Biomaterials for Bone Tissue Engineering Applications
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Module 1
Lecture 5

Ok, in this module, I will discuss the biological cells, I will introduce the biological cells as well as tissues, now this part of the course, in particular this module we will only talk upon some of the key, some of the key terms, as well as also discuss some of the salient aspects of the cell structure without getting into significant details like what a biologist otherwise would cover. So these are the bulleted points that I am going to talk upon one by, ah, one after the other, so we will first define what is cell.

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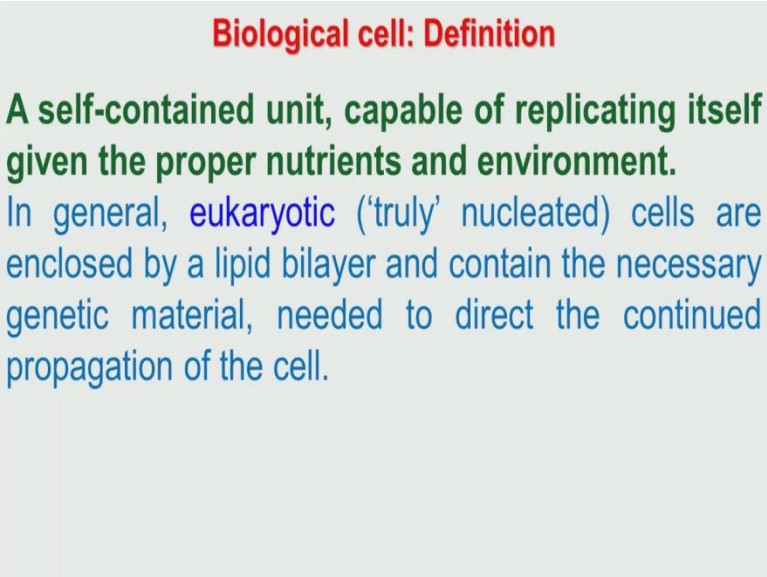

So Biological cell is defined as self contained unit capable of replicating itself given the proper nutrients and environment.

So let me explain some of these terms, phrases that I have used in this particular key definition, so this one is so this particular term like self content and so on, so self content means it has all the organelles which can be used to ah, which can perform certain specific functionalities, ah for example ribosome in cells can synthesize proteins, Mitochondria is used as a power house, I will go through that next, right, then I will explain it more and it is also capable of replicating itself that means it can propagate from 1 to 2, 2 to 4, 4 to 8, like cells can undergo cell division and if

the cell division is there, if cell division is possible only when, when proper cellular micro environment exists, that means the cell is provided with proper nutrients and environment.

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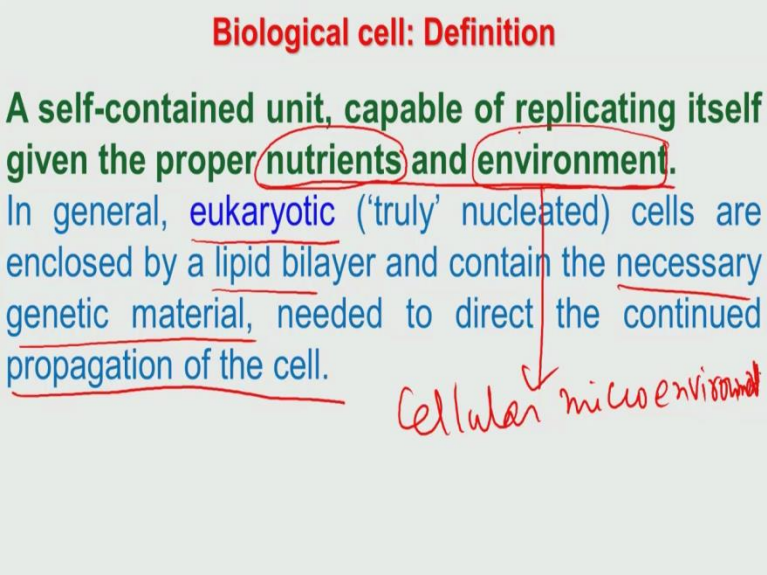

So in general a nuclear, so in general the cells can be classified as two classes, one is Eukaryote, (EU) means fully, so EU characteristic means, fully nucleated cells which are enclosed by lipid bylayer and also contain the necessary genetic material, so essentially that nuclei, genetic material includes the nucleic acids like DNA/RNA needed to direct to continue propagation of the cell.

