Introduction to Biomaterials

Prof. Bikramjit Basu

Prof. Kantesh Balani

Department of Materials and Metallurgical Engineering

Indian Institute of Technology, Kanpur

Module No. # 01

Lecture No. # 05

Cell Communication – I

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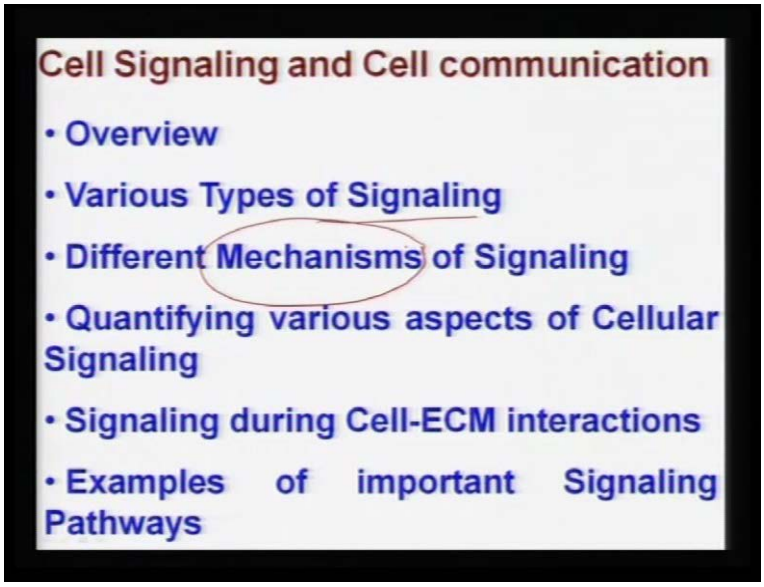

So, in this lecture, we will be discussing the process of cell signaling and how it is relevant for the communication between the two cells. So, over all, these particular cell signaling and cell communication is very important as far as the different cell processes are concerned. For example, cell migration, cell differentiation, cell divisions, cell proliferation. So, all these cell based processes; one of the essential state is the cell signaling. So, if the cell signaling does not take place in a very appropriate manner, many of these cell ware processes cannot take place in, under in vitro or in vivo conditions.

In this perspective, that overall structure of this presentation will include, first, I will go through of some of the overview of the cell signaling process that will be followed by the

various types of signaling. Then, I will come to the different mechanisms of signaling. So, these mechanisms means like, you know how these signaling processes take place. How these signals are generated from the source cell and it is communicated to the target cell. What are the Mechanisms?

Then, fourth aspect is the quantifying various aspects of cell signaling; that means, that the student should have an idea about that what is the time scalar, what is the typical concentration over which a signal can be very effective and what is a concern that concentration should be maintain. Then, fifth one is that you know, I will give you some examples, particularly for the Cell-ECM interaction and another and different other cell signaling pathways.

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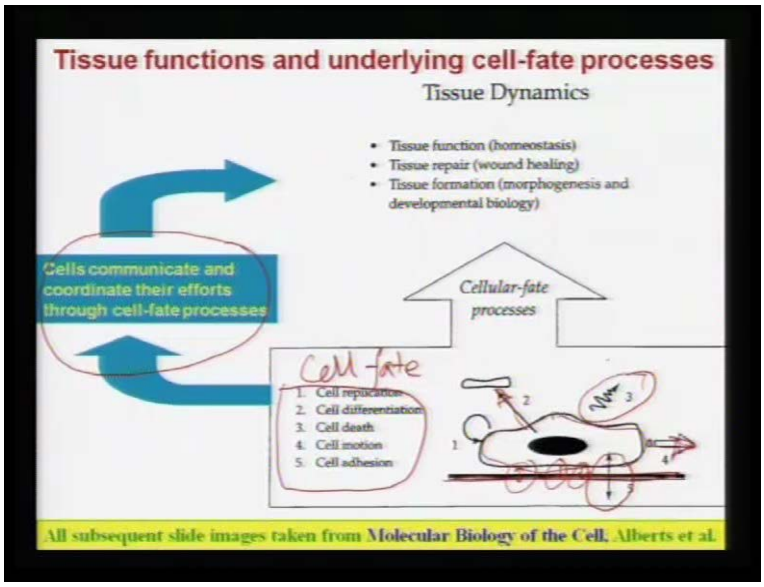

So, this is the overview of what I just discussing two minutes ago, that is, the tissue functions underlying cell-fate processes. Now, coming to the tissue dynamics, like tissue function like homeostasis, like as I will discuss later, that you know that all these cells survival, cell differentiation and cell motility, all these things are essential components of the homeostasis process.

The second one is tissue repair, that is, the wound healing process. Third one is tissue formation like morphogenesis and developmental Biology. Now just few minutes back, I mentioned that these are the cell-fate processes, like, this is the cell-fate process, like cell

application; that means, cell division, cell differentiation like different gene expression or different cell functionality.

Cell death processes like necrosis or apoptosis, cell motion like cell motility and cell adhesion. So, all these processes cannot take place, unless a given cell will receive particular and appropriate signals from another cell. So, that is, what is mentioned that cells communicate and co-ordinate their efforts to the cell-fate processes.

So, all these cell communication process, the ultimate outcome is that cell-fate processes. So, this is typical eukaryotic cells like nucleated cells. And, you can see that this process two means, that this particular cells can be going to another state. That is, the differentiated state; then, cell application means these cells will increase in number cell adhesion. Means, that it is an biomaterial biomaterial subset and on which, there is protein absorption, as I was mentioned before. This protein absorption is important for the cell adhesion process.

Cell motility means like a child walks on the ground. Similarly, how the cells will walk on the sub state or a given biomaterial sub state. So, like that cell motion and this how these cells, like may be, I had mentioned earlier, that you know cells have also filopodia lamellipodia, like you know a human being has legs and hands. So, similarly, the cells, these two lamellipodia extension of filopodia extension, the cells can walk on the material sub state.

And number three process is the cell death. Like you know, cells will no more be surviving because of the some problem, either due to necrosis wrap-up process.

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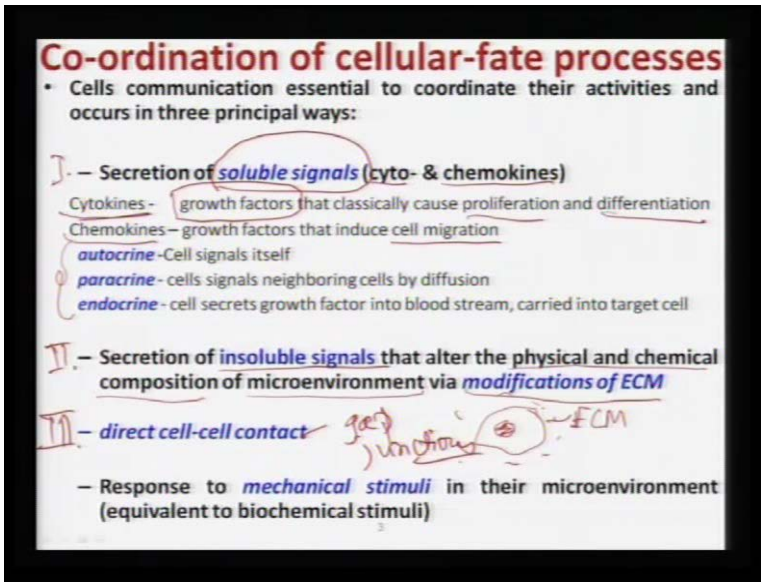

Now, co-ordination of cellular fate processes. So, cell communication essential to co-ordinate their activities and occurs that take place in three principle ways. Number one is that, secretion of the soluble signals. So, here the soluble signals means that a source cell will generate or will essentially secrete some kind of soluble signaling proteins. So, signals means essentially, they will generate some bio-molecules or protein molecules or some other molecules and these are known as signals. So, it can be cytokines or it can be chemokines. So, what is cytokines? Cytokines are actually growth factors. Now, growth factors, this term need, require, request definition. So, growth factors are essentially, that cause growth factors essentially cause proliferation and differentiation.

So, these are like cytokines. Chemokines means, growth factors that induce cell migration. So, essentially, cyto means that is essentially related to proliferation and differentiation. Chemokines means, which is essentially related to the cell migration. Now, again there are three types of, you know soluble signals for signaling processes. Number one is that autocrine. Autocrine means, same cell, signals to itself for the division cell division processes, like a mother cell is dividing in to two daughter cells. So, therefore, a same cell can send the signal to the itself. So that, you know cells can divide to make two daughter cells.

Paracrine means, cells signals to neighboring cells by diffusion. Endocrine means, cell secretes growth factor into blood stream and carried into target cell. I will show these

things, these three things in a few minutes. Then, this is the soluble signals. The second one is that, so, this is the number 1. Number 2 is the secretion of the insoluble signals that alter the physical and chemical composition of microenvironments by a modifications of ECM.

So, insoluble signals. So, first of all, there is a difference between soluble signals and insoluble signals. Then second thing is that, these cells, they cause the changes to the physical and chemical composition of microenvironment; that means, if there is a cell here, eukaryotic cells, this is nucleolus and this eukaryotic cells. Microenvironment means, this is called ECM that is extracellular matrix.

So, these insoluble signals cause some modifications to extracellular matrix. So, this is called another type of signals. Third one is the direct cell to cell contact. So, that express through gap junctions. Now, what is gap junctions? I will come to that in a few minutes. So, this is a direct cell to cell contact. The forth one, is the response to mechanical stimuli in their microenvironment equivalent to biochemical stimuli. So, the first three different principles ways of the cell communication. I repeat that is, through soluble signals, second one is insoluble signals; third one is the direct cell to cell contact.

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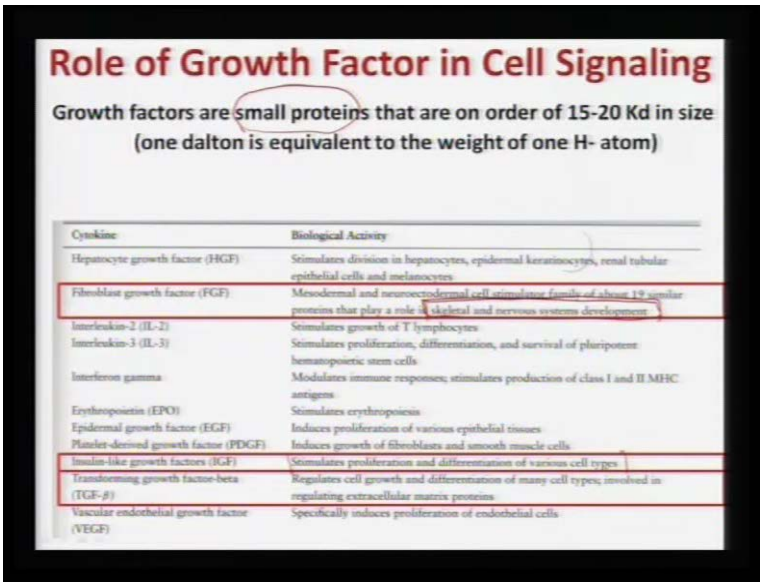

Now, as I said that, what is the growth factors? So, growth factors are essentially small proteins there are that are on the order of 50-20 kilo Dalton. So, K d stands for kilo Dalton; that means, 15-20000 Dalton.

Now, what is Dalton? 1 Dalton is equal to the weight of 1 hydrogen atom. So, therefore, growth factors are nothing, but, the small protein molecules which are, which has the molecular weight of 15-20000 kilo Dalton. So, this is the growth factors. Now, there are different types of growth factors. See, if it is not a course on biology as such, I like you to would remember the at least two or three different growth factors, which are important in the process of cell-fate processes.

Number one is the fibroblast growth factor that is F G F, number 2 is the insulin like growth factors that is called I G F and the third one is that transforming growth factor that is called T G F. Now, what is the functions of biological activity of these three growth factors? Now, fibroblast growth factors, they are essential biological activities; mesoderm and neuroectodermal cell stimulator family about nineteen similar proteins that play a scale role in skeletal and nervous system development.

So, these particular growth factors that is fibroblast growth factor, they essentially help in the development of skeletal and nervous system development, nervous systems. Insulin like growth factor, I G F that stimulates proliferation and differentiation of different growth factors. So, therefore, as you can see in the context of the cell-fate processes, I J F is more important because, I G F essentially stimulates, it stimulates or it facilitates cell proliferation and cell differentiation, transforming growth factor beta that regulates cell growth and differentiation of many cell types involved in regulating its cellular matrix proteins. So T G F, T G F actually regulates cell growth and cell differentiation of many cell types.

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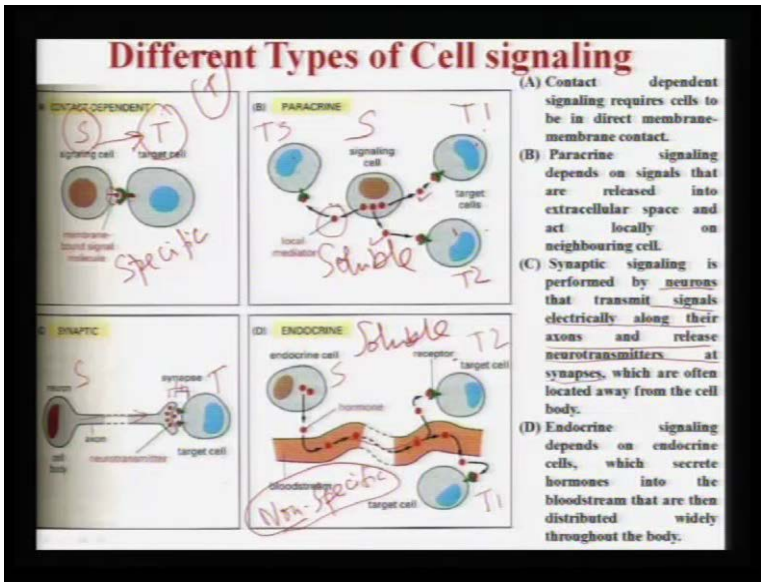

So, as I said in the last slide, that you know, there are three different types of growth factors are important. I repeat, F G F is fibroblast growth factor, I G F is the insulin growth factor and T G F is transforming growth factor. Out of that, I G F is the main growth factor, which is important for the cell proliferation differentiation. Now two or three slides back, I mentioned, that you know, what are the different types of cell signaling process. Like, one is that contact dependent, that is, that through gap junctions and then three other types of signaling, that I have mentioned, that is, through soluble signals.

So, if you remember correctly there are three ways signaling takes place. One is that soluble signals, second one is that insoluble signals, third one is direct cell to cell contact. Now, under the category of soluble signals, there are three different ways this signaling can take place. One is that autocrine, one is called paracrine and third one is called endocrine. Autocrine means, cells signals to itself. Paracrine means, cells that signals and then, it will go to another target cell and then it is the Paracrine. Endocrine means, cells secrete the signals molecules that will be carried away to the blast stream and then, it will be transported to another targets cell. That is the Paracrine.

Now, here you can see, that is, the first one is the contact dependent. So, this is your signaling cells S and this is your target cell T. So, from signaling cell, the signal

molecules you can see, membrane bond signal molecules that is the red one, and this is your target cell, green one is the your target cell surface receptor.

So, they will come and then, they will attached, they will get attached to the cell surface receipt on the target cell. Then, they will cause some changes in the target cell T. So, this is like contact dependent, like two cells are in direct contact. And among various cell types, the cell type that comes to a mind that where this contact dependent signaling takes place, is the endotheliosis. Endothelasasis are like cells, which are arranged one after another in a very dense manner. Paracrine this is your signaling cell S. Now, there are two three different target cell. This is two, this is one target cell, this is another target cell, this is T 1, this is T 2 and this is T 3.

Now, these all are eukaryotic cells. As you can see, now this signaling cell, they produce or they generate two or three different type of signaling proteins, For example, now one of the signaling proteins can be transported to this T 3 by via local mediator; that means, it cannot directly be transported via protein molecule directly to another targeting cell. So, this red one will get attached to the local mediator and then, this local mediator.

So, this local mediator can be transported to the target T 3 cell. Similarly, different local mediators are present. As you can see, this is another local mediator and this is another local mediator. So, the signaling cell from S to the target cell T 1 T 2 T 3, the transport of the signal molecule takes place via the local mediator.

So, this is the process of the Paracrine signals. Endocrine signals means, this is your Endocrine cell S, that is, the source cell. Now, this is your target cell, this is T 1 target cell, this is T 2 target cell; all are Eukaryotic in nature. Now, endocrine cell, this secrete of some signal, then it will go to the hormone and then, to the blood stream. This is your blood vessels or blood stream, it is going on and it is transported to the blood blood stream and then, it goes to target cell. So, this is the T 2 and T 3.

So, essentially, all you can see here, that from S, the target cell goes to T 1 and T 2 and then, it can be transported. So, this is called endocrine signals. Then, another one that has been mentioned here is that synaptic signals. Synaptic signaling is performed by neurons. Neurons means, it is actually important in neural tissue engineering or in general

neurons. So, this is transmit signals electrically along their axons and release trans neurotransmitters at synapses.

So, here you can see, these are like synapses here and these synapses, there is a neurotransmitter. So, these signals are such it is transported electrically and then, it is going to another target. So, this is your source cell and this is your target cell t. So, from S to T that, through neurotransmitter this these signals is transported. So, direct cell to cell contact, this is little bit more details on the direct cell to cell contact. Like eukaryotic cells, they express proteins on their surface that lead to direct cell to cell contact enabling, highly specific communication. Highly specific communication means, if you go back here, the signal molecules that is generated here, from this S cell, this same signal molecules can be transported either T 1 T 2 or T 3. So, it can be in nature non specific.

But, when these two cells are in direct contact, you know that these signal molecules, which are generated from this S cell, it will directly go to the T cell because, just because, it is physically in contact with each other. So, this is called highly specific communication. Highly specific communication means, this signaling communication is exactly for these T cell, target cell; not another target cell which are located here. So, then in contrast soluble and ECM signaling is non specific. Soluble and ECM signaling means, as you can see, this is called soluble signaling or this is another soluble signaling, that is paracrine and endocrine.

So, these signals, this source cell does not know where their signaling proteins will finally arrive at, whether it will arrive at T 2 or whether it will arrive T 1. So, that means, this is like nonspecific. So, I hope you are getting my point that, these are specific means, just because they are contact dependent direct. So, source cell to target cells, this is called specific interaction. Nonspecific interaction means, like source cell, it is going to two target cells and this is called nonspecific interaction.

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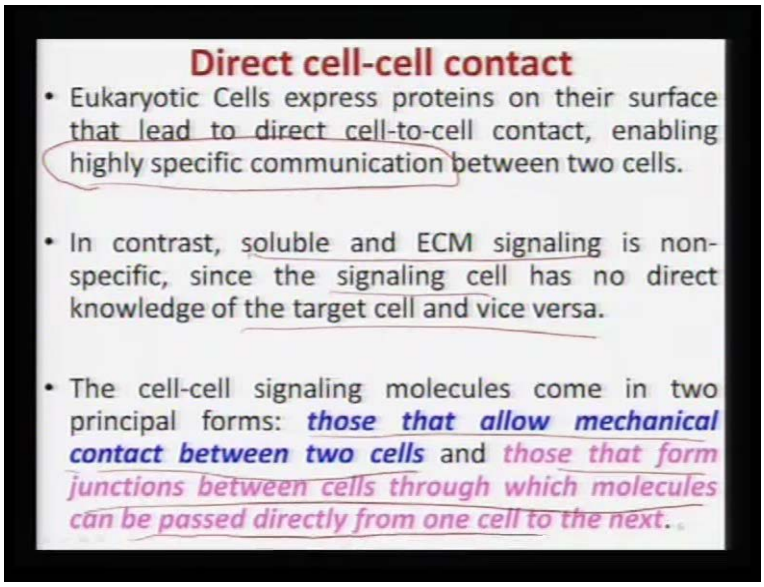

So, it is nonspecific is the signaling cell has no direct knowledge of the target cell and vice versa. Has no direct knowledge means, signaling cell does not know which target cell there signal protein will finally arrive at. Then, third point that has been mentioned here is that, cell to cell signaling molecules come in two principle forms, like those that allow mechanical contact between two cells. Then, secondly, those that form junctions between cells, through which molecules can be passed directly from one cell to the next cell.

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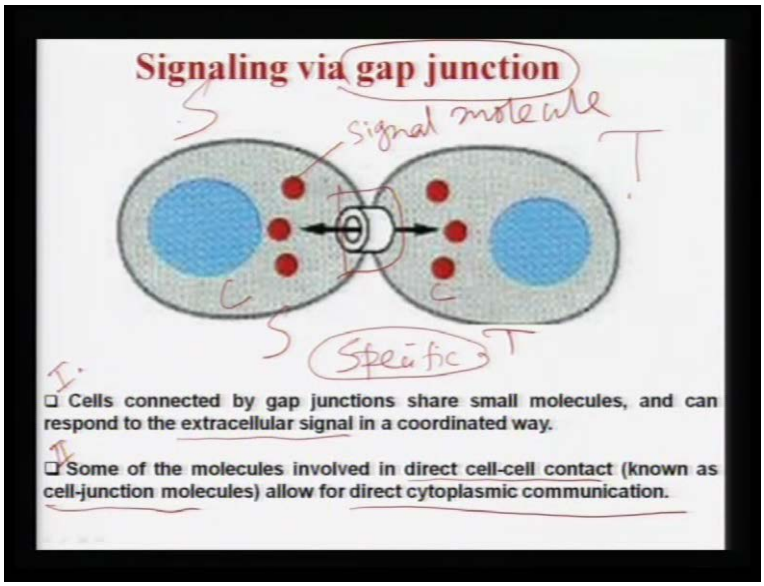

So, this is what I was mentioning there, that, so, this is signaling via gap junction. So, this is direct contact and this direct contact here, you can see these are the signal molecules. So, these signal molecules, it can go from source to target and this is highly specific in nature. Highly specific in nature, why because these, if this is source and this is target, source cell exactly knows this is target cell, where the signaling molecules will directly pass through. These junctions here, it is known as the gap junctions.

Now, these gap junctions, the communication through gap junctions only takes place in cell type liking endothelial cells, where multiple cells are lined up one after another. So, there is direct physical contact between the two types of cells. So, here it has been mentioned that two points; point number one that, cells connected by gap junction shear small molecules and can respond to the extracellular signal in a coordinated manner. So, can respond to the extracellular signal in a coordinate manner. Second point is that, some of the molecules involved in direct cell to cell contact known as cell junction molecules, allow for direct cytoplasm communications. So, like molecules were in direct cell to cell contact, so, this is your cytoplasm C; this is cytoplasm is C here.

So, these signals molecules, if it is transported between this or in a reversible manner can directly communicate to the cytoplasm.

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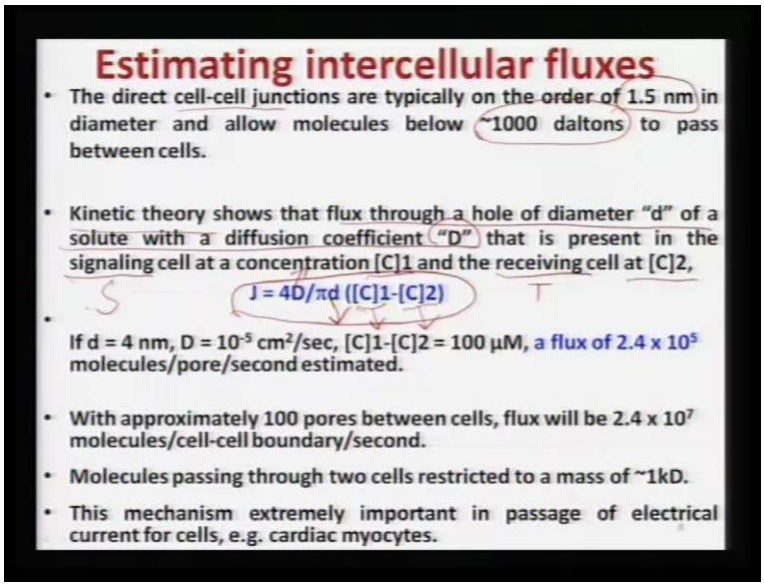

Now, coming to the some quantification, estimating that intercellular fluxes. Now, how to quantify that? What is the typical fluxes that will be generated in this intercellular, in this signaling process? Now, let me go back. So, intercellular fluxes means, like we need to quantify that, how much signal molecules that will be generated at the source cell surface here, and how much flux that will be generated that will be going to the three different cells, which are mentioned here. So, that means, what I am trying to tell you is that that, what would be the flux of the target, what is the what will be the flux of the signal molecules that will be transported from one signal cell to multiple target cells.

So now, for the particular case of the cell to cell junctions. Typically, cell to cell junctions, they are they are dimension is around 1.5 nanometer and therefore, that allow below 1000 Daltons. So, most of the growth factors if you remember, they are very small protein molecules and which a size of 15-20 kilo Dalton, means like 15-20000 Dalton. So, therefore, all these growth factors can be easily transported between these two cells and these 1000 Daltons to pass between cells. Now, Kinetic theory shows that that the flux through a hole of diameter D of a solute, with a diffusion coefficient capital D that is present in the signaling cell at a concentration C 1 and the receiving cell as concentration C 2. So, this flux is j is equal to 4 D, D is the diffusion coefficient here; small D is the cell to cell junction diameter.

C 1 is the concentration in the source cell; C 2 is the concentration in the target cell. Receiving cell means, this is essentially target cell and signaling cell means, this is essentially source cell. You understand what I am saying.

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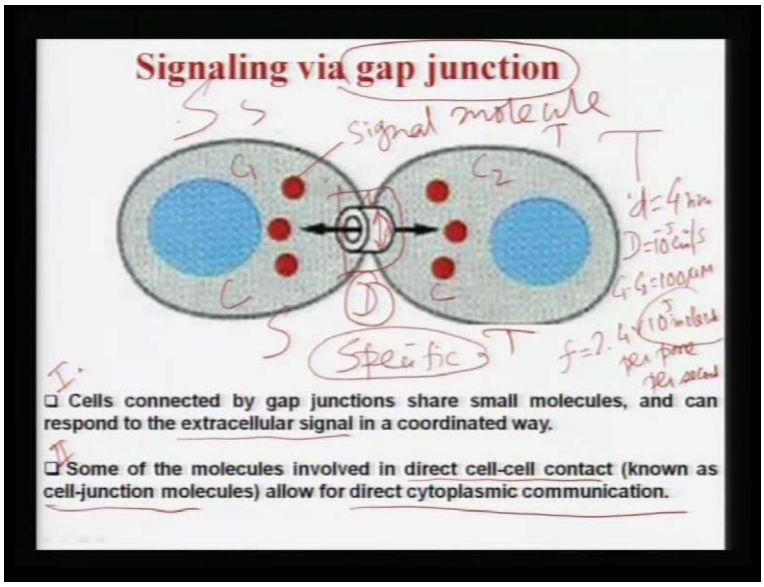

Let me go back to this. So, what has been mentioned here, suppose this is source cell and this is your target cell. Here, the concentration is C 1, here the concentration is C 2. Capital D is your diffusion coefficient that how the signaling cell will be passing through and this diameter if it is small D here. So, then the flux that how much flux that would be transported, that has been calculated by a simple fix law is j is equal to 4 D pi over D C 1 minus C 2. So, always the flux will be from higher concentration to the lower concentration.

So, now if you see that, if you consider that the D is 4 nanometer, capital D is 10 to the power minus 5 centimeter square per second. Like you know, typical diffusion coefficient C 1 minus C 2 is 100 micro molar, then what could be the typical flux. This typical flux would be 2.4 into 10 to the power of 5 molecules per pore per second.

So, let me go back to this slide and let me explain to that. So, this small D has been assumed as, suppose this is 4 nanometer. So, if you calculate here, D is equal to 4 nanometer. Your capital D diffusion coefficient is 10 to the power minus 5 centimeter square per second and your C 1 minus C 2 is typically like 100 micro molar.

Then, this flux which is F, it is like 2 into 2.4 in to 10 to the power of 5. So, flux is around 2.4 in to 10 to the power of 5 molecules per pore per second. So, let let us let us try to understand these numbers. So, these numbers means; that means if there is, this is

the particular one gap junction. So, through this gap junctions, from one cell to another cell around 10 to the power 5 orders of molecules will be transported each second.

So, that means, if you now considered that cell culture time frame is typically few hours like 24 hours to 72 hours. Typically, when cells will adhere initial stages that takes space may be past 6 hours. So, for 1 hour means 3 or 3600 second. So, in 1 hour how much molecules that will be transported 2.4 10 to the power of 5 multiplied by 3600 multiplied by n, that capital n is the number of ports that are present among the two different cells.

So, you can see that the amount of molecules that will be transported which will be huge number, it is like around 10 to the power of 8 molecules per pore in 1 hour and 8 molecules per pore means, depending on the cell type, that number of pores you can consider. So, it can be 10 to the power 9 10 to the power 10 order of molecules that will be transported per hour.

So, that is the huge number of cells. If they will be transported, those cell, sorry, huge number of molecules, those will be transported between two cells. Now, you can see typical in culture medium, you you you culture at least 10 to the 4 to 10 to the power 5 number of cells. So, huge number of cells are cultured on a material substance. So, now, you can understand this entire process, that your total number of cells is 10 to power 14 into the 5, your molecules that will be transported between two cells, which is of the order 10 to the power 8 10 to the power 10. So, such a large number molecules are involved in your cell adhesion process, cell proliferation process, cell differentiation process.

So this, you as students, one should have a feel for the numbers that will be responsible for certain biological processes. It is not the exact number like 2.4 , but, it is the order of magnitude, like 10 to the power 5 or 10 to the power 8 or 10 to the power 10 molecules that are responsible or that is this molecular transport is responsible for certain processes.

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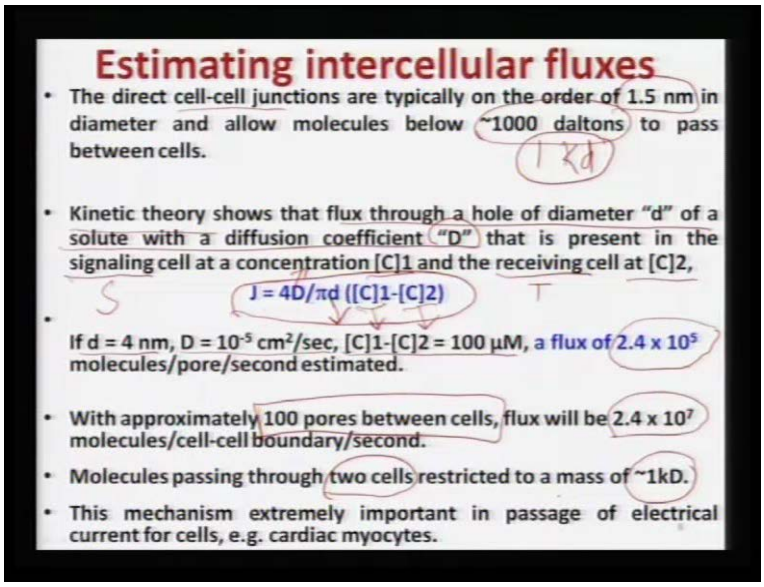

So, this is what you know, in the initial slide, I mentioned that one should quantify the total number of, kind of what should quantify the intercellular flux, that how much flux is there in the, how these fluxes can be calculated.

So now, here it has been mentioned. Next point is that, with approximately 100 pores between cells, between the two cells, if there, if you consider a 100 pores, flux will be of their order of 2.4 into 10 to the power 7.

So, that means, 2.4 10 to the power per second per cell to cell boundary per second that would be the molecules that will be involved in this cell signaling process. And molecules passing through two cells are restricted to 1 kilo Dalton, that I have mentioned here, that is 1000 Daltons.

So, extremely small molecules, they can be only transported by direct cell to cell counter. If the molecules are larger in size, then, what would be the alternative way? Then, it can go to Paracrine or Endocrine. Like you remember, this Paracrine and Endocrine; that means, this is the Paracrine, like they will be soluble signals, they will be soluble in soluble in ECM or Endocrine means they will be soluble in bloodstream and they will be transported to two or three different target cells.

This mechanism is extremely important in passage of electrical current for cells. For example, cardiomyocytes or cardiacmyocytes. So, cardiomyocytes are the cells, which

are typically cultured on the heart tissue replacement, or, if you want to develop some cardio vascular patches. For example, P L J carbon nano fiber composition, other similar composites.

If one has to evaluate, that whether this materials are suitable for the patches to be developed for the heart, so there, in order to find out the cytocompatibility, people normally cultured the cardiomyocyte cells.

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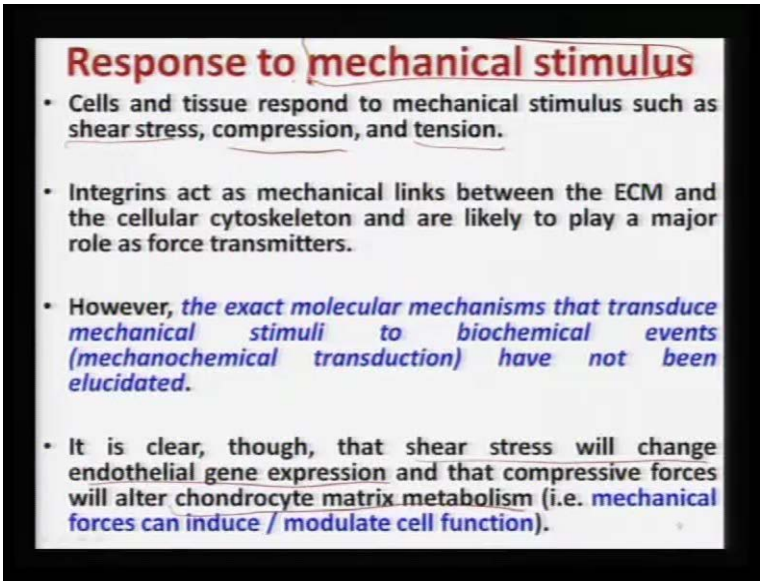

The other thing that, I mentioned in the overview slide is that, there are three types of signaling. One is the soluble signal, one is that insoluble signal, third one is the direct cell to cell contact, fourth one is the mechanical stimulus.

Now, mechanical stimulus means, the cells and tissues respond to mechanical stimulus like shear stress or compression or tension. Now, people from material sense background, they always think that you know shear stress compression and tension is should be of the order of megapascal or gigapascal.

But, one has to understand the cells are extremely complain, they are not very stiff. Typically, the cells elastic modulus is very low, if the order order of kilopascal like 10 to the power 3 Newton per meter square. So, therefore, the shear stress and compression and tension; these stresses also should be of similar order like kilopascal or extremely small force.

Like, so here, you have to understand, if you are consider 10 to the power 3 Newton per meter square therefore, that your force, that one should apply to understand the behavior of the cells under force, under compression or tension. This force should be of the order of piconewton or extremely small load. Otherwise, you cannot, otherwise you cannot study these cells.

Then integrins, act as mechanically between the extracellular matrix and cellular cytoskeleton and likely to play major role as force transmitters. Third point is that, exact molecular mechanism, that transducer mechanical stimuli to biochemical events have not been elucidated yet.

Fourth point is that, it is clear though that shear stress will change endothelial gene expression; that compressive forces will alter chondrocyte matrix metabolism. Now, endothelial cells are the tissues, are the are the cells of the endothelial tissues, which actually prevent the different body parts and chondrocyte cells is the cells, which are useful for cartilage.

Now, when you have this cartilage fracture or cartilage breakage, so there, you need to you need to seed the chondrocyte cells on the cartilage matrix. So that, you know, the cartilage can grow. So, here it has been mentioned that their shear stress will change. Endothelial gene expression and compressive forces will alter chondrocyte matrix metabolism.

So essentially, these examples essentially illustrate that how mechanical stimulus, like in terms of the tensile shear or compression stresses; they can locally change the cell-fate processes like gene expression or metabolism, etcetera.

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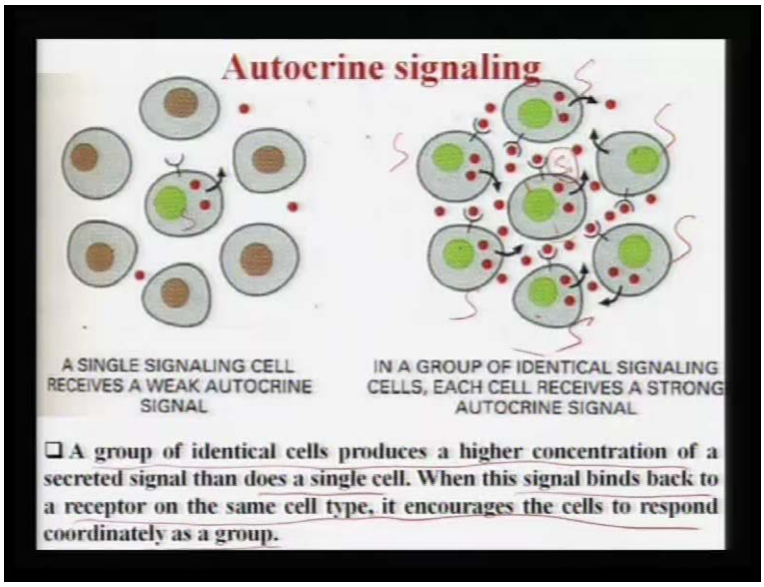

Now, autocrine signaling means, that cells, they will signal to itself. So, here you can see, this is your source cell. A single signaling cell receives a weak autocrine signal and this is a, in a group of identical signal cells, each cell receives a strong autocrine signals.

So here, suppose, this is your source cell, but, all these cells they are like similar to sources. For example, you are culturing only fiber blood cells. So, all the cells are of similar nature fiber blood (( )). So, these are like L 99 cells.

So therefore, here in case of the autocrine signals, all, a single cell which is at the center of around six cells here, they will receive similar type of signals, which will be generated by all these different type of all these six cells, which are of similar nature, which are of also fiber (( )) nature.

So essentially, that what is the physical implications of that a group of identical cells, they will produce a higher concentration of a secreted signal than does a single signal. When this signal binds back to a receptor of the same cell type, it encourages the cells to respond coordinately as a group.

Now, when this autocrine signaling happens, like you take an example that you know, suppose, you do not know whether material is crypto compatible. So, then you are siding all fiber blood cells like 10 to the 4 10 to the 5 fiber blood cells, you are culturing or you are siding on the material.

So, that means, there you can expect autocrine signaling. Why because, individual L 99 cell will receive signals from a few other neighboring L 99 cells. Therefore, the strength of the signals or the concentration of the signal molecules also, will be higher and that is what I am trying to convey to you.

So, therefore, in a group of cell, a single cell, it lets, take an example, which is star here. The single cell, will receive the signal molecules from all the similar cell type, which are in its neighborhood and the concentration or the strength of the signal also would be higher.

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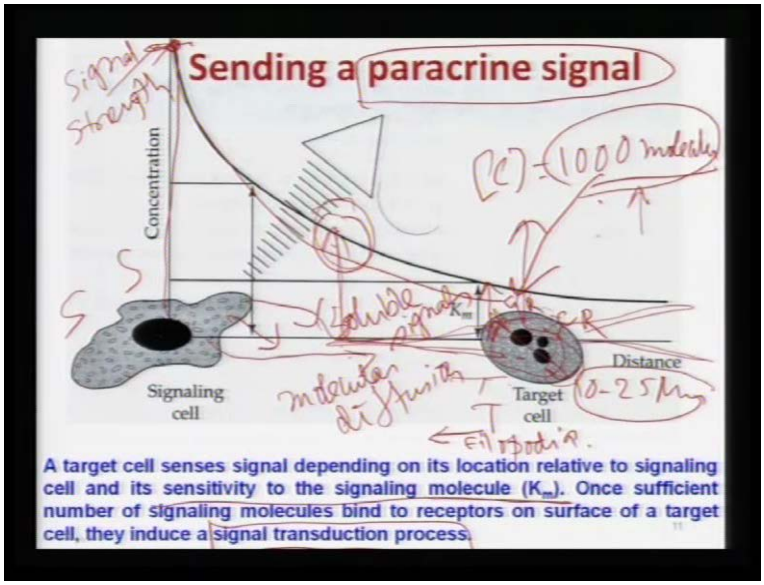

Now, sending a paracrine signal. So, this is your signaling cell or source cell; this is your target cell. Now, signaling cell source or target cell at the signaling cell surface, your signal strength or concentration. So, this is also in biological language it is called signal strength.

So, this signal strength is the highest, but, then, it will go to this target cell via molecular diffusion process, right. Molecular diffusion process means, the signaling cell that it is generated. So, these are like all soluble signals because it is not autocrine, it is paracrine signals.

So, these are like soluble signals. Now, once the soluble signals will be transported from source cell to target cell, then what will happen. That with distance, this concentration

also will decrease. So, at the target cell, your concentration is the this one and your source cell your concentration is this one.

So, signal strength will also decrease because your signal concentration or concentration of your signal molecules decreases. So, therefore, a target cell senses signal, depending on its location relative to signaling cell and its sensitivity to the signaling molecule. Sensitivity means, that how well this target cell is equiv, in terms of the cell surface receptor. So, these are like cell surface receptors, in terms of the cell surface receptors.

So that, the signal molecules, once it is transported from source cell to signal cells, they will be able to bind to the receptor of the target cell. So, that means, that is known as a sensitivity in biological language. Once sufficient number of signaling molecules binds to receptors on surface of target cell, they induce a signal transduction process.

Now, what is signal transduction process? That I will explain to you after few minutes. So, from this from this point, it is very clear to you that source signal, source cell or signaling cell, your concentration of the signaling molecules will be at the highest level. Once it will go through the molecular diffusion process to the target cell, then this concentration will decrease.

However, these, whatever molecules that will be transported by molecular diffusion to target cell, the target cell also should have a good affinity towards binding to this molecule, signal molecules. So, that has been mentioned by the sensitivity to the signaling Molecules K m.

Now, sensing a concentration gradient. Now, given a cell size, typically 10 to the power 10-25 micron and no concentration of signal molecules, sometimes greater than 1000 molecules, it is difficult for a cell to sense a concentration difference across a cell body.

So, what is, what it means here typically, the cell size, this cell size is around 10-25 micron. So, that is the diameter of the cells. Now, if the signal molecules here, suppose the concentration that is reaching here 1000 molecules; that means, whatever the molecules that are generated at the source cell, only 1000 molecules are coming here in the target cell. Sorry.

Now, this 1000 molecules which are coming here in 10 10-25 micron size cell, so, what has been mentioned here, that it is difficult for a cell to sense a concentration difference across the cell body. Means, what it means that, inside the cells also there are lot of molecules are present. Suddenly, the cell has to sense that 1000 molecules, which are coming by transporting from the molecular diffusion from source cell, it is difficult for a cell to sense this 1000 molecules.

So, what it does, it says that that a cell can extend podia to travel the distance over which over which it is determining a concentration gradient. What it means? This means, that this target cell T, it will extend its filopodia or lamellipodia. It is like, you know stretching a human being hands or legs.

So, once this lamellipodia or filopodia, there is a leg kind of thing extension is there. So, this lamellipodia can sense more higher number of concentration here, because the concentration gradient is down this way. So, are you able to get it, what I am saying? What I am saying, the cell itself will not be able to sense, but, cell will cell will extend its filopodia to some distance.

So that, this filopodia can sense a larger number of signal molecule concentration, which is present. In continuation with the discussion that I had in earlier lecture, so, let me let me repeat what I said in the last lecture. That you know, that there is a two cells; one is the signaling cell, one is target cell.

Now, if the target cell is located far from the signaling cell, so that, the concentration of the signal molecules that will be sensed by the receptors of the target cell is very small. For Example, 1000 molecules or. So, then what will happen, this target cell will extend its legs or hands just like a filopodia lamellipodia.

Now, once this filopodia will be extended towards the signaling cell, then it can sense a higher molecule concentration or higher concentration of the molecules. So, that there will be more effective binding of the signal molecules to the cell surface receptors. So, this is what exactly happens in case of the target cell, which is which is located far from the signaling cell.

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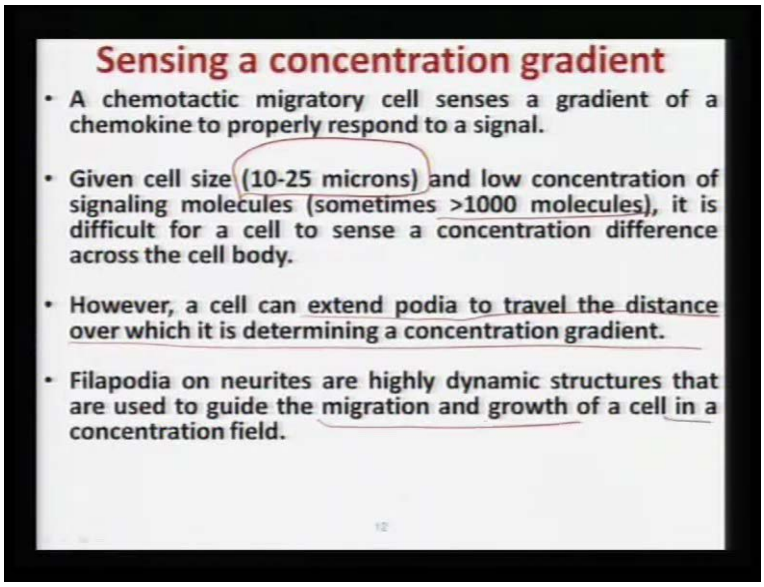

Now, filapodia on neuritis are highly dynamics structures that are used to guide the migration and growth of a cell in a concentration field.

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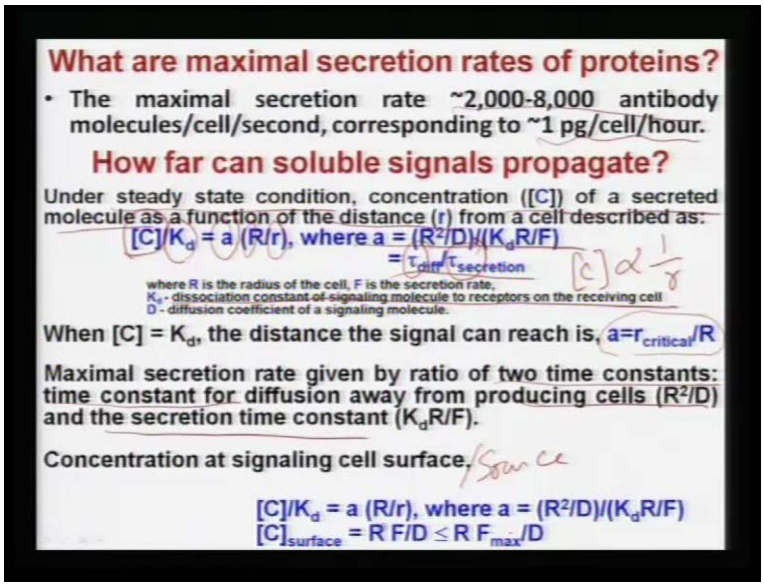

Now, what are the maximal secretion rates of the proteins? The maximal secretion rate is around 2000-8000 antibody molecules per cell per second. Then, these corresponds to typically 1 picogram per cell per hour. 1 picogram means, 10 to the power minus 12 gram.

Nanogram means 10 to the minus 9 gram; picogram means 10 to the minus 12 gram. So, one individual cell, what it means, one individual cell can secrete 1 picogram of the signal molecules per hour.

So, if you go back to the slide, this particular slide, so, particular this source cell. So this, will this, can this secrete 1 picogram of signal molecules per in 1 hour. So, if you culture 24 hours, so, how much picogram of this signal molecules that will be generated by individual cell; it is 24 picogram.

Now, if you culture 10 to the power 5 cells, then, what would be the total number of signal molecules that will be generated? Typically, 24 multiplied by 10 to the power 5 picogram, 10 to the 5 into 10 to the minus 12 is 10 to the minus 7. So, that means, it will be 2.4 into 10 to the power minus 6. So, total signal molecules that will be generated would be 2.4 into 10 to the power minus 6 gram, right in 1 hour.

So, 2.4 into 10 to the minus 6 means 2.4 microgram. So, from picogram to microgram, that is, like a few micrograms of the signal molecules that would be generated. Remember, this signal molecules are extremely smaller in molecular weight, like you know 1000 kilo Dalton, 1000 Dalton or so.

So, these small molecular weight signal molecules that will be secreted by the source cell. The next question that I have posed here, that, how far can soluble signals propagates? That means, what would be the typical distance D or what is the typical distance S. For example, D or S over which these signal molecules can be propagated.

Now here, there has been very simple calculation that has been done based on the molecular diffusion theory. So, under steady state conditions, concentration C of a secreted molecule as a function of the distance R from a cell. So, function of a distance R from a cell means, this is your K d. So, K d is the dissociation constant of a signal molecule to receptors on the receiving cell.

C is the concentration of the molecule, small A is, that another one is the tau diffusion by tau secretion and capital R is your cell size and small R is your distance. So, what you see here; the concentration is inversely proportion to the R that is, a distance from the cell.

So, what it what it means, higher the distance, lower will be the concentration, is it clear. So, what I am saying here, this concentration is inversely proportional to small R; small R is the distance from the cell. So here, in this line, in this particular diagram if you look at here, so, this small R is this. So, this is your small R distance, that is, the distance from the cell and this is your concentration C, here at the source cell.

So, if C proportional to R means, the way it will go, the further you will go, you will sense lower concentration of the soluble signals, are you getting my point. So, therefore, that larger the distance, smaller is the concentration and a is that R critical by small R.

So, maximal secