Biomaterials for Bone Tissue Engineering Applications
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Module 5
Lecture No 21

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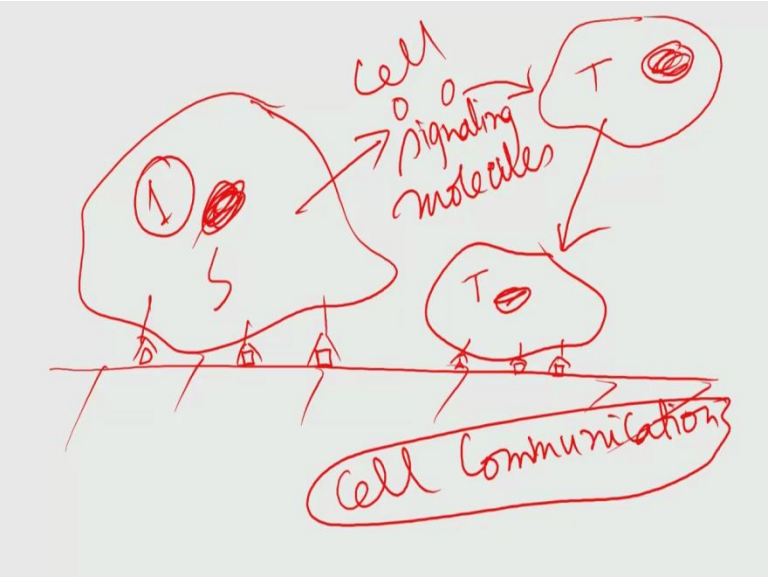

So in the last module we have discussed about the cellmeter interaction and in the cellmeter interaction one of the themes that I have mentioned is that, when a biological cell interacts with a biomaterial substrates, so you have an eukaryotic cell and eukaryotic cell has well-defined nucleus and then you have cell surface receptors which interacts with the proteins absorbed on a material substrates and then after that I mentioned that once the cell adheres on a material which is compatible in nature which is compatible at the cellular level then cell sends out the signaling molecules and the signaling molecules essentially communicate with these cells in the neighborhood and so that these cells now would come and try to adhere and try to interact with the protein molecules absorbed in the material substrate in the same way that it has that cell number 1 has done and if it this is number 2 then the cell number 2 would also do in the same way.

So this essentially means that the cell signaling is, this essentially means that this cell signaling is essential as a communication pathway and therefore there is a need to understand that how the cell communication take place in different manner. So cell communication is essentially

represents a very complex cellular network, by which the cell conveys certain formation to the cell in the neighborhood to another cell in the neighborhood so that it will convey certain instructions for the target cell, to follow further and these instructions are generated from the source cell.

So here it is the source cell and this is the target cell and this target cell after receiving the particular instructions which are being conveyed through the cell signaling proteins, so this is the cell signaling molecules or soluble proteins. So this then they will this target cell will perform certain desired function.

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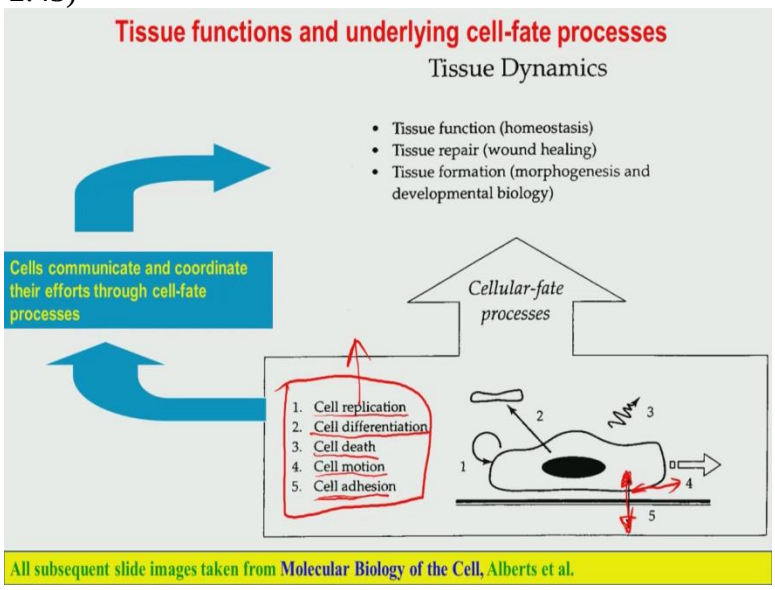

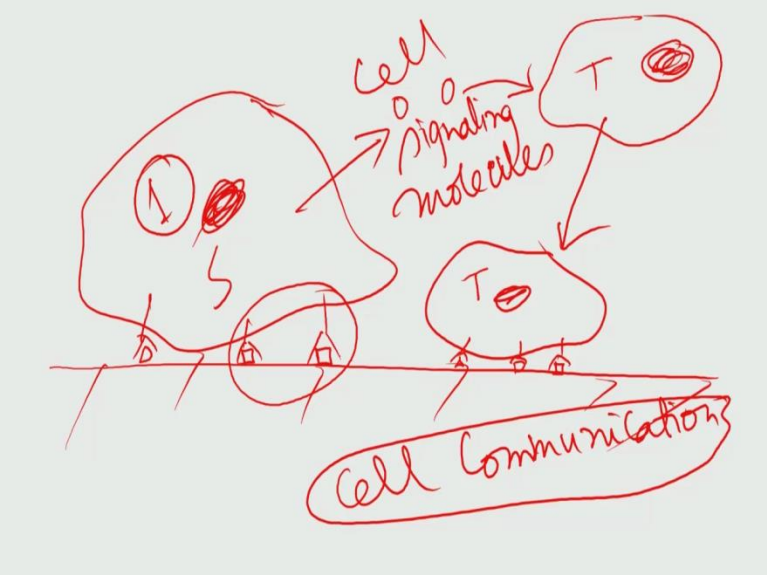

Now what is the function that we are talking about and these functions are essentially cell fate processes. Now each of the cell fate processes may be mentioned very briefly in some of the earlier modules. Replication means, so mother cell will give rise to two daughter cells and so on, so 1 to 2, 2 to 4 differentiations. The word differentiation means different gene expression that means a cell will be transformed to a more mature cell type.

Cell death means certain signaling molecules are removed or certain signaling process is removed from the cellular micro environment so that cell can undergo program cell date as well as necrosis that is that accidental cell death. Cell motion means the cell would kind of work or crawl on the material substrate and this is the cell migration. Cell adhesion means that is the formation of focal adhesion complexes or the focal adhesion points which is nothing but a combination of a few cell surface receptors integral protein as well as absorbed protein assembly protein complexes.

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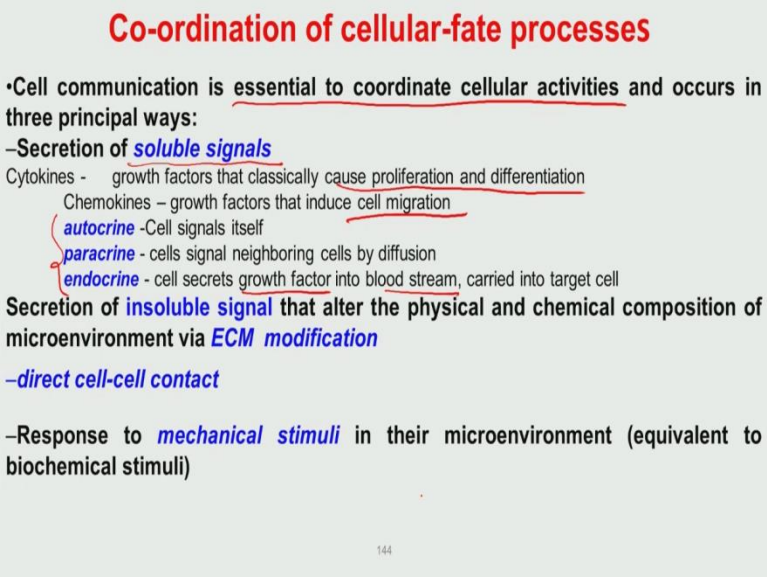

So whenever a cell communication is essential to co-ordinate the cellular activities and this can take place in primarily by 3 ways one is that secretion of soluble signals and these signals are known as Cytokines when these are the growth factors that cause proliferation or differentiation or chemokines that can do cell migration itself. And what are the 3 types of soluble proteins? One is that Autokine that is that cell signals to itself, Parakines means cell signals to neighboring cells by diffusion and the Endocrine like cell signals growth factor and this growth factors are transported by blood stream into the target cell.

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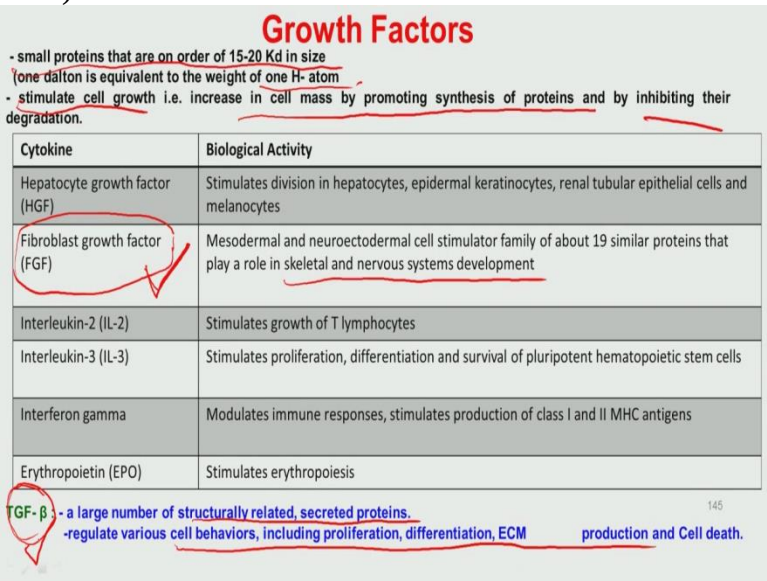

Now this so this growth factor is one of the important things in cell biology and they are defined as small proteins that are of the order of 15 to 20 kilo Dalton in size and 1 Dalton is equivalent to the weight of 1 hydrogen atom. And these growth factors essentially stimulate stimulate the cell growth and that is by increasing the cell mass by promoting protein synthesis or innovating their degradation.

Now this particular table shows a few growth factors or list a few growth factors but in actually cell biology there are not a large number of growth factors which are important but out of that in the context of biomaterials research that some of the growth factors which are important is the fibroblast growth factor that is FGF which essentially plays a role in skeleton and normal system development then you have these hepatocyte growth factor and also the another growth factor which is important in biomaterial research that is the TGF that is the transforming growth factor beta and this is a number of structure this represents a structurally related and secreted proteins.

And what is the role of TGF beta? The TGF-beta essentially regulate proliferation and differentiation as well as extracellular matrix production etc. So as a beginner or as a researcher in the biomaterials field, I would recommend one to remember at least 2 or 3 growth factors which would include FGF that is fibroblast growth factor and another one is called transforming growth factor beta that is TGF-beta.

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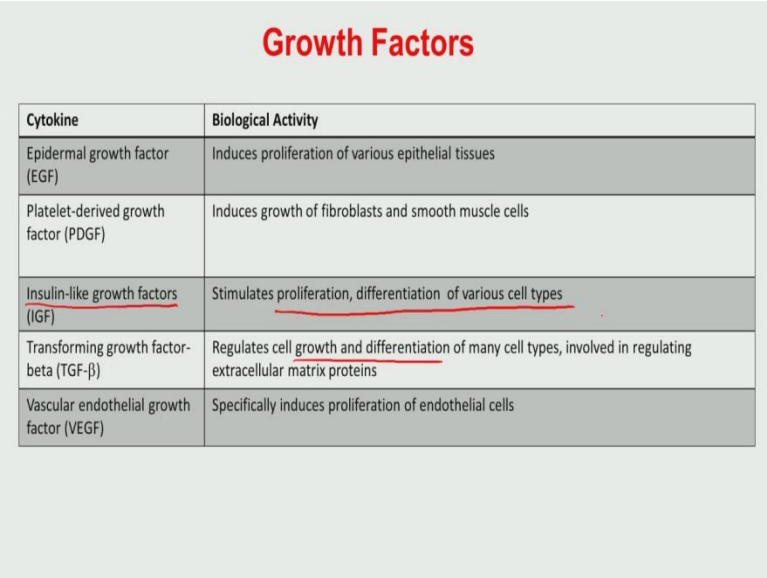

So TGF-beta as I said that it regulates cell growth and differentiation. Then you have the insulin growth factor that is the third one which is important and that is stimulus differentiation and stimulus proliferation differentiation in some of the specific cell types.

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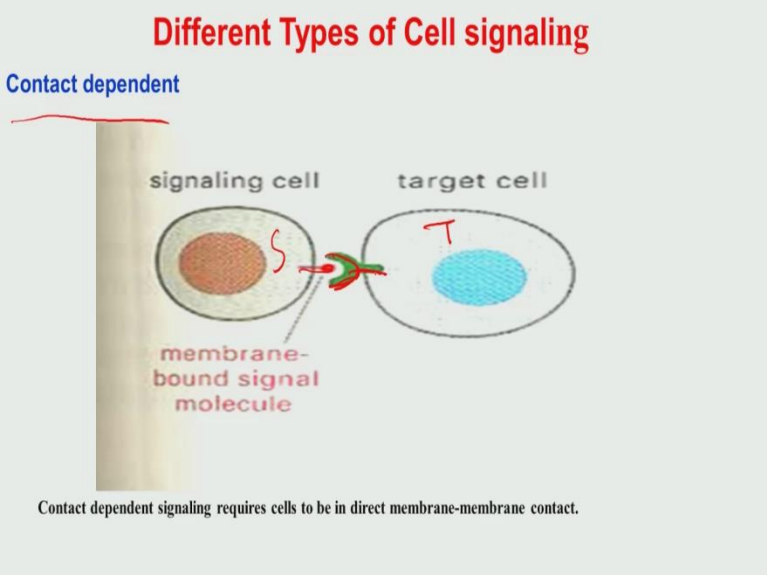

Now coming to the different ways that cell signaling process can take place. So one of the ways is the contact dependent so this is particularly through gap junctions. So this is your signaling cell or source cell and this your target cell. So and this is the membrane bounds signal proteins which are being which are linked to the cell surface receptors for the target cell.

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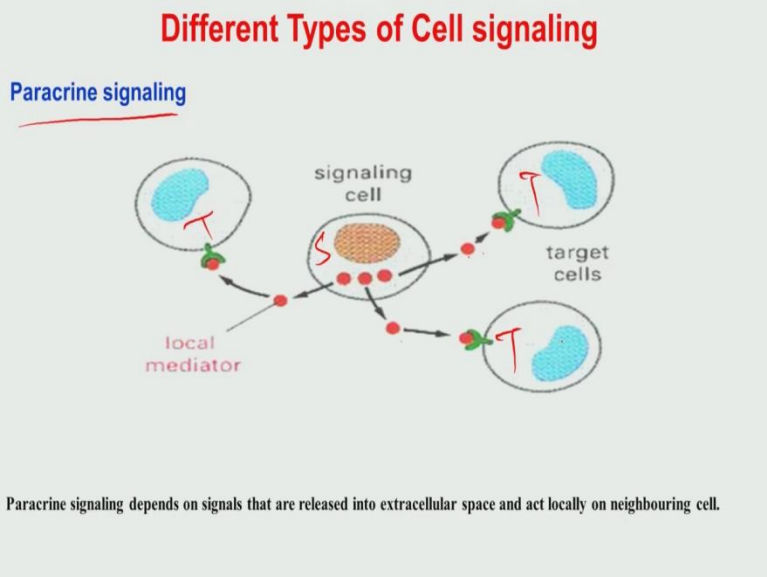

Now paracrine signaling means from one of the source cell suppose you have that now a few target cells in the neighborhood, so signaling cell will release the signal molecules or local mediators and then they will be transported to all the target cells by the process of diffusion.

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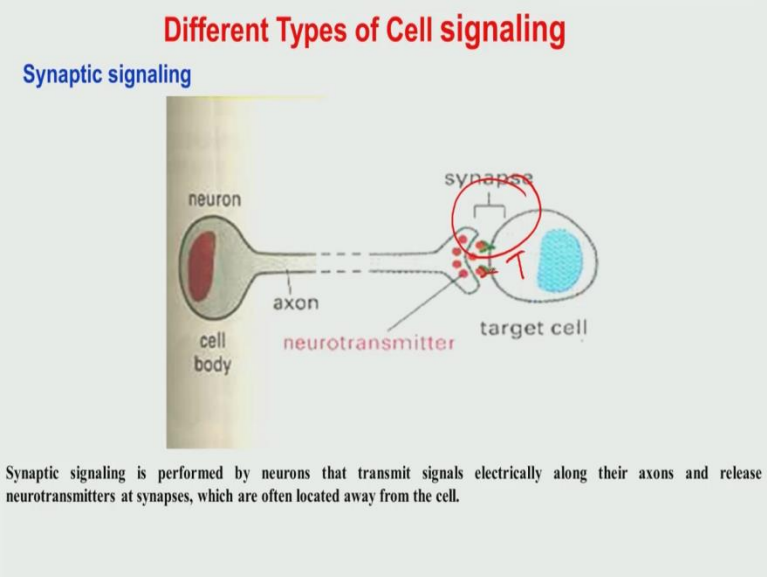

Third one is the Synaptic signaling that is relevant for these neurons and in the case of neurons you have the synapse and then you have the target cell here, so it is very easy for the neurotransmitters to be attached to the cell surface receptors of the target cell itself.

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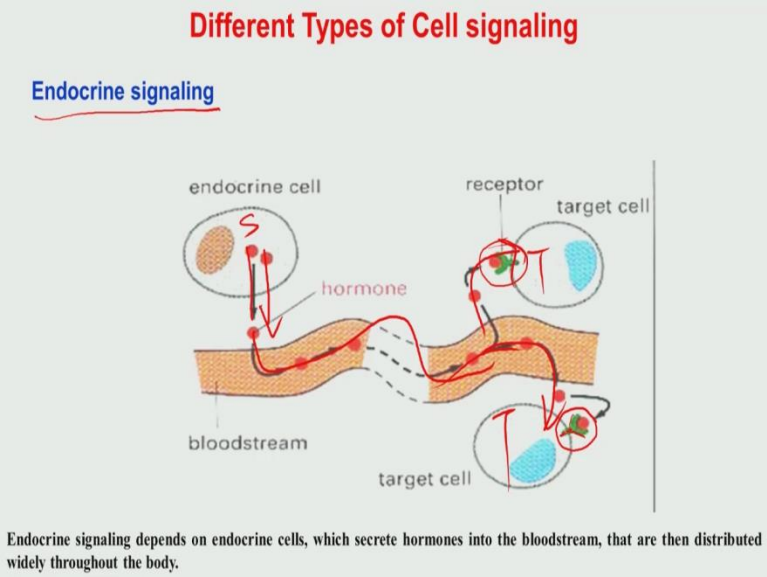

Fourth one is endocrine signaling. As mentioned before endocrine signaling means that you have one of the source cell and these signaling molecules are released to the blood stream. So it is transported through the blood stream to the couple of target cells here. And however in all these cases you can see that commonalities commonality is that how the signaling molecules get hooked or gets attached to the cell surface receptors or the target cell.

