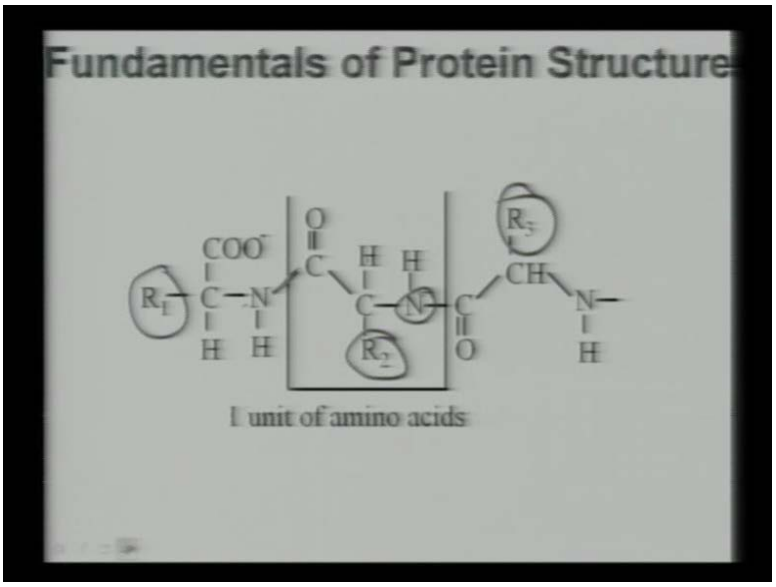

Now, this is important, now protein structure; now what is protein structure? At the individual level, protein structure contains one unit of amino acids and this is the typical composition of amino acids. You have this organic radicals here, R 2 and you have this hydrogen in those few ends and one end you have a nitrogen N and other end, you can have single bonds and then you can, it is connected to another part of this, another mar unit of the protein structure.

So, this is a large macromolecular structure and essentially, R 1, R 2, R 3, that can be three different organic radicals or the subunits and depending on this composition of the R 1, R 2, R 3, you can have different type of protein molecules also.

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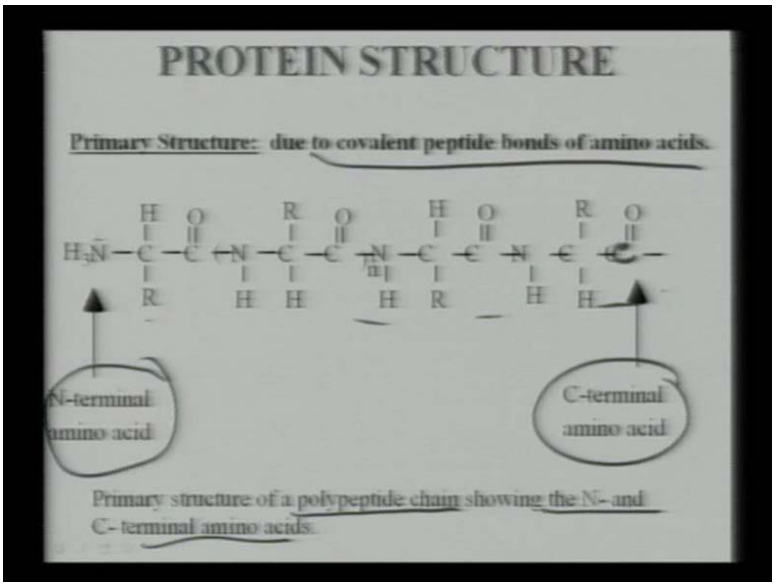

So, proteins, as I said, that it has N-terminal amino acids and you have C-terminal amino acids. N-terminal means, it ends with N; C-terminal means it ends with the typical carbon atom and you have both, single bond and double bond in this structure. And this is a polypeptide chain, that shows the N and C-terminal and it has covalent bonds across the individual chain. So, individual chain, like you know, different polypeptide units, they are bonded together with individual chain and a complete protein structure, protein molecule have both, N-terminal and C-terminal here.

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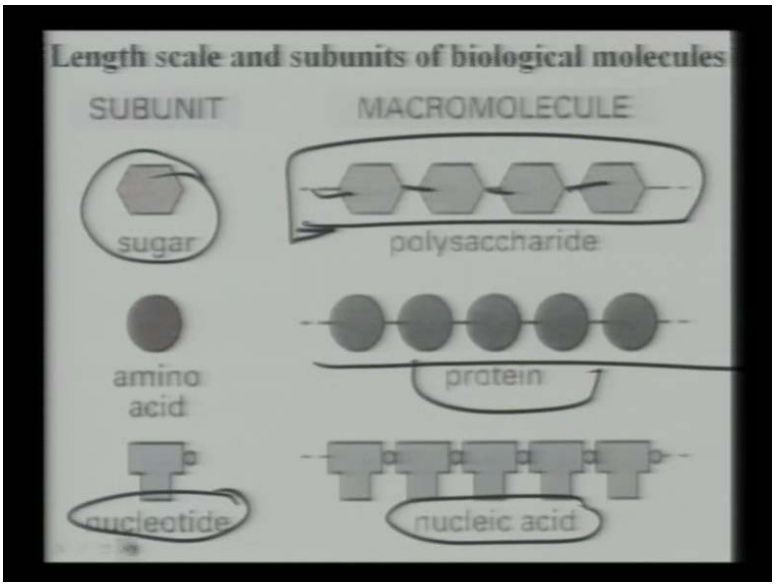

Now, this shows, kind of, more kind of shape or morphology of the protein molecule like subunits, like sugar subunits, like hexagonal structure and then, you have the polysaccharide. This polysaccharide structure is composed of this hexagonal structure added to each other by covalent bond, you have the amino acids. When they are covalently bonded, they form the protein molecules, you have a nucleotide. Then, when they are covalently bonded, then it is called nucleic acid.

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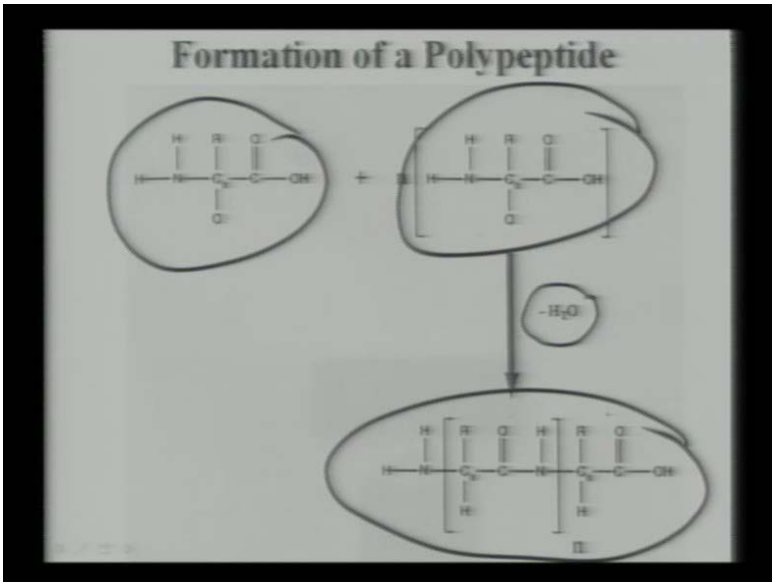

And how this formation or this polypeptide chain there take place, that chain formation is possible? Now, you have this particular organic configuration. Now, when it, when it is added with this N number of such mar units and from this N number of mar units, 1 is to molecule is extracted out and if due to the loss of this water molecules you create a typical protein structure, which is little bit a complex organic formula.

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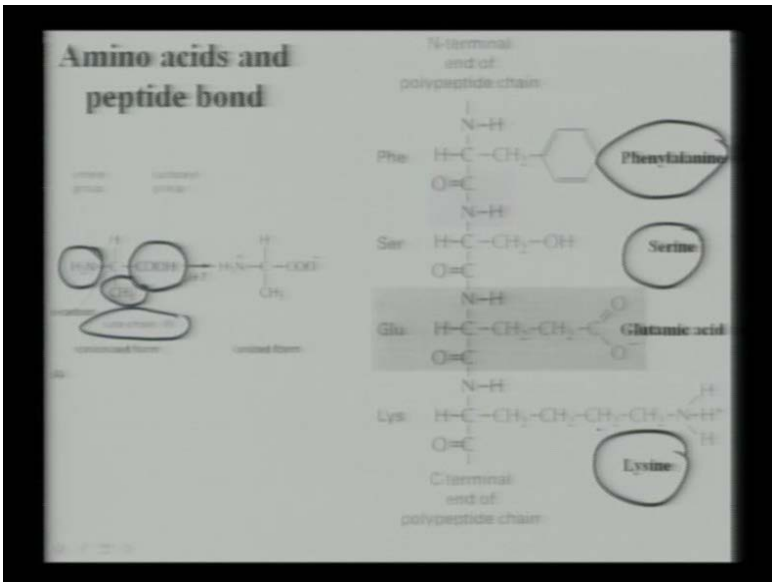

Now, amino acids in the peptide bonds, you have this COOH, that is the carboxyl group. Here, you can see and you have that NH 2, that is the part of the amino group and you have also the side chain that is the side chain is that R 1, R 2, R 3. Remember, in the basic protein description, that R 1, R 2, R 3 can be different things. Here, one of the R is that amino acid, that is, that, and that another side chain is R, is that CH 3 group, that is, the methyl group.

Now, as I said, depending on what is the composition of the R 1, R 2, R 3, you can have different protein molecules, that is the fundamental. You cannot remember all this protein molecule names and their structure, but the fundamental thing is, that in a protein molecule you have R 1, R 2, R 3, different organic side groups is possible and depending on what is the side group, you can have phenyl alanine, you can have serine, you can have lysine. So, this can have a very complex type of structure of the amino acid.

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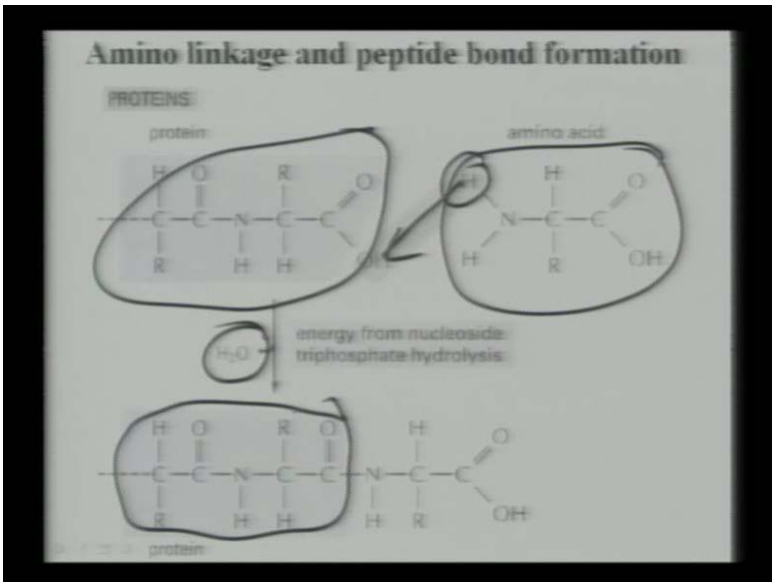

Now, amino linkage and peptide and peptide, peptide bond formation, what it shows here, that you have one amino acid here and you have this, one of these mar units here. Now, this H reacts with the OH and as a result, this H 2 O molecule is taken out from the system. Now, energy from nucleotide and triphosphate hydrolysis and because of these H 2 O molecules leaving the system, you create this yellow shaded region, which is like a peptide bond, essentially peptide bond formation. And protein molecules, they are (( )).

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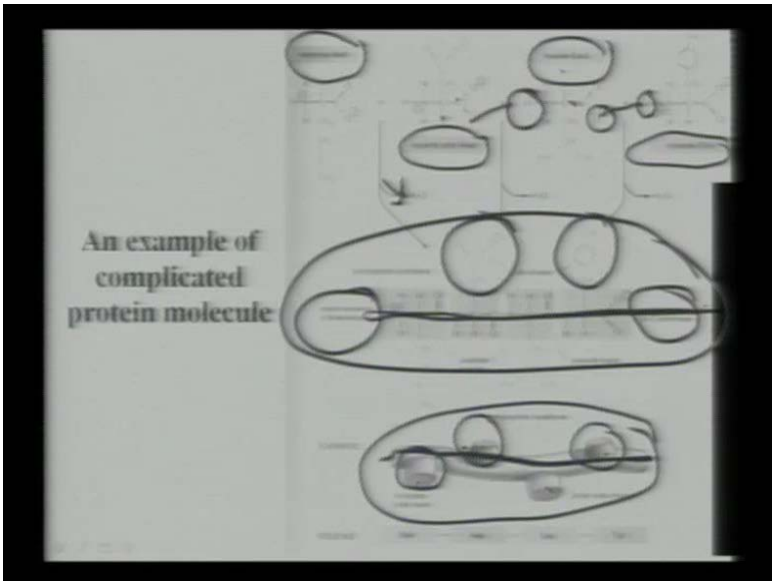

An example of the complicated protein molecule, what you can see here, the sequence of the protein molecules, this is also important. Many times one can, many times in the lectures you can say, that protein molecules configuration changes. Configuration changes means, like how this protein molecules, how this is your polypeptide backbone. It is just like any organic polymer, it has backbone structure and if you have the side groups, now this side group can be different depending on, whether it is a different kind of protein molecule, whether it is a non-polar side chain or whether it is a polar side group. And the orientation of these side chains, that determine what kind of protein molecule configuration it will have.

So, if the protein molecule configuration essentially means, that either you can, this position of orientation of the side groups, how does it change and how this sequence of this different polar groups they change because of some biological, because of some condition in biological changes. Now, this is like a very complicated protein molecule formation, how this is formed? Like you can start with that methionine, methionine is met and then you can, you can add this aspartic acid, that ASP, then leave sign, that is LAU, and trio sign, that is TRI.

Now, each case what is happening? Then, when this two are added, the H 2 molecule is released. Now, these two are added, then again H 2 molecule is released and that is possible because you have the weight and you have the H hydrogen, so that forms O H of H 2 O again. These two and it is added, then O H group is released, H 2 O is released and as a result, these three, these three, four comes together, subunits and then, you can have again, C terminal and N terminal.

And this is the polypeptide backbone structure and you have side chains, that can have different side chains, it can have benzene ring, it can have methyl groups, you can have methyl groups, all these ethyl groups, but that is, remembering all this sequence, that how a complicated protein molecule forms is not important, what is important is the understanding overall how this protein structure can form, like you have different R 1, R 2, R 3 group, they can come together and every time one protein molecule, if these forms, then you have to understand, that one H 2 molecule has to be released.

And if that H 2 is cannot be released, then protein molecule cannot be formed, simple, because you need to ensure, that your backbone chain is a polypeptide chain and it has

one end is the carbon, one end is the nitrogen and typically, according to the convention your N-terminal is always on the left hand side, your C-terminal is always on the right hand side, it is just a convention.