So there are certain things which is kind of implicitly mentioned here like, and I will describe it in much more details in later part of this course, like nutrients and environments, so nutrients are essentially used to, essentially required for the survival of the, or cell survival, environment also cellular micro environment means essential it is cellular micro environment means like cell must receive certain ah, cell must receive certain signaling molecules or for each cell to survive it must receive a combination of signaling molecules, and in the absence of signaling molecules the cells cannot survive. Now what is cell signaling mechanisms and what are the signaling molecules which are essentially proteins, those things I will discuss it later, ok.

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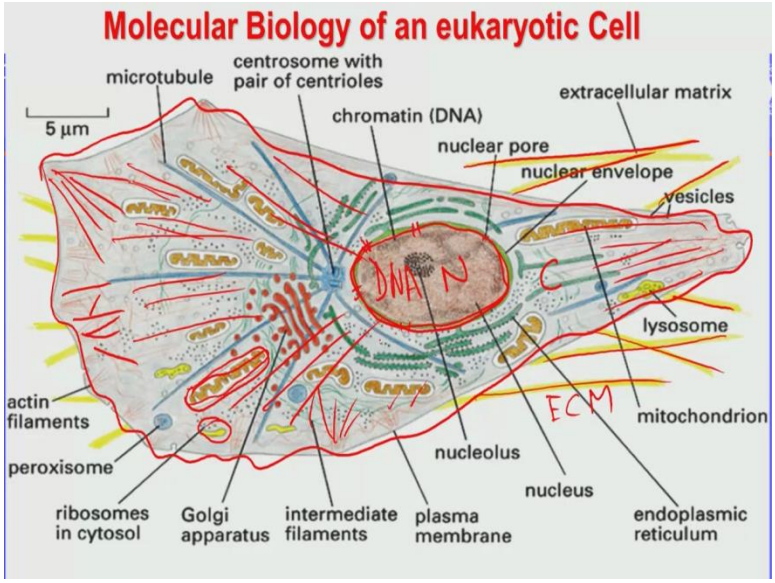

Now if you move to the next slide, what I was telling you some 2, 3 minutes ago and let me explain with some little bit more specific details in this slides, so these kind of horse shoe type of structure you see this is mitochondria, so this is known as the power house of the cells, now you can also see here certain ribosomes, and this is the ribosomes in the Cytosol, these are the ribosomes essentially are the organelles which above which are used for synthesizing proteins,

Now other things very important is that if you see that very regular type of cell membranes factor, now although it shown so simplistic manner in this particular cross section slides of the animal cell, but in reality the cell membrane is very important and in eukaryotic cells it is a double layered structure, also individual organelles as a marking here like a Mitochondria then you have the Golgi body as well as well as the other organelles, they also have a very well defined double layered structure, so this double membrane structure is kind of very characteristic of different organelles of different cellular organelles.

Ok, the other things that you can see rather quite clearly, so this is almost like a sample region, this is the nucleus, now nucleus contains DNA that is Deoxyribonucleic acids, and also nucleus is also separated by, separated from rest of the part of the cytoplasm by very well defined double layered structure and this nuclear membrane also has certain pore structure, and this pores are essentially formed that nuclear pore complex, so later on I will explain to you that in adult healthy cells or in a, as a matter of fact in all the cells the DNA can undergo transformation to

RNA which is known as the transcription process where RNA can go to synthesize to proteins and this is called translation process, so typically translucent process takes place in the nucleus, transcription process takes place in the cytoplasm often eukaryotic cell.

The story is little different in case of prokaryotic cells which is like more primitive cells and Unicellular cells, the other phase is important as It is shown in this particular slide you see that these are like yellow kind of fibrous structure, so this is what is known as the ECM (Extra Cellular Matrix). Now extra cellular matrix contains collagen, elastin and various other biological micro molecules and these extra cellular matrix plays certain key roles in determining the cell functionality like the way cell would migrate or cell would, or cell motility would take place on an extra cellular matrix or in a bio metrical subscript.

The other things should draw your attention is that this is the cytoplasm of the cells and you can see the cytoplasm as certain fibrous structure, so there are three components of cytoplasm, and these fibrous structures although shown more clearly towards the end of a, end of a this particular cross section image, but you have to believe me that this particular cytoskeleton structure explains very neatly throughout the entire cross section of the cells and the cytoskeleton structure has three constituents, one is action filament, and then one microtubules and third one is intermediate filament, so I repeat cytoskeleton in a eukaryotic cell has acting filament, intermediate filament as well as microtubules.

