Biomaterials for Bone Tissue Engineering Applications
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Week- 04
Lecture- 15

So in the last module we have discussed about the cell signaling process. Today we will start on the discussion on the different cell fate processes.

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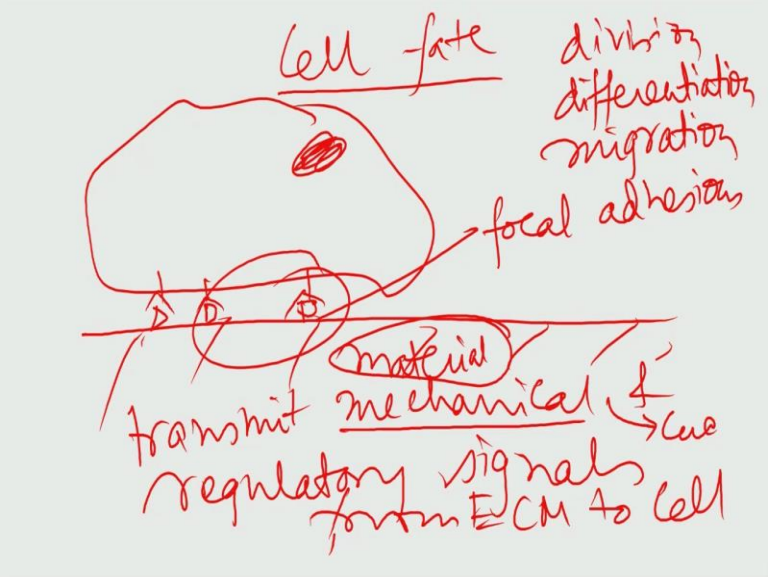

So, if we recall that if we recall that cell fate processes essentially means that you have a (bio material) you have a material substrate on which you have a eukaryotic cells, truly nucleated cells and you have some of the focal adhesion, focal adhesions on the, on the material substrate so essentially some of the macro molecular assemblies are formed, so focal adhesions the bio logical role of focal adhesions is to transmit mechanical and regulatory signals to the interactin cells, mechanical and regulatory signals to, signals from ECM that is the outside extra cellular matric space to cell.

So this is one way that signal is being transferred to the eukaryotic cell, another thing is that, that this (bio) material substrate has certain elastic stiffness, so this elastic stiffness essentially can, essentially is a, acts as a mechanical cue, so depending on what is the mechanical stiffness of the material this eukaryotic cell would now want to do several of these things, which I will try to explain here in this slide.

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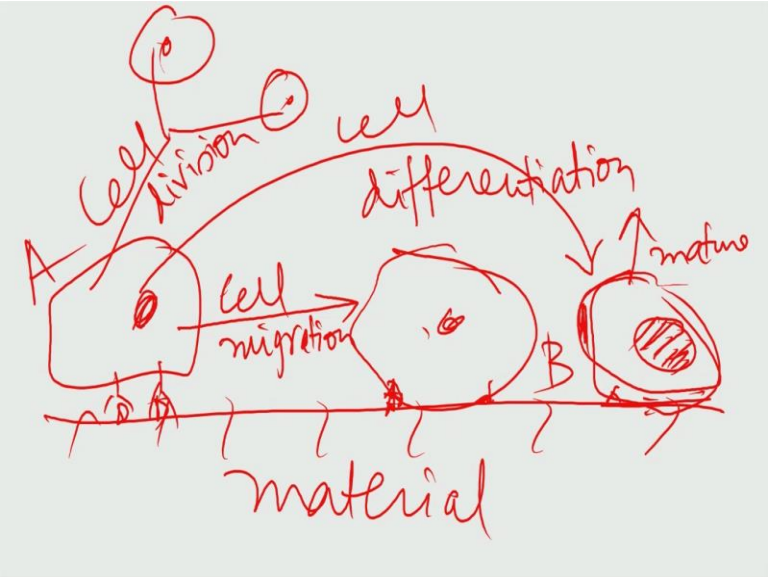

So this is a small eukaryotic cell, so you have these focal adhesions and all so it can decide to it can decide to migrate or to undergo cell motility that means that it will go from place A to place B here on the bio material substrate itself, so you have this cell focal adhesions here. Or it can decide to undergo cell differentiation, okay? So I am I am (ba) drawing this eukaryotic cell intentionally with different nucleus which is hatched here, so this is your differentiated cell or more mature cell type, so it can undergo cell differentiation or cell migration. Or it can undergo 2 daughter cells. So you have 2 daughter cells so that means it can decide to undergo cell division. So at least 3 cell fate processes has been mentioned here. So I repeat 1 is division, second 1is differentiation and third 1 is migration or cell migration.