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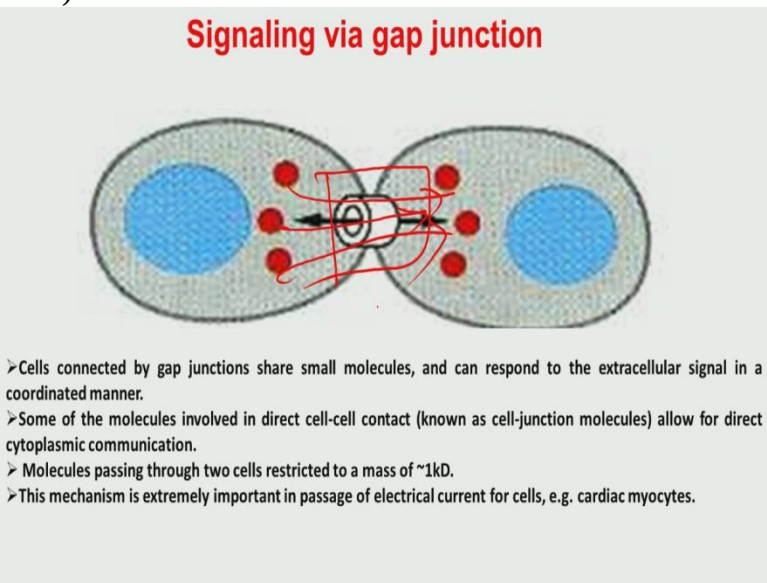

The other one which is more important for this signaling gap junction that is more useful for the cell, which which are the tightly spaced cells like in a endothelial cells or epithelial cells the cells

they are very tightly spaced cells and there the signaling molecules can simply be transported from one cell to another cell by the gap junctions here.

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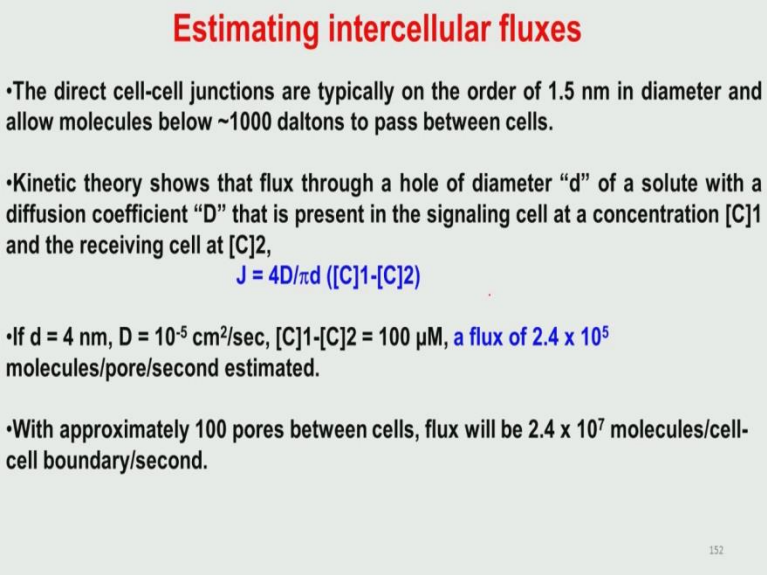

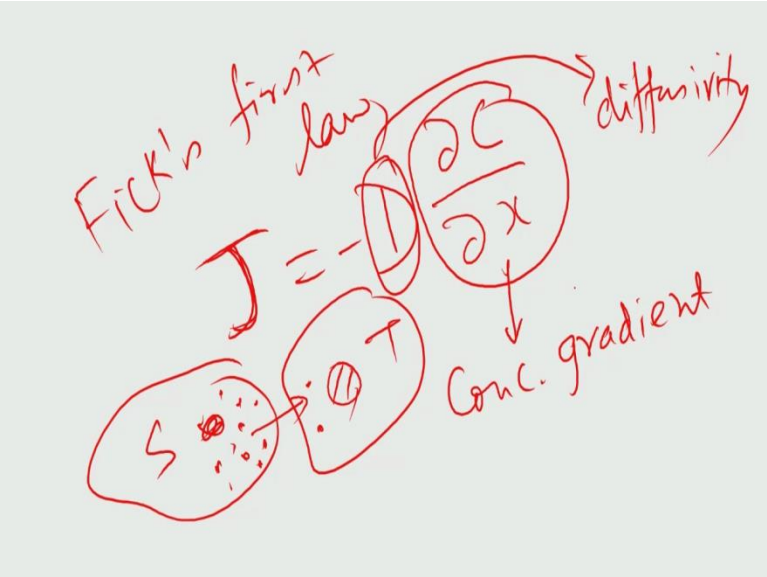

Now how to find out that what is the intracellular fluxes? Intracellular fluxes is essentially means that how many so how many signaling molecules can be can pass from one source cell to target cell per unit time. And these fluxes if it is driven by simply diffuse purely diffusion related so therefore Fick is two laws are important and Fick is two laws the 1st law essentially tells that flux

is equal to - D. D is the diffusion co-efficient Del-C upon Del-X. So Del-C upon Del-X is the concentration gradient.

So if you have the more number of signaling molecules in the source cell, so if you have the source cell here which is is' and if you have a target cell here and if the target cell has a different nucleus let us say, so from the source cell to the target cell if the signaling molecule is diffusing and suppose you have large number of signaling molecules in the source cells so certainly there is a concentration gradient between source cell and target cell.

Therefore diffusion will be driven by the Fick is first law. And Fick is first law is very common in the field of science and which is essentially the flux of the signaling molecule is equal to - D. Capital 'D' is the diffusivity or diffusion co-efficient multiplied by the concentration gradient or change of concentration in the special in X direction or spaces.

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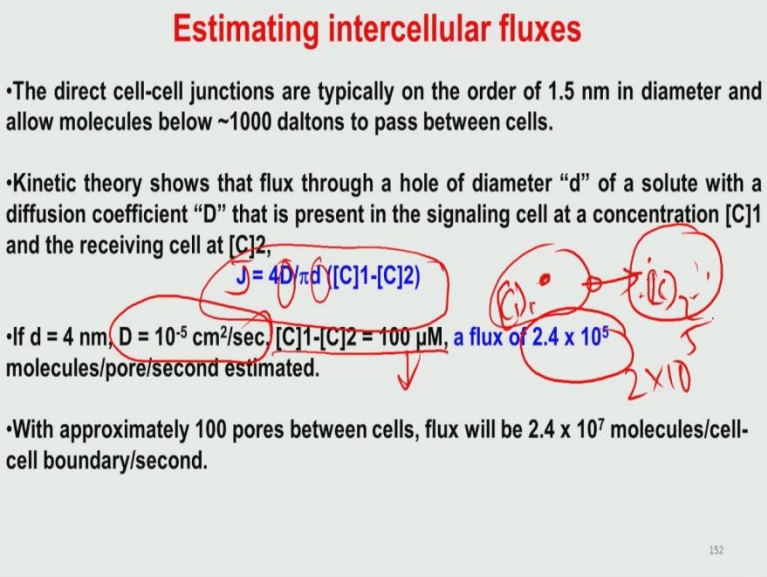

So if you follow this simple equation then you can find out that in this particular case the source cell has a signaling concentration is C1 and then the target cell has a signaling concentration which is C2 then you can find out 'J' is equal to 4D upon pi d so 'D' is the diffusivity and small d is the diameter of the gap junction through which signaling molecules diffusing away from the source cell to the target cell, multiplied by C1 - C2.