Now these three different components of the cytoskeleton, they have different range of their mechanical properties in terms of their elastic stiffness or deformation delivery under stress, why I am emphasizing so much on this cytoskeleton structure because cytoskeleton also supports or provides mechanical support to other cellular organelles. So from that point of view the understanding of the cytoskeleton structure is quite important.

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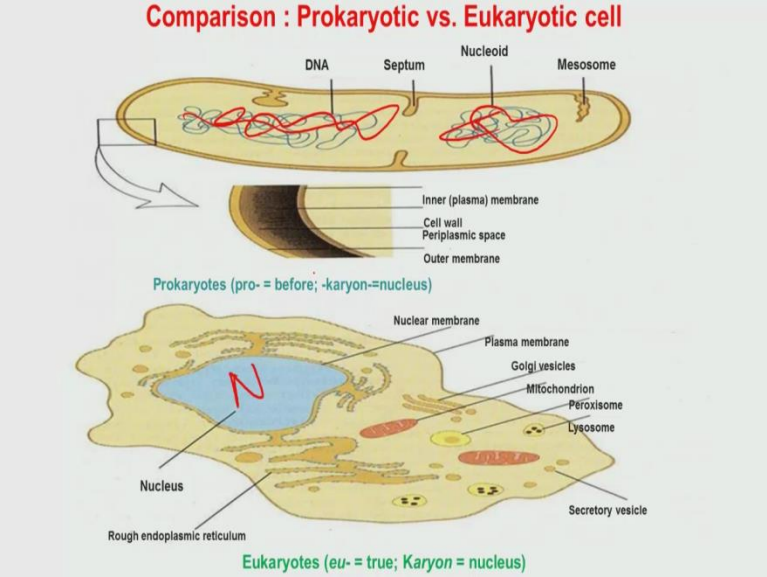

So just to give you some difference between the Prokaryotic that is Promens before and Karyopyknosis Prokaryotic cells and eukaryotic cells. Now Prokaryotic cells are not very, are not very well developed cellular structure, where as eukaryotic cells represent the most matured and more well developed cellular structure. In prokaryotic cells there is no well defined nucleus like what you have in an eukaryotic cell, instead DNA as you can see in the very coil form is distributed or dispersed in the cytoplasm of prokaryotic cell.

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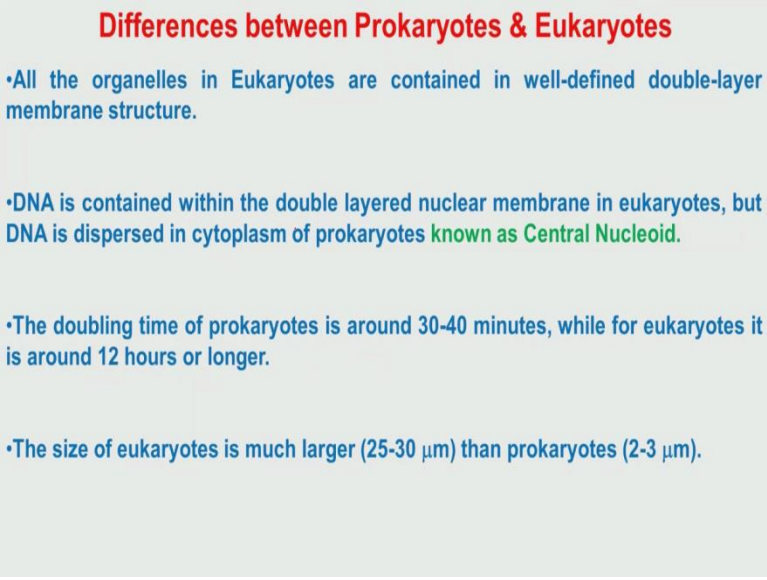

So this is that major difference between prokaryotic cell and eukaryotic cell, other difference is that the doubling time of Prokaryotes is around 30 to 40 minutes. That means the prokaryotes is typically divides much faster than the eukaryotes which are like a full nucleated cells. And for eukaryotic cells like fully nucleated cells doubling time is somewhere between, somewhere around 12 hours or longer, the other thing you might have noticed in last slide, I think there was a, micron scale bar is not there, otherwise the size of the eukaryotes is much larger around 25 to 30 microns than prokaryotes, that is 2 to 3 microns.