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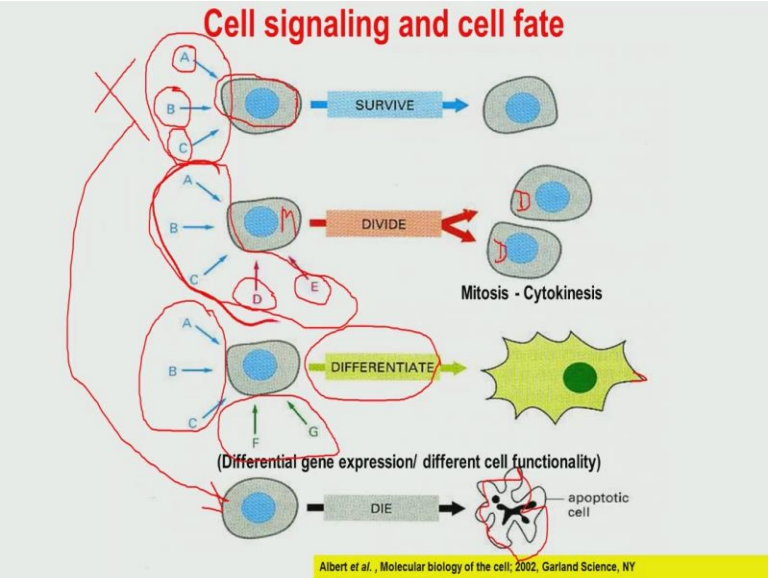

So depending on the, depending on the combination of signaling molecules, if you go back to the last to last slide in the last module, so depending in the combination of different signaling molecules which are present, in the extra cellular matrix or which a particular cell, which a given cell receives, it can decide to undergo cell division or differentiation or migration. So having said this let me now discuss each of this topic with little bit more details and we (d wud) we would like to develop some understanding that how this cell fate processes take place in a (b) in biological system containing biomaterials and different cell types.

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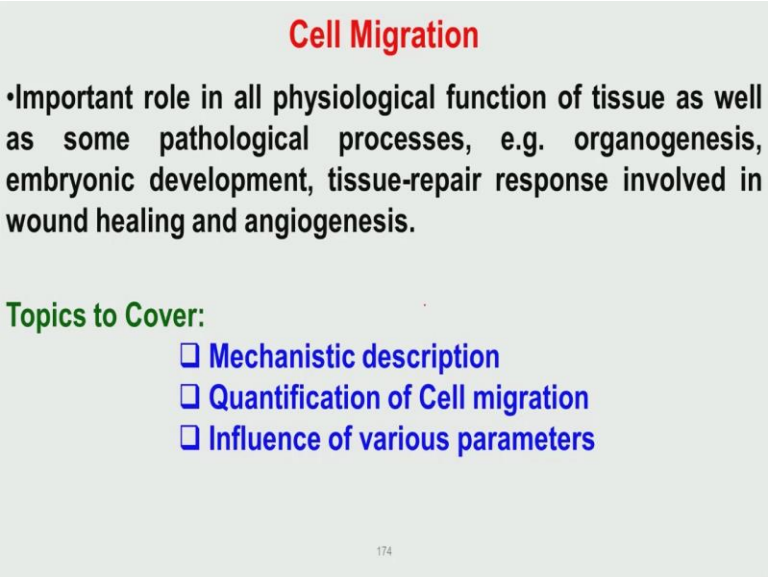

So, first 1 is a cell migration. Cell migration is essentially as I said, cell migration is also described as cell motility, so these are like synonymous terms. And essentially it is like crawling and walking of a biological cell on a material substrate and it is very similar to a baby walking on the floor, similarly when a biological cell walks or crawls on a material substrate it is called cell migration. So it plays an important role in various physiological function of tissue as well as some of the pathological processes. Some of these has been mentioned in this slide like tissue repair response or wound healing or angiogenesis, so all these typical pathological processes demand the cells to migrate locally from 1 place to another which may be separated by some 100 to 1000 microns.

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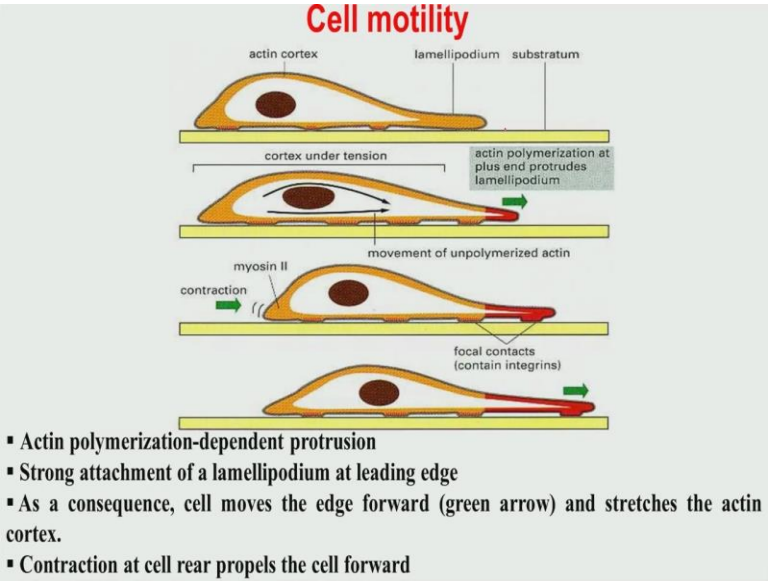

So what is the first stage of this cell migration, the first stage is the protrusion on membrane, lamellopodia so this slide essentially shows schematically a cell is adhering on a material substrate, this is your focal adhesion, so this is 1 focal adhesion, this is (1) second 1 focal adhesion. So the cell is trying to move in this x direction and this is your forward end and this is your rear end so that is the back end.

So, this is your nucleus here on this, and then you have the cytoskeleton, is extended all through the cells. Now this particular inset essentially shows that there is a process of depolymerisation of the actin filaments is taking place, so this is the process of depolymerisation. So, depolymerisation leads to several of the monomers from the actin filaments and that kind of getting accumulated at the forward end of an advancing cell.

Now this small dotted arrow essentially indicates when these monomers again will be repolymerised, it will advance along these dotted line or the forward end of the cell will advance along the dotted line. So there are several podia in the cell, 1 is called filopodia; the second 1 is called lamellopodia. So the, essentially the role of this, all these filopodia and lamellopodia is to assist the cell in their locomotion or assist cell locomotion or assist cell motility on another elastic substrate. So this has been also explained here in terms of text. So it is the, first 1 is this so first 1 is the protrusion of the membrane lamellopodia and advancing towards certain directions.

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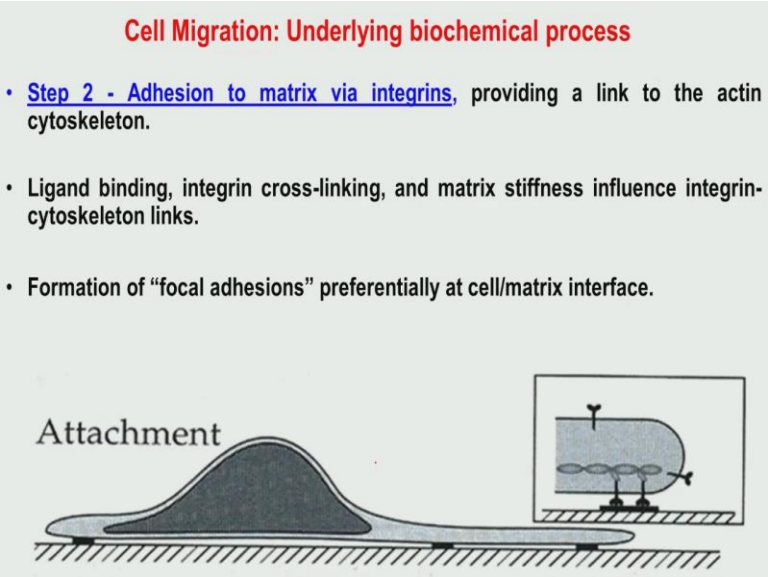

Second 1 is the adhesion to matrix by integrins, so that is what it means. Then once this dotted line, remember in the last slide I have shown this dotted line, along this dotted line a cell advances by few microns, then this advancing edge of the cell, this is your advancing edge, so this advancing edge of the cell will, will only be stable, if an only if that advancing edge also establish focal adhesion with the underlying material substrate. So the formation of the focal adhesion is key as far as the stability of the particular cytoskeletal part of the cell, and that has been shown.

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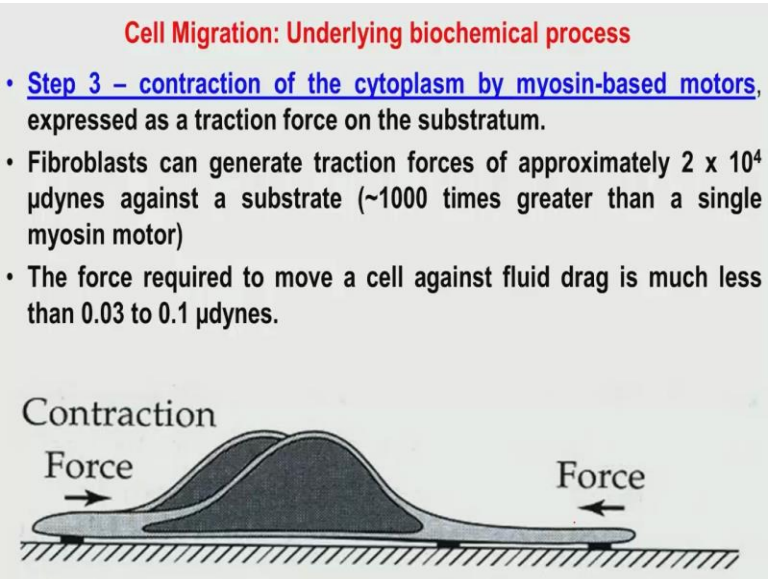

So this is your new focal adhesions that has formed that forms, and this focal adhesion once it forms and then (attach) then it helps the cell to attach to the substrate much more firmly at the advancing edge and there will be a contraction force at the rear end, and these contractions forces are approximately like 10 to the power 4 microdynes micro means 10 to the power minus 6. So 2 into 10 to the power minus 2 dyne, so dyne is your gram per centimetre square.

So essentially I am talking about this traction force is of the order of point 2 into 10 to the power minus 2, that means point 02 dyne and 1 dyne is gram per centimetre square; so this is a CGS unit. So, essentially you can see that how small is that force that actually (gen) is generated as a traction force here.

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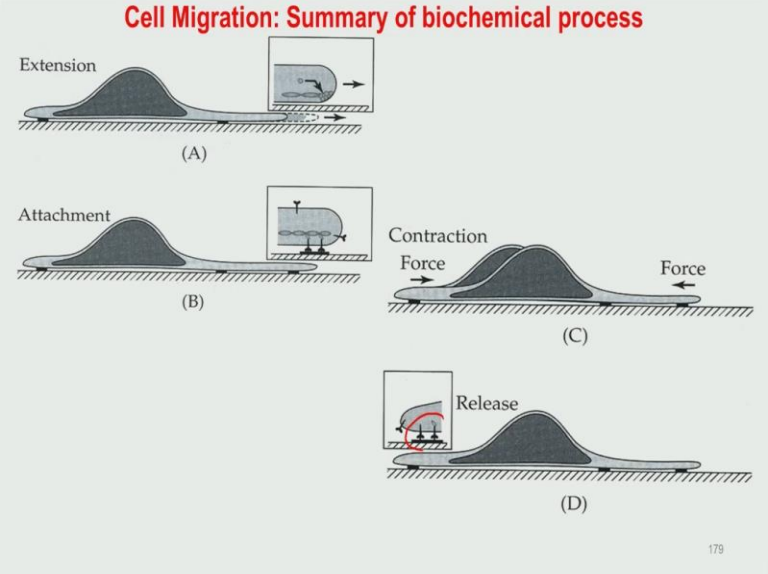

And this (traction) and this traction force, because of that presence of this traction force what is the next stage it does, that it released the this focal adhesion at the rear end is released, so this is that stage 1, this is stage 2, this is stage 3, and this is stage 4. In the stage 4 what will happen, these focal adhesions, this A phase are now released or they are now kind of lifted off from the matrix, helps the cell to migrate along this x direction, okay?

So this entire bio mechanical process, bio chemical and bio mechanical process involved here in the cell motility is explained in this particular slide. As you can see that the cell was initially at this position A, cell has now moved this position B. Remember this A to B distance can be of the order of just micrometer, micron range. Simply because as I will give you some numbers within

the next few minutes, you will be able to realize that the speed at which a cell migrates on any elastically stiff substrate varies in the range of 10s to 100 micro meter per hour. So essentially in one hour a cell can move in a particular direction only by 10s to 100 micro meter in the length scale.

So therefore the way it appears here exactly it happens in the real life scenario. So, I repeat that cell initially was at position A and it has forms these 3 focal adhesion, this is that micro molecular complexes. Now slowly that cells is now advancing along this direction, along the arrow. This involves actin polymerization, (actri) as well as protrudes lamellopodium and then after that, then there is a contraction force here and that allows this particular focal adhesion to be released, so this focal adhesion is released at the rear end. And once this focal adhesion is released at the rear end it is lifted off and then this helps the cell to progress 1 step ahead. S,o this entire process allows the cell to go from place A to place B and along this along this green arrow, along the screen arrow.

The another thing that is important in this cell motility is that some cells, they can grow or they can (they can) migrate in 1 particular direction for at least few hours but some cells, they often change the direction (at) along which the cells moves. So this changing direction, that how frequently or how soon that this cell changes its direction; that is determined by persistence time. So I will go to that some few numbers I will just show you.

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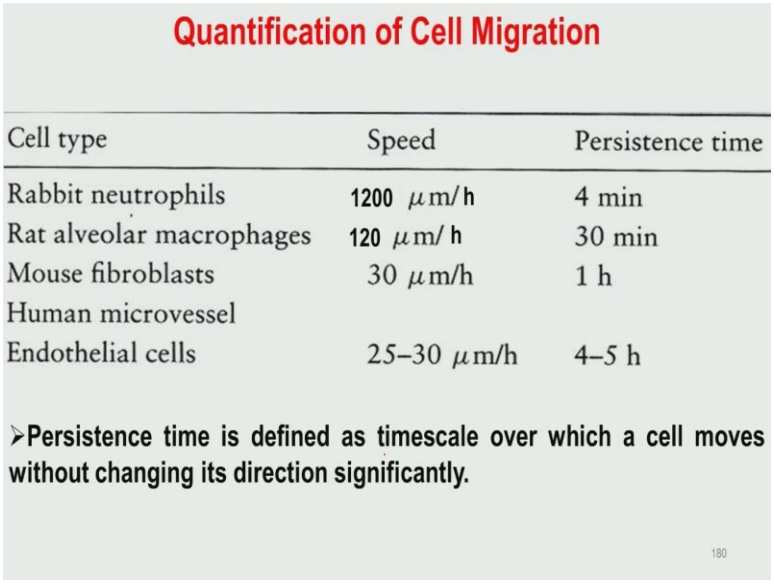

So persistence time is defined as the time scale over which a cell moves without the changing the direction significantly. So this persistence time, I have mentioned a few cell types in this particular slide. Let us take the example of mouse fibroblast cells that is the L 9 to 9, this cell line is quite widely used in the bio materials research. So therefore let me circle this particular cell line, the numbers related to his particular cell line.

The cell migration speed of L 9 to 9 cells is 30 micro meter per hour. That means in 1 hour the cell can move only 30 micro meter. The cell size of this L 9 to 9 is somewhere around 20 to 25 microns. That means, what it means that in an hour a cell can move over a length scale which correlates with the size of the cell itself. So therefore you can very well realize that how slowly a cell moves on a material substrate.

The second thing that what you what you see this (nu) another number that is persistence time which is like 1 hour, 1 hour essentially means is fibroblast cells can move on particular substrate in a particular direction, in a linear direction only for 1 hour, after that it may likely to change the direction and migrate in another, in another direction or it can change the direction.

Some of the other numbers like Endothelial cells, endothelial cells again their cell migration speed is similar to L 9 to 9 mouse fibro blast cells 25 to 30 micro meter per hour and persistence time is little longer, it is 4 to 5 hours. Persistence is fairly (sm) fairly short, it is for the rabbit neutrophils which is like 4 minutes, simply because it also has an extremely high speed

compared to L 9 to 9, the speed is like 1.2 millimeter per hour, that means it can go over a mili meter in a given in 1 hour time frame.

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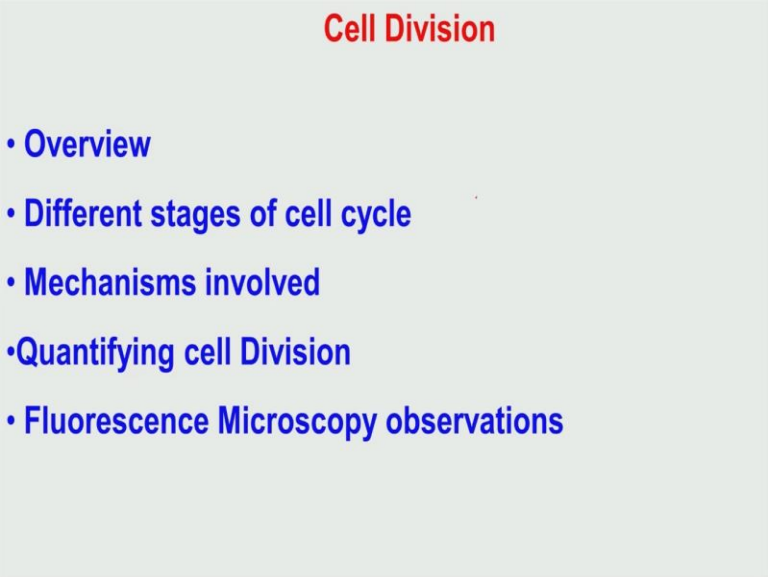

So this is enough of cell migration. Let me go to that another 1, this is cell division. So cell division essentially means that a mother cell divides to 2 daughter cells. I will just show you that some of the different stages of cell cycle as well as mechanisms, different mechanisms involved, how to quantify cell division and so on.

So the cell division is very important because once a cell adheres on a material substrate, so this is your material substrate, this is your cell, so 1 of the things that when the, when you are growing, when you are seeding the cells on a material substrate, or when you are culturing the when you are culturing the cells in a typical culture flask, like a typical biologist they do, whether the cells are viable and in its perfect growth pattern, that is monitored by counting the number of cells after different time scale in the culture.

Same thing, when (the) when a cell will adhere that 1 of thing that we need to know, that after their adhering on a material substrate, whether the cells are able grow in an uncompromised manner like it would otherwise have grown in a typical culture flask. So if you have a typical culture flask, so this is a typical culture flask like you have culture medium, you are growing the cells.

So here if you grow the cells, the cells will grow very fast like 1 to 2, 2 to 4, 4 to 8, whether this kind of growth pattern is compromised simply because it is now not being grown in a free manner, in an isolated manner in a culture flask instead it is now grown on a particular material substrate. So for those kind of analysis you have to understand that essentially how a biological cell divides and grows.

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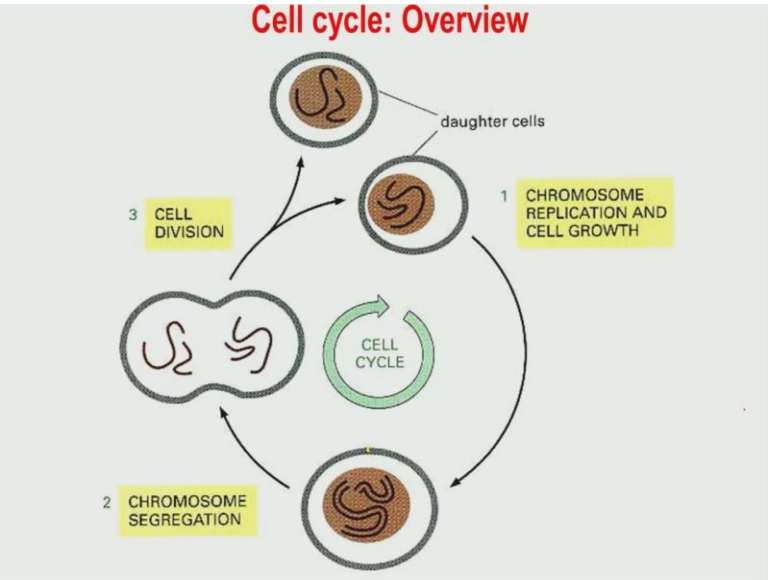

So this is an overview of (the) of a mother cell undergoing the cell cycle, cell division to 2 daughter cells. What you see that this is a, when this cells, cell division starts, it also starts with the chromosome replication and cell grows, like cell increases its size. And then after that there is a chromosome segregation is taking place, so it is now showing certain indication that some furrow formation and some indication that cell is likely to divide to daughter cells.

Remember why I am saying cell is likely to divide because all the cell division process also depends on whether the appropriate signals are present in the cellular micro environment, so around the , around the cell. So this is your cellular microenvironment and whether in the cellular, whether the cellular microenvironment contains the signaling molecules, that way I have mentioned in the last module during the discussion on the cell signaling process. And once this signaling that sufficient signals are present then cell will be divide, cell will finally divide to 2 daughter cells.

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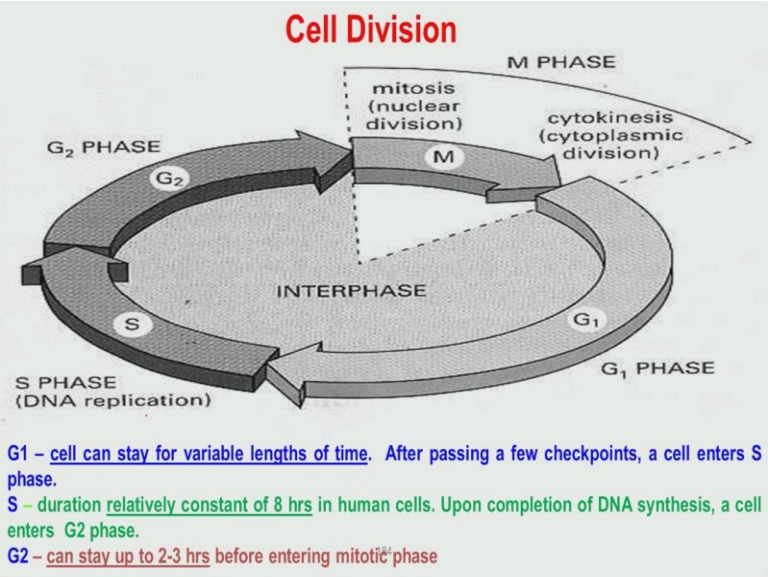

Okay, so this is a typical eukaryotic cycle you have this, so there are something called (G no) G1 phase, that is the gap phase. Then you have a S phase that is the synthesis phase and then you have a gap2 that is the G2 phase, and you have an M phase that is the mitosis (phase) that is a nuclear division phase. And then after that it goes to cytokinesis that is the cytoplasmic division. So mitosis, nuclear division, cytokinesis that is the cytoplasmic division and that takes place in the M phase.

So if you consider a kind of a clock, the way it has been shown the major part of the clock is now contains the interphase that is the G1 plus S plus G2 phase and then the way it has been shown here, it is very clear to you that G1 plus S plus G2 is more than 80 (per) 80 percent of the total cell division cycle time. So at the G1 phase, a cell can stay for variable lengths of time and after passing a few check points the cell enters the S phase and then S phase it is typically human cells; they stay for 8 hours and G2 phase another 2 to 3 hours. So S plus G2 is almost like 10, hours it is roughly.

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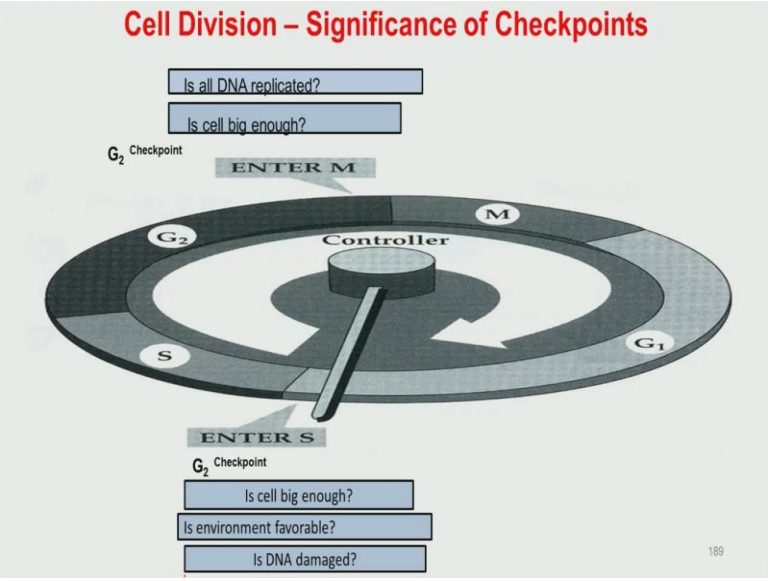

So, so that what, what are the different check points and what is the role of this different check points. Check point number 1 that is C1, what you see that a cell will try to (enter) or wants to enter from G1 phase to G2 phase. So (G1) as it tries to enter from G1 to G2 (phase) G1 to S phase, the certain questions need to be answered by the cell. That is, is the cell big enough, is the environment favorable, is the DNA damaged? So what it means is that, that whether the protein synthesis is taken place while cell is staying at G1 phase.

Environment favorable means whether the signal molecules are present or sufficient signaling is taking place, that means the signal molecules to be there for both cell survive as well as cell division both. So you need to have certain extra signaling molecules which will (insta) which will influence the cell division process. If the DNA of the cell is damaged then cell cannot go to S phase. It will remain in the G1 phase.

So another thing that I must mention is that G1, to S or G2 to M or S to G2, that, for that matter of fact these are like irreversible process. Irreversible means once a cell goes from G1 to S it cannot come back from S to G1, so G1 to S is an irreversible phase. Similarly when a cell goes from G2 to M again it is a irreversible process. It cannot come back to G2. So this is something called commitment, commitment means once a cell goes from G1 to S that means cell is committed to go to S phase and this commitment will not be it cannot be reversed like it cannot come back to G1.

Now for example, let me explain these things with little bit, little bit more time, for examples if the, some of the questions that are asked here, the answer is not favorable then what will happen, a cell would stay in G1 phase and after sometime the cell would activate its own suicidal mechanism to undergo apoptosis; apoptosis means programmed cell death. Similarly when a cell wants to go from G2 to M phase again cell has to answer to these 2 different questions; is all DNA replicated; is the cell big enough? And if the answer is yes to both the questions then only cell will be committed to enter to M phase.

So this is your G2 check point, and that is (another) so this is your G1 check point and this is your G2 check point. So these 2 check points are very important and they determine the level of commitment of the cell to undergo the cell division process. And finally once it goes to M definitely the cell would go to 2 daughter cells finally, so 1 mother cell will lead to go to the 2 daughter cells.

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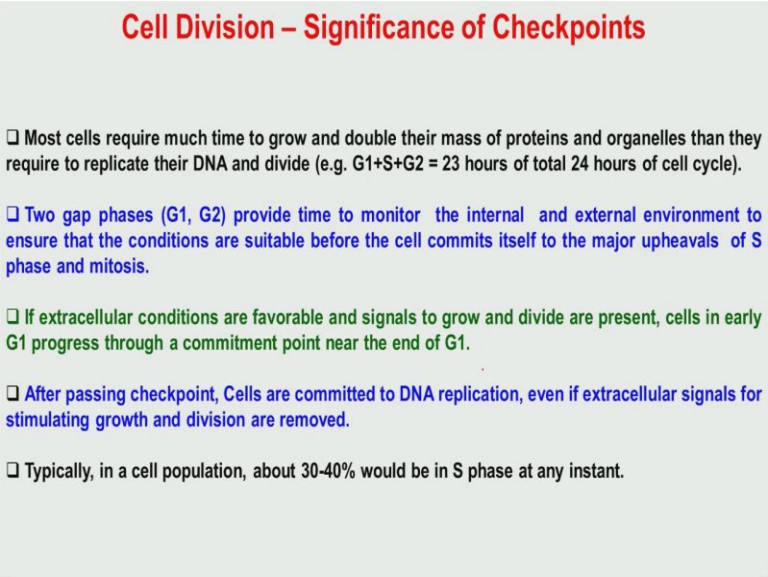

So these, all these points that has mentioned this has been summarized in this particular slide. So most cells require much time to grow and double their mass points than they require to replicate their DNA and divide. To substantiate this point there are some numbers as mentioned here, so total time taken in the G1, S plus G2 is 23 hours and typically cell cycle if, for any given cell type is 24 hours, so it is more than 90% of the time a cell stays at G1, S and G2 phase.

Second 1 is 2 gap phases that is G1 and G2 provide time to monitor the internal and external environment of the cell to ensure that the conditions are suitable before the cell commits itself to the major upheavals of S and G2. And if the extracellular conditions are favorable and signals to grow and divide are present then only the cell is committed to go to G2 near the end of G1 and goes to G2 and once it is committed it is an irreversible process and if, and they will, once it is (go) once it goes from G2, to M phase even if now the signaling molecules are removed from outside the cell or in the cellular neighborhood or cellular microenvironment, cell is now committed to division, and that cannot be stopped, essentially once it crosses the check point from G2 to M, no matter whatever is the cellular microenvironment, cell will now divide into 2 cells 2 daughter cells.

Typically in a given cell populations, 30 to 40 percent of the of the cell are always in S phase. Now how to quantify this what kind of cells what is the fraction of the cells, it is in G1 or S or G2 or M phase. Typically these things can be done by fluorescent activated cells sorter analysis or FACS analysis and the other things that in this FACS analysis, they do, they will quantify the (nu) fraction of the cells that is in (G no G no G) G0 G1 or S or G2 and M phase.

So at any given point if you see when you grow when you grow this culture, at any given point if you find the fraction of cells which are at the S phase increases and then fraction of the cells at G2 and M phase decreases or they remain constant, that means you can (indi) that means you can understand that there is some problem with the cell division process, that cells are (not) cells are somehow getting stuck at the S phase so that is why S phase, it increases and they are not able to go from S phase to G2 phase or G2 to M phase.

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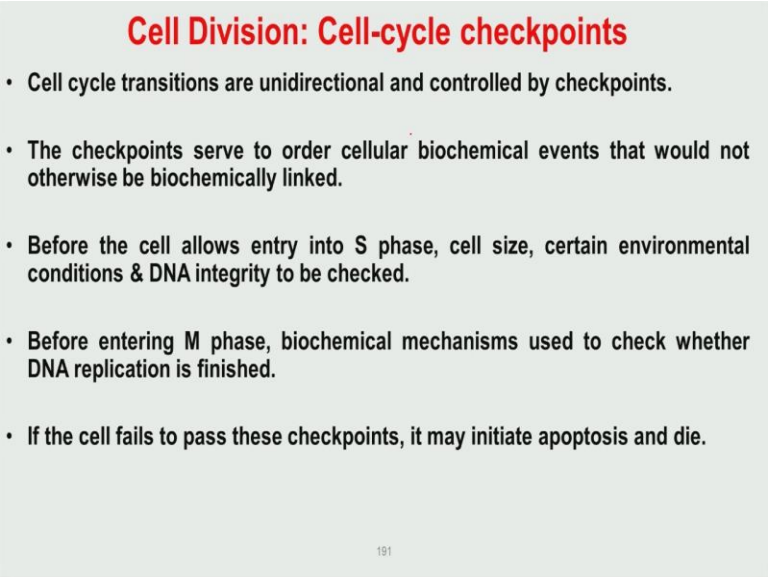

So these are some of the other points that is also mentioned and these are summarized again that is that, if a cell fails to pass these check points, it initiate apoptosis and die, and these all the checkpoints, the significance of these check points are essentially summarized in this point and this point that is going from 1 phase to another phase and the cellular bio chemical evidence that would not be otherwise bio chemically linked so this is the check points, that is that why these checks points are are important in the cell division process; this particular statement explains that. Other things that I have mentioned and I repeat that cell cycle transitions are unidirectional and therefore it is irreversible in nature so it goes only in 1 direction.

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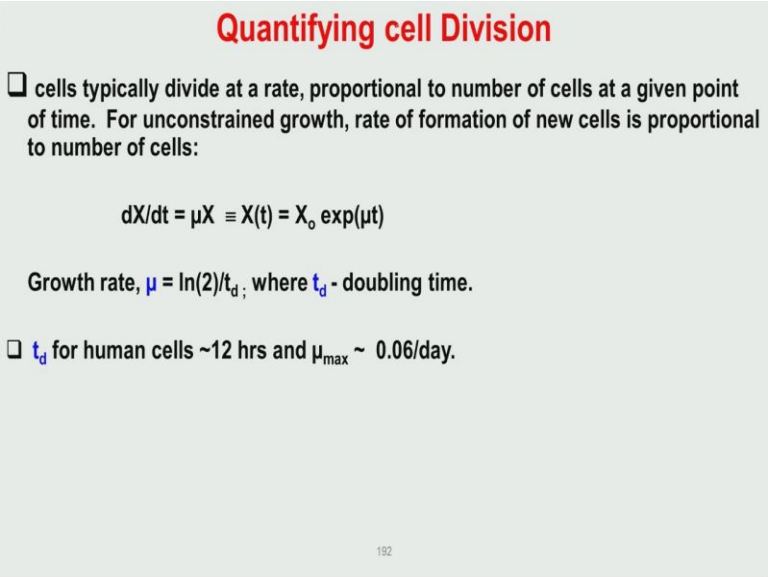

How to quantify the (s) rate of cell division, this has been mentioned here, let us say at any given point of time if the total number of cell is x so dx by dt is equal to mu x and this is a simple equations, mathematical equations you can solve and you can get these values, like at any given point time t, xt is equal to x not that is at time t is equal to t not, exponential mu t so mu is your growth rate that is the log natural 2 divided by td, so td is the doubling time. For most of the human cells the doubling time is 12 hours therefore mu max, maximum is point 06 per day. So I think I will stop here and then we will start with the next module.