So C1 - C2 if you take for example this 100 micro molar and if you take a simple diffusitive values of 10 to the power of - 5 cm square per sec, so from this simple equation you can estimate that a flux of 2.4 or or let us say 10 to the power of 5 orders, 10 to the power 5 molecules per pore per second of flux that will be transported.

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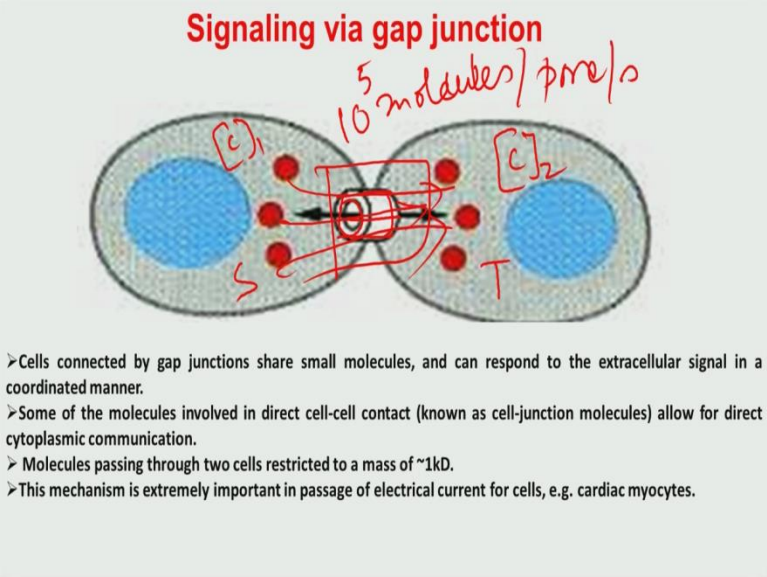

So what I am saying is that, if you take an example there is a single pore here through signaling molecule, with a concentration C1 at the source cell is diffusing to concentration C2 at the target cell, so what the simple estimate based on the Fick is first law tells you that 10 to the power 5 molecules per pore per second that will be if you transported from source cell to target cell.

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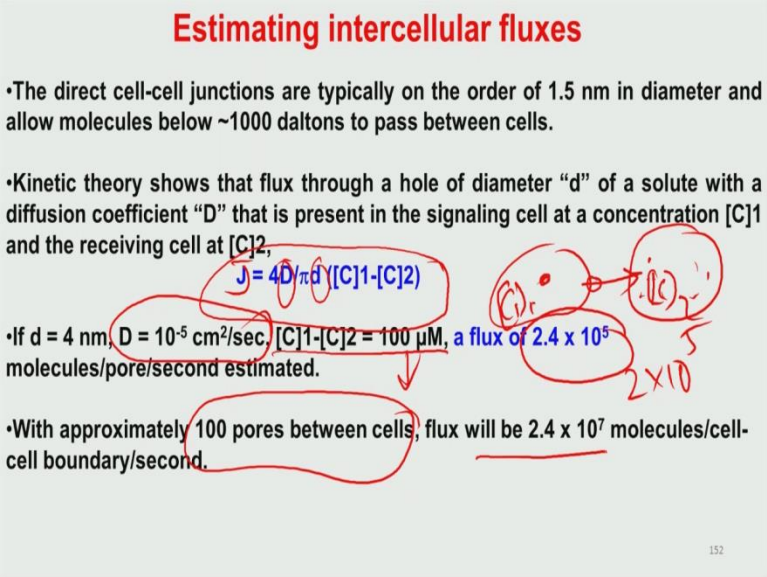

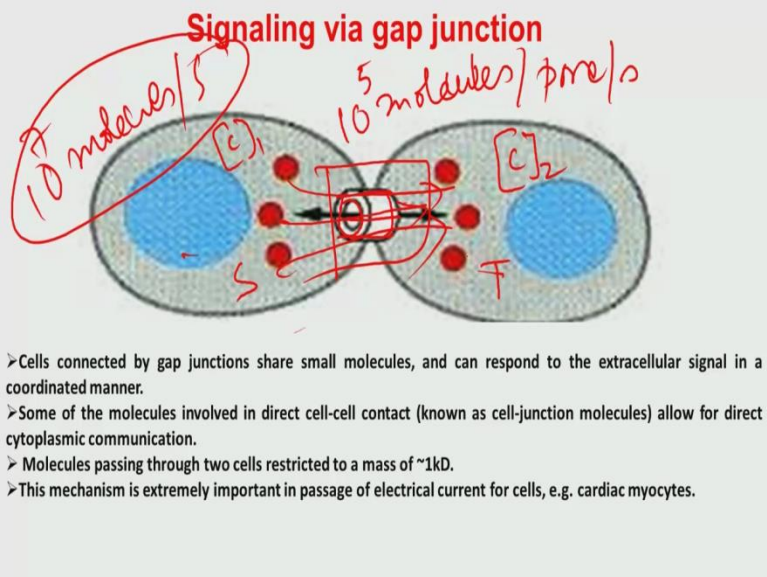

Now you have to now imagine that typical cell surfaces a large number of pores and through which the signaling molecules can easily pass from one cell to another cell. And this number of pores suppose if it is 100 pores per cells then flux will be 2 into 10 to the power of 7. So total flux in that case it is 10 to the power of 7. And these 10 to the power 7 cells so 10 to the power of 7 molecules per second, that will be transported between these two cells between source cell and target cell. Now this this very simple estimate also tells you that the huge number of traffic or huge traffic of the signaling molecules will pass from one source cell to one target cell. So this

kind of numbers are important so that you can understand that how signaling molecules or how signalling processes can influence the way cells will function in this neighborhood.

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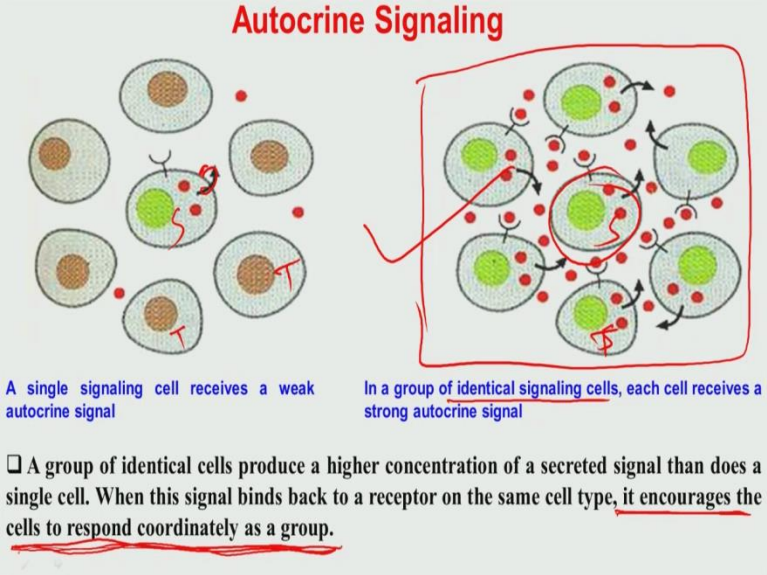

Ok. Now autocrine signaling essentially tells you that this is your source cell and this is your target cell. So a single single signaling cell receives a very weak autocrine signaling. So this is like autocrine signalling. And in a group of identical signaling cells like when source cell and when so sell and target cell this is central is your source cell and all in the periphery are your target cells. Now if all the cell types are the same, both the source and target cell then much higher concentration of the signaling molecules can can can can pass through from one source cell to all the target cells neighborhood.

And therefore it encourageous, that is the the bottom line, it encourages the cells to respond more coordinately as a group. So this kind of scenario is biologically much more relevant and tats is why it is been mentioned here that a group of similar types of cells when they will signal to each other and therefore their coordinated action will be much likely to take place more easily than a source when the source cells and target cells are of different cell types.

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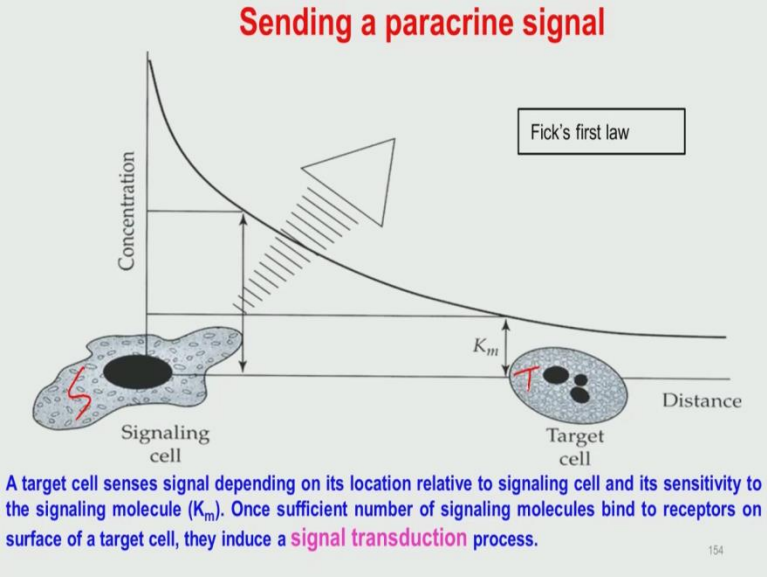

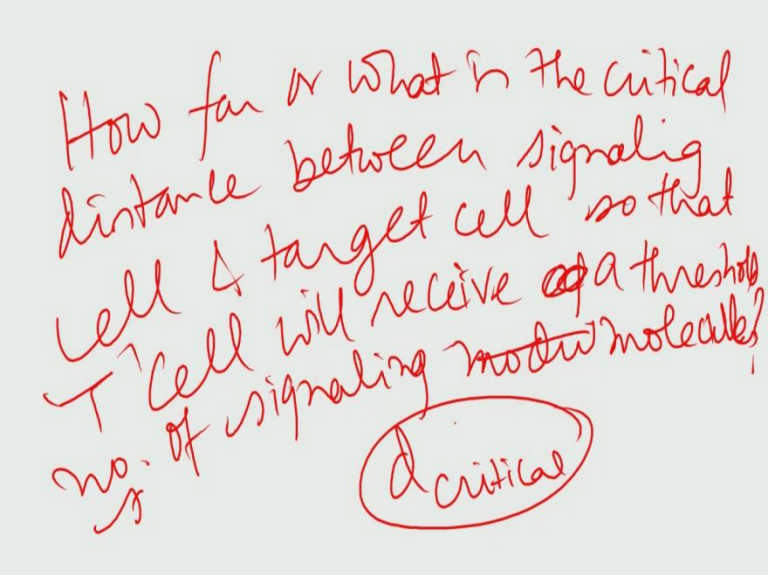

Now other things which is important and which may require some discussion at this stage is that you have a signaling cell source cell and you have a target cell here. Now 2 questions that we need to address, how far or what is the critical distance between signaling cell and target cell so that target cell is 'T' cell what I abbreviated as target cell will receive a threshold number of signaling molecules signalling molecules. So that is the one thing as to what is the critical distance. So this is the'd' critical which we are considering here.

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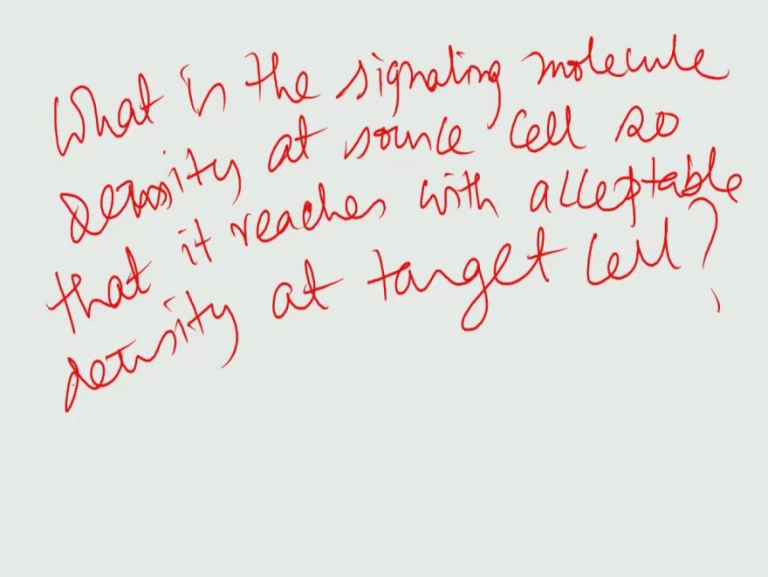

Another important question that we have to address that what is the signaling molecule density at source cell so that it reaches with acceptable density at the target cell?

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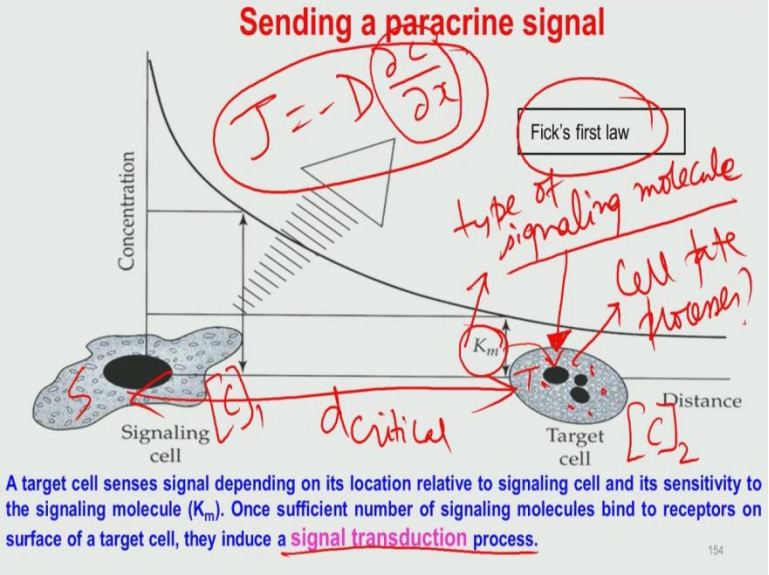

Let me first explain physically what this question means. This question means, So you have 2 you have 2 cells, one is the source cell and one is the target cell. Then certainly the source cell has a larger concentration of signaling molecules C1 and target cell has another different concentration which is essentially lower concentration in C2. So the question that we are trying

to address what is the ‘d’ critical value so that this concentration (Km) at the target cell of that specific molecule is still sufficient to activate certain cellular fate processes.

I repeat, what I said that let us hypothetically take 2 cells, one is the source cell and one is the target cell. So the source and target cell cannot be separated by significantly large distance so that even if the diffusion process is activated and then there is a certain number of signaling molecules would be able to reach the target cell but it may be lower than the critical concentration of signaling molecules which is required by the target cell to activate certain signal transduction mechanism within the target cell or to activate certain cellular fate processes within the target cell.

So there therefore the entire processes are biophysically possible if and only if the signaling cell and target cell are at a critical distance and also there should be large enough concentration gradient, that should be established or that should be set up between the signaling cell and target cell so that the value of (Km) should be above the threshold level of the sensitivity of the target cell, towards that specific signaling molecule type. So that (Km) also depends on the type of signaling molecule that is the threshold concentration threshold concentration for specific signaling molecules which would be just sufficient to activate certain signal transduction processes or to activate certain self fate processes in a target cell.

So these 2 things needs to be critically considered while analyzing that, how a paracrine signal can activate certain signal transduction process in a target cell. So summarizing this signaling mechanism again that Flick is first law essentially provides a basis for the cell signaling processes in the in a group of cells and while Flick is first law tells us that, this is I repeat again that Fick is first law tells you ‘J’ is equal to - D into Del-C upon Del-X so - D capital ‘D’ is the diffusivity of the particular signaling molecule in a typical cell culture medium and capital ‘J’ is the flux of the signaling molecule per second, Del-C upon Del-X is the signaling molecule concentration gradient which is set up between the signaling cell and the target cell.

And the 2 questions I have tried to address in this particular slide. The question number 1 is what is the minimum distance between the signaling cell and target cell wo that the the signaling molecules would be diffused to the target cell and the concentration at the target cell of that specific signaling molecule should be more than (Km) where (Km) is the threshold concentration

of that specific type of signaling molecule which would be sufficient to cause certain signal transform mechanism in the target cell itself? So I hope I have clarified these 2 things.

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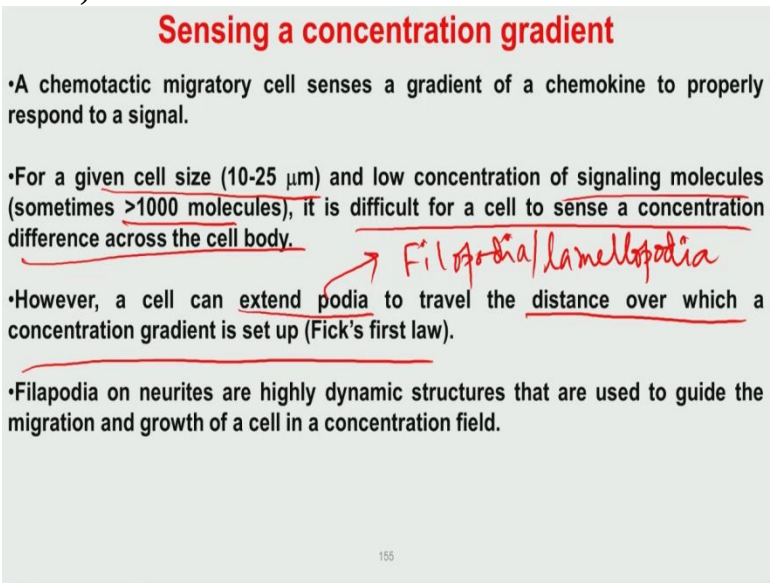

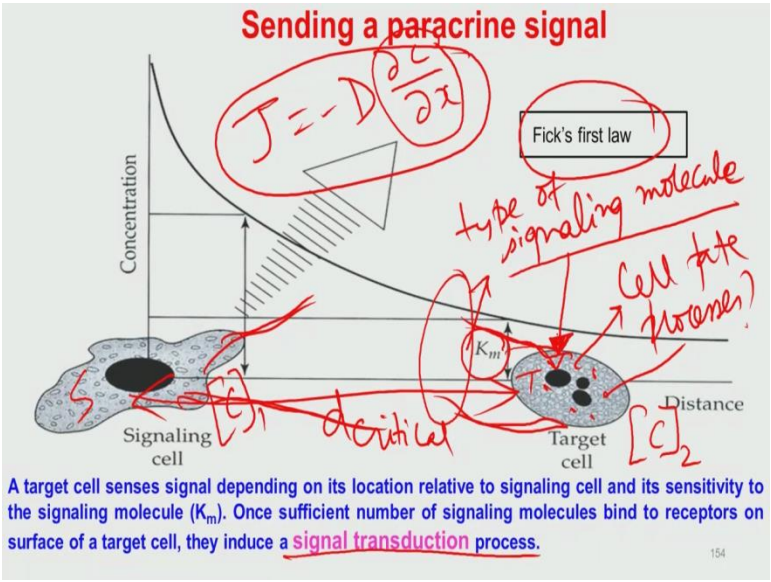

So this some of the things that has been mentioned in this slide, for a given cell size of 10 to 25 micron low concentration of signaling molecules sometimes greater than 1000 molecules it is difficult for a cell to sense a concentration difference across the cell body. However the cell can extend podia so what are the different podia? One is the kind of filopodia, and other can be

lamellapodia. So these are like cellular structures which also help the cells in the migration process to travel the distance over which a concentration gradient is set up.

So for example from here the cell filopodia or lamellapodia can extend just to see from here the cell target cell can extend filopodia lamellapodia so these are like cellular structures to sense this particular little higher bit of signaling molecule concentration simply because at the cell surface signaling molecule concentration is low enough, so that signal transduction process is unable to be activated.