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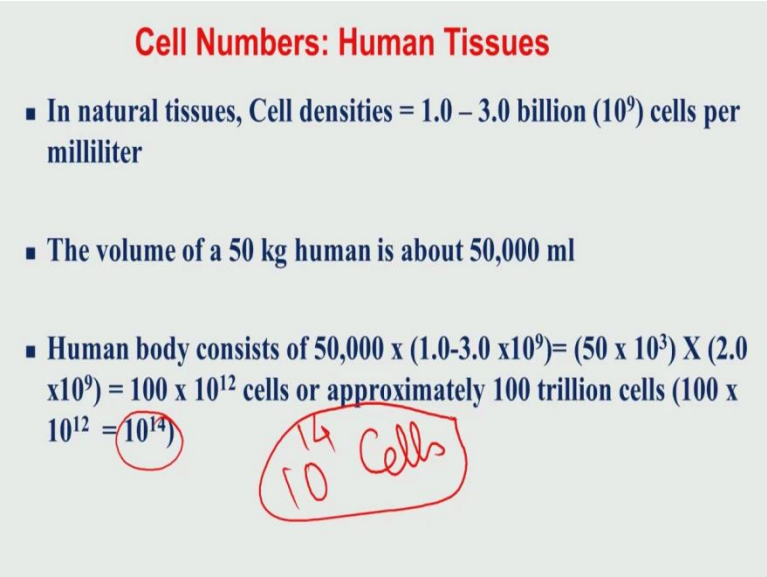

The first two differences I have already explained to you a few minutes back, now coming to the cell size as I said that cells are typically 20 to 30 micron in size and in natural tissues like in an adult human, the cell density is typically is 1 to 3 billion cells per milliliter, now if you consider a normal healthy person weighing 50 Kilos, therefore will have around 50,000 milliliters of, milliliters of, and therefore the human body contains somewhere around 10 to the power of 14 number of cells, now this numbers as you see that it also depends on the body weight of a humans, of a person but roughly this order of magnitude of this numbers are important so that you have an idea, that how many number of cells typically are present in human body.

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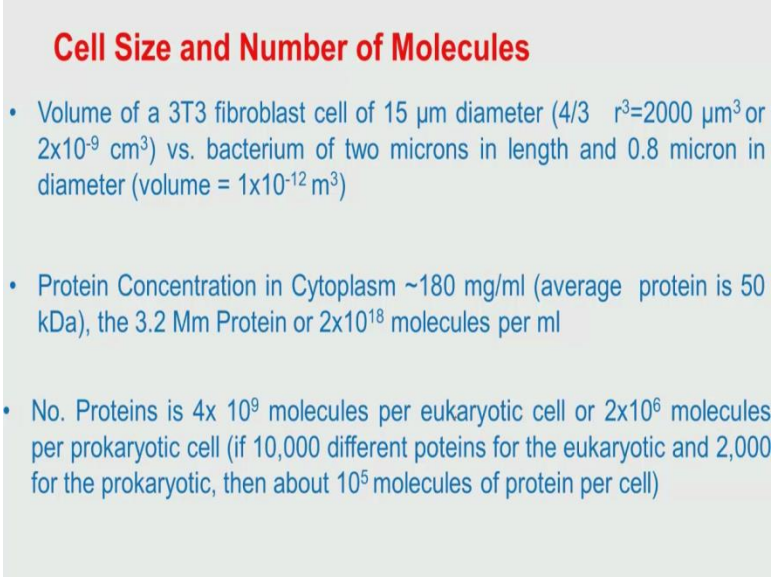

Other things one has to also consider that depending on the size of this, whether it is eukaryotic cell or prokaryotic cell, you have to see that what is the total number of protein molecules, which are proteins which are present in the eukaryotic cells, now if you go by this very simple calculation which are mentioned in the slide, what I want to draw your attention to this numbers here, so typically eukaryotic cell has a protein molecule, Cystolic 10 to the power of 9 molecules where as in a prokaryotic cell, this protein molecule number of proteins is little less simply because this prokaryotic cells have a much lesser cell size compared to eukaryotic cells.

So I repeat you a 10 to the power of 14 number of cells, typical eukaryotic cells and in terms of proteins you have a 10 to the power of 9 proteins in eukaryotic cells whereas around 10 to the power of 6 proteins in prokaryotic cell, so this, some idea of this numbers, or order of magnitude of these numbers is important so that one can, one can rationalize that once a cell is placed in a culture medium with proteins and so on, so how these proteins play an important role or mediate the cell material interaction.