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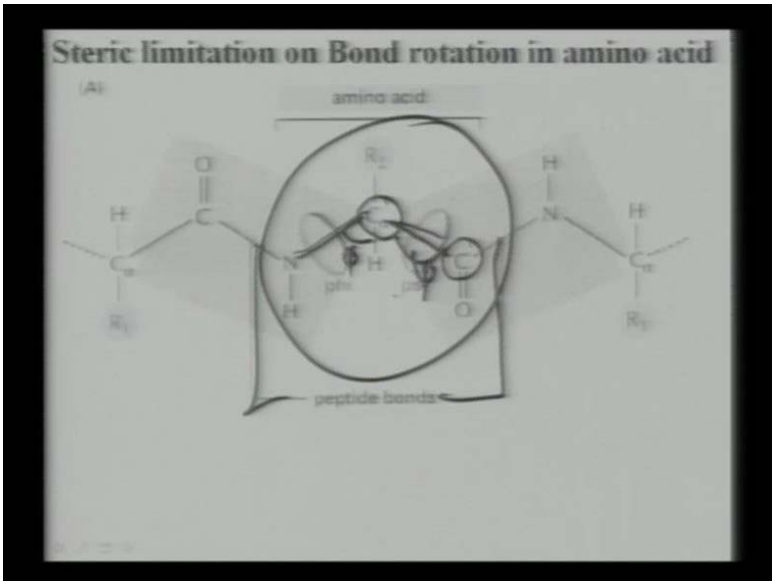

This is also schematic illustration of the bond rotation in amino acids. Now, this amino acid of protein chains, also protein molecules, you can see, that you know, there is C alpha, that is one particular carbon atom orientation and it is connected to another carbon atom and it is connected to another, C, nitrogen atom and this is your, essentially the peptide bond.

Now, bond rotation means, now this is, that individual amino acid, now bond rotation means, this bond angle, they can rotate depending on what is the scenario, what is the sequence. And if they can rotate, now this one configuration if they rotate to this side, then configuration also changes, do you understand? And once it rotates, it can always come back to the original configuration also. So, what I am trying to say here, this phi and psi angle, this phi and psi angle value is not a fixed value, they can dynamically change depending on the biological conditions that the protein molecules will experience in a particular biological environment.

So, overall, if you, if you, if I want to summarize, that you know, protein molecules structure, it has a polypeptide backbone chain, it has it can have different R 1, R 2, R 3,

different organic side groups and this side can be either benzene ring based or it can be, have simple side groups, like methyl or ethyl group.

Now, then, the other thing, that you have to remember, that when two different types of amino acids are coming together, they form a protein molecules, then, that these addition of the two amino acids is only possible when they will able to release the water molecule. So, that reaction must release water molecule, otherwise that polypeptide chain is never formed. The other thing is that this bond angles between the carbogen connected to nitrogen and carbon, carbon-carbon bonds or carbon-nitrogen bond; that bond angle is not a fixed value, it can change during that any biological or pathological processes and it can cause this change in the configuration of this protein molecule. And that protein configuration changes take place very dynamically during any biological environment.

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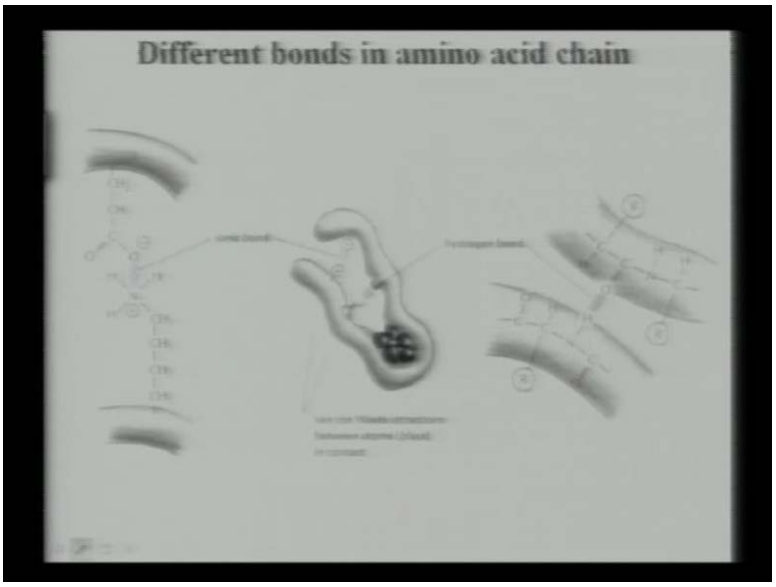

So, I think I will stop here and we will start from this in the next class.