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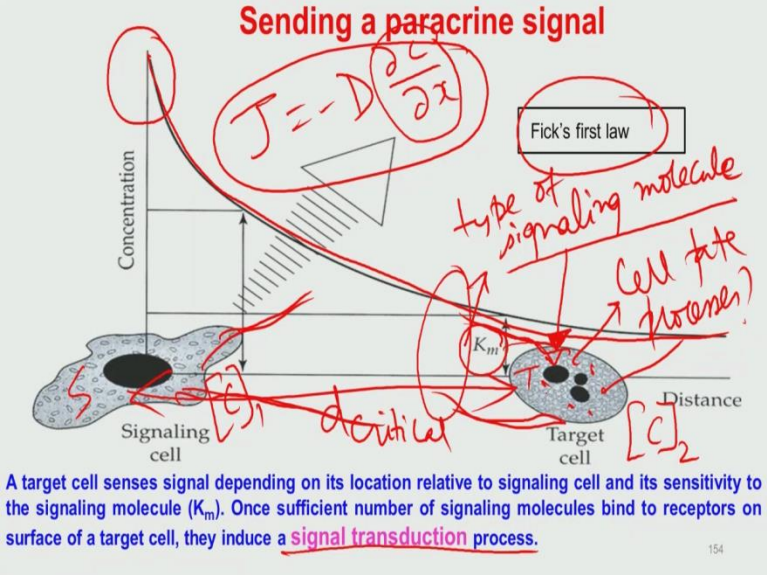

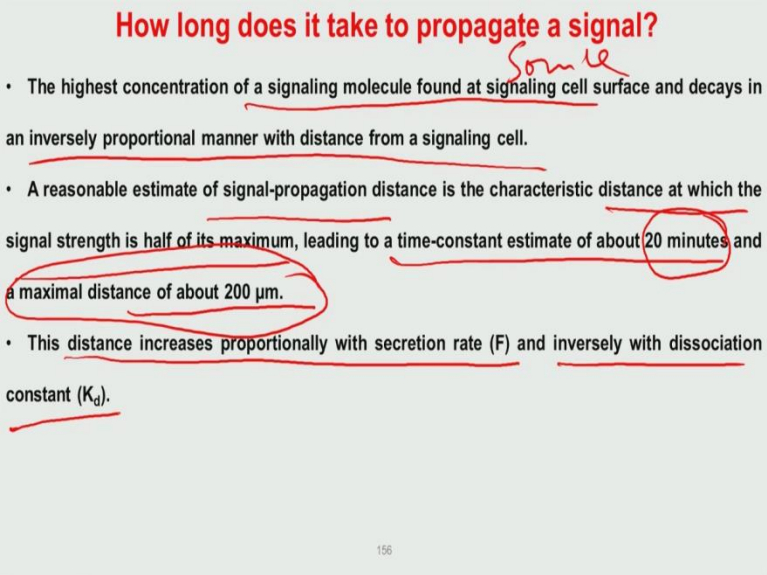

So the highest concentration of the signaling molecule is always found at the signaling cell surface or the source cell surface and is inversely proportional to the with the distance from the signaling cell to the target cell. And a reasonable estimate for the signal propagation distance is the characteristic distance at which the signal strength is half of its maximum. Signaling strength means here the signaling molecule concentration at the source cell. As you can as you see that it asymptotically decreases as you go from the source cell to the target cell.

So anytime I mention the signal strength that means that is the signaling molecule concentration at that particular cell. And time constant of typically the signalling process is 20 minutes. So this is essentially the time constant comes from a typical electrical engineering circuit concentration . In the cell signaling as I said before, that it also represents a complex communication network between a source cell and target cell. So therefore this kind of network also can be be following the electrical engineering principle can be solved to get a time constant which is of 20 minutes and a maximum distance in the cell signaling to effectively transfer is about 200 micron.

So what it means is that if a signaling cell and target cell is separated by a distance of 500 micron, then the concentration of the signaling molecule at the target cell, is will be significantly weak enough not to cause any change in the cell fate or cell functionality or cell fate processes of the target cell. However since the critical distance as I said is 200 microns, critical estimate as I said this 200 micron if the target cell is located within or it is separated from the signaling cell by a distance of 100 micron which is less than the 200 micron then target cell will receive or the signal strength at the target cell will be significant enough for the signal transduction process to take place in the target cell.

I hope I have explained enough physical significance or physical implication of this number. So the other things that has been mentioned in this slide is that this distance increases proportionally with secretion rate. What distance I am talking about? That is the maximum distance which is taken as 200 micron and inversely with a dissociation constant (Kd). So dissociation constant means that once once the signal molecule is generated it is being attached to the cell surface receptors of the target cell. Now if it is dissociated from that signaling process would be little weak.

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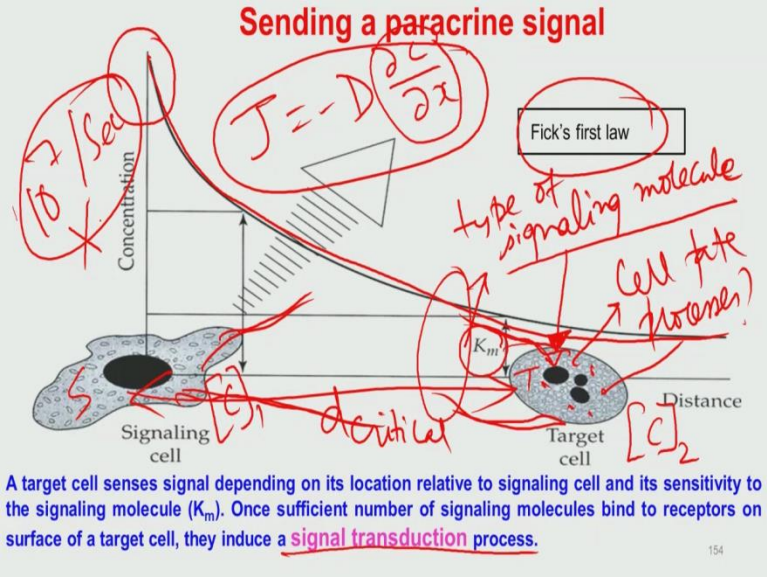

Now to summarize only up to this part of what I mentioned here that this signaling molecule once it is done by the gap junction not by this kind of autocrine signaling then what we have estimated that 10 to the power 5 or 10 to the power 7 molecules per second signaling molecules are generated from source cell to target cell. But when signaling molecules are further apart then this number may not be correct. So this is correct 10 to the power of 7 number of molecules per second is more for tightly spaced cells. Not like when 2 cells are physically separated by a certain specific distance.

Second thing I have mentioned in this slide that one can estimate or one can arrive at this 10 to the power 7 molecules per second number from the fundamental Fick is first law which states that J is equal to capital - capital D del-C upon del-X. Third thing I that have mentioned that there is something called critical distance of separation between signaling cell and target cell and there is something called threshold signal strength at the target cell.

Now threshold signal strength can be some few 1000 molecules per second here and that critical distance of separation can be somewhere around 200 micron. Anything larger than the separation distance more than 200 micron then target cell will not receive critical number of signaling molecules, so that signal transduction process will not get activated in the target cell. So I will come back to this discussion further in the next module.