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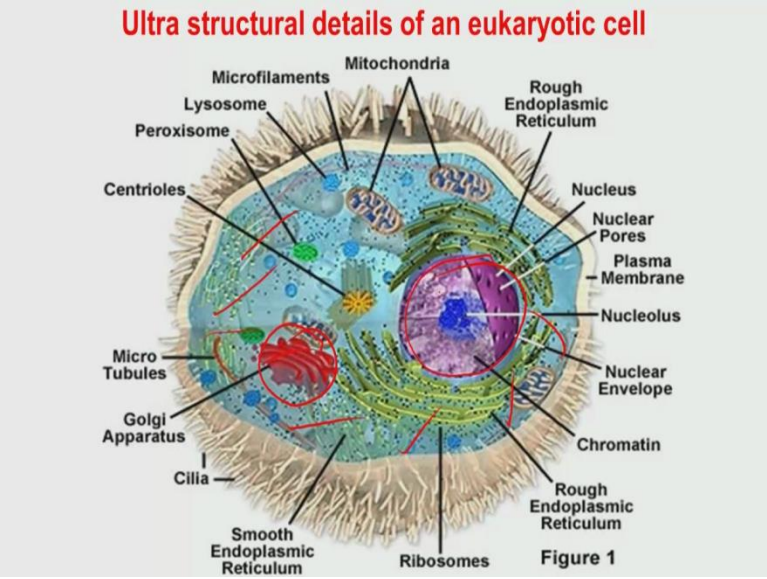

This is another fantastic image of a animal cell cross section where each of these organelles are essentially staying with different fluorescent dyes and as a result you can see trough endoplasm reticulum is staying in more or less green Golgi apparatus appears is red, mitochondria also you can stain it by micro tracker that is one of the dyes typically the nucleus is stained by Dapi stain and actin filaments also stained with a different kind of dyes.

So essentially what is, what these microscopic image tells you that it is indeed possible to carefully stain each of these specific organelles using certain fluorescent or certain fluorescent specific dyes so that one can image different organelles appropriately under a microscope.

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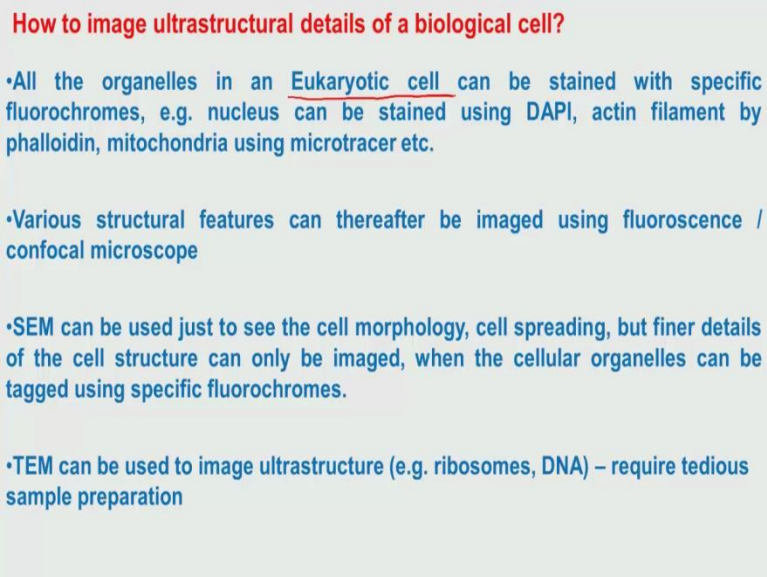

So this has been, this brief discussion has been summarized in this slide, so as I said that all the organelles in an eukaryotic cell can be stained with specific fluorescence nucleus by Dapi, actin filament by phalloidin and mitochondria by microtracer, and then the structural features can therefore be imaged, can thereafter be imaged by chromosomes are confocal microscope, scanning electron microscope, that is abbreviated as SEM, this can be used to see the cell morphology or cell spreading, but how the final details of the cellular ultra structure can be imaged, can only be imaged when the cellular organelles are tagged using specific fluorochromes.

And therefore to appropriately image the cell structure one has to use different kind of microscope that is process microscope or confocal microscope, another microscope which is used not that widely or not that extensively that is the transmission microscope, it can used to image some of the ultra structures like ribosome structure or DNA structure but which requires very tedious sample preparation and very careful sample preparation and therefore many researchers they regularly use fluorochromes or confocal microscope in conventional biological sciences as well as in the field of biomaterials.

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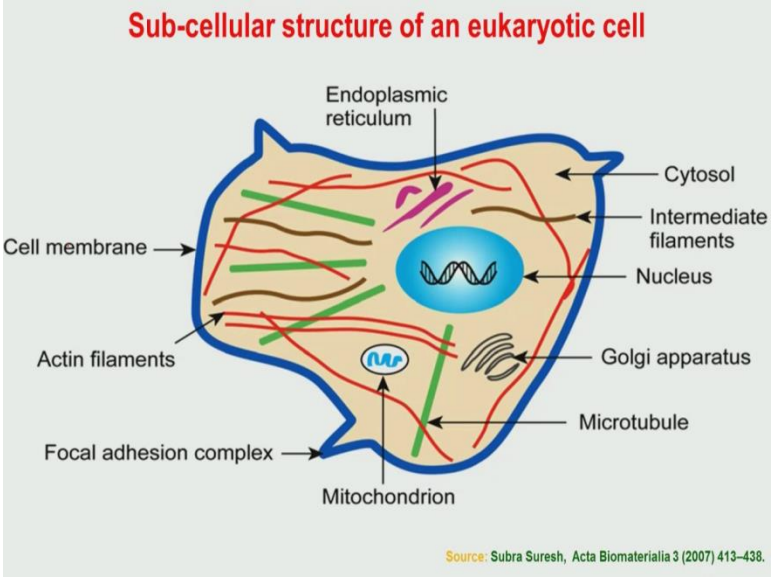

Around 5 to 10 minutes back I was explaining you that you know how these cytoskeletons structure they appear in a eukaryotic cell , now here this particular slide shows some of the details and essentially you have this microtubule which has a different diameter than the actin filaments which is shown here as red and also this another one, so you have a intermediate filaments also shown is a with a different colors like black colors so microtubules, intermediate filaments as well as the actin filaments, they essentially represent different protein complexes and also they have different morphology as well as sizes, so from there one can distinguish in a carefully imaged cellular structure that how these three important elements of a cytoskeleton would appear in real life.

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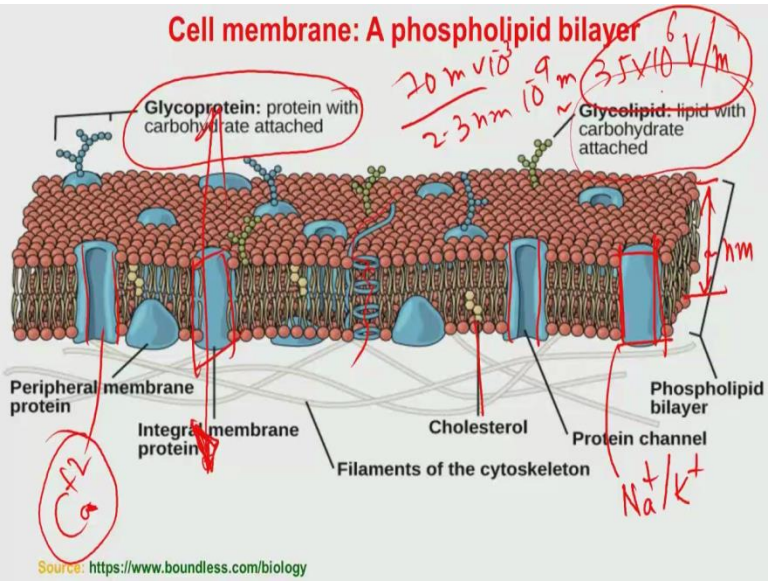

I was also explaining sometime back that although it appears very simple as a cell membrane, cell membrane has various characteristics structure what you call as phospholipid bilayered structure, so in this phospholilpid bilayered structure you have certain trans membrane protein also, so trans membrane protein means the protein which extends from intra cellular region to extra cellular region, like which extends from cytoplasm of the cell to outside space of the cell, these are trans membrane proteins.

You also have some of the specific proteins which are attached to the cell membrane, one of them has been shown here glycoprotein, which is protein with carbohydrate attached and another one is called glycolipid, so lipid with carbohydrate attached. You also have the cholesterol and this is just inside of the cells, and you have a peripheral membrane protein, so it is like a very nice two bilayered structure, it is a very kind of sandwiched type of structure, and there are several transmembrane proteins are all also shown and also you have a certain channels in the cell membrane structure.

Now these channels are very specific channels and many researchers in a biological sciences department they do extensive research on the characteristics and properties of these channels, so one of these channels can be sodium potassium channel, sodium potassium channel essentially has some specificity that It can allow only sodium intake exit or only the potassium intake or exit, at the same way cell membrane also has a voltage sketched calcium channel, so this channel

is essentially will send certain voltage differences across the membrane and accordingly they will open up only to allow the transport of the calcium ions.

I repeat there are two types of channels in the cell membrane one is called sodium potassium channel, another one is called calcium channels, now this particularly this calcium channels, they are voltage gated calcium channels, now these channels extremely sensitive to the voltage difference, so at a particular voltage difference this channels will allow the specific transport of only calcium ions, similarly some channels are there in the cell membrane which are shown differently in the terms of their morphology, particularly these type of channels, these type of channels, so these channels are essentially can be sodium potassium channel, again they have, they can specifically allow the transport of the sodium potassium through these channel into cell or outside the cell.

Here I must mention that any given time point there is a kind of a balance in terms of the sodium potassium calcium ions between the intra cellular region and extra cellular region and the difference in terms of these ion concentration between the intra cellular region and extra cellular region causes certain transmembrane potential across the cell membrane and these transmembrane potential is of the order of the 70 milli volt or so on. But you have to also understand that the sequence of this cell membrane is very small, it is like few nanometers like 2 to 3 nanometers or something like that.

Now although this transmembrane potential is very small 70 milli volt, but if you consider the voltage difference across the cell membrane it is 70 milli volt divided by 2 to 3 nanometer, the nanometer means 10 to the power of minus 9 meter, milli volt means 10 to the power of minus 3 volt, so therefore you can immediately see that it is kind of turns out to be 35 into 10 to the power of 6 volt per meter that is the, roughly that is the transmembrane potential voltage difference, so it is essentially this value represents very significant voltage difference across the cell membrane simply because of the difference in the tiny concentration are in the extra cellular or in the intra cellular region.

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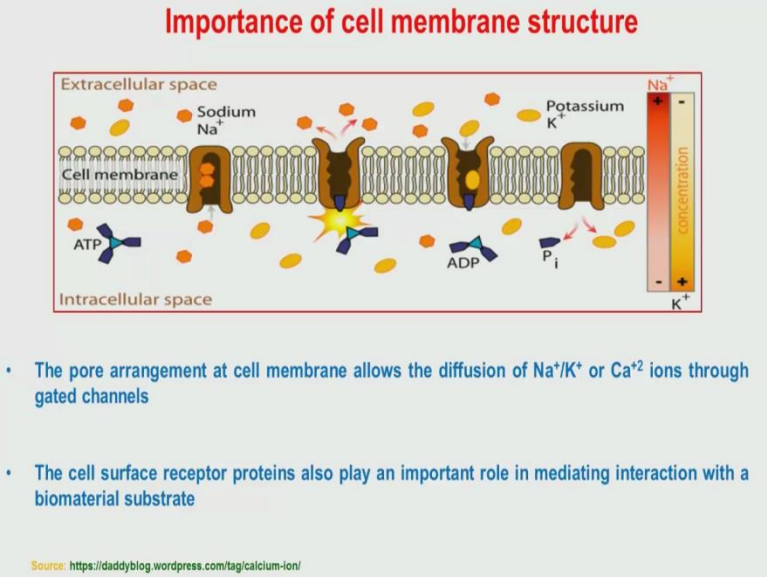

Ok, this is what has been mentioned to you just few minutes ago that importance of the cell membrane structure you have sodium ions, you have potassium ions, and the sodium ions can be transported through some of the specific channels, potassium ions can be transported to these channels, the specific pore arrangement of cell membrane allows Sodium Potassium Calcium ions and the cell surface restructure proteins are there, they play an important role in mediating interaction with a biomaterial substance.

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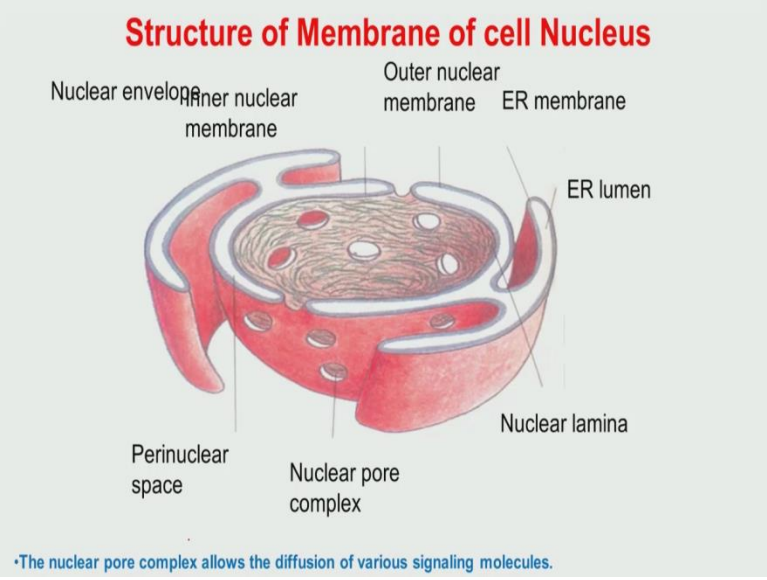

So other specific characters speaks of a nuclear membrane as I mentioned in the beginning of this module, so nuclear membrane is also a characteristic bilayered structure, you see this is the inner layer and this is the outer layer, and this inner layer and outer layer they have a very specific arrangement and the way you see that there are certain holes in the way I am drawing, these are certain holes in the nuclear membrane structure and these holes they essentially fall on what they call nuclear pore complex.

So this nuclear pore complex is one of the things that also allows the transport of the proteins or certain cellular signaling molecules into the nucleus from the cytoplasm. So as you can see from last 15, 20 minutes or so the cells have the unique cellular organelle characteristics which also allows the transport of various ions, proteins and biological macro molecules.

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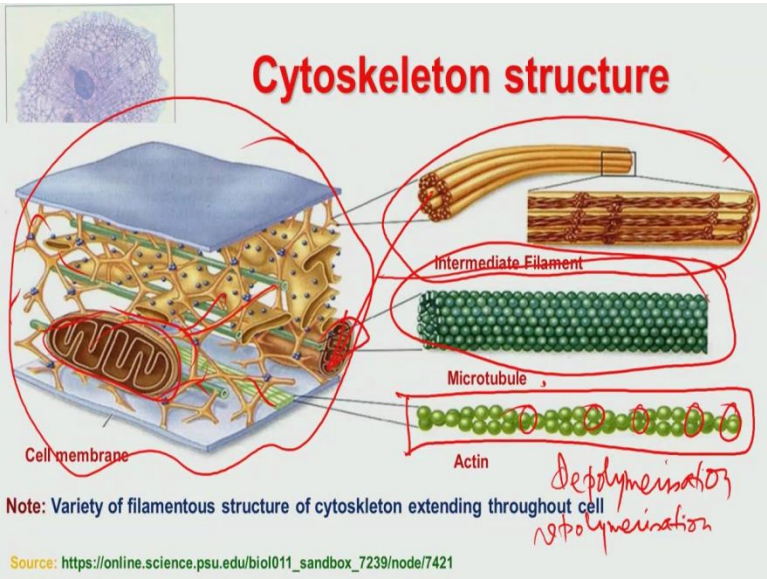

So this gives little bit more details about what I have shown you in this particular slide that what is the sub cellular structure eukaryotic cell or in other words, what is the cytoskeletal structure of the eukaryotic cell because skeleton means like a human skeleton you know that human skeleton is cellular skeleton essentially means that is the skeletal structure which supports, which provides mechanical support to all the cellular organelles in the cytoplasm, therefore one has to understand that what is the key characteristics of these kind of skeleton structure that makes it so special in terms of providing mechanical support.

Now you can see that I will start with the bottom one that is for the actin filament, as I said that this essentially represents a protein complexes or polymeric long chain and here these polymeric chains are arranged in a specific manner along the back bone chain and these actin filaments during the sulfate processes they can undergo de-polymerization and de-polymerization essentially means that these actin filaments can break down to individual monomeric molecules, individual monomers.

Now if it is required these monomers can come back together to make again actin filament to molecule and this is called re-polymerization. So the sequence of these pre-polymerization and re-polymerization takes place in a cellular structure. The second structural element of cytoskeleton is intermediate filament as you can see from the schematic of these two, sorry microtubules, as you can see from the schematic of this two microtubule and acting filament, micro tubule has a much larger, relatively larger dimension in terms of the tubule diameter compared to the actin filament.

Third one is the intermediate filament, intermediate appears to have a intermediate size between that actin and the micro tubule, now this has been shown very nicely in this part of the cell membrane structure where you can see this is the mitochondria and this is a different filament structure in the cytoskeleton which is very nicely shown and this is your intermediate filament and some of the microtubules are shown here in the form of a large tubular structure and also you have a actin filament structure which is shown her.

So you are seeing this green color, slightly green, like light green color this is your actin filament, you see dark green color large tubular structure this is your microtubule and you also see that intermediate filament with a characteristically different morphology compared to other two filament structures, I think I will stop now and I will come back to this more discussion in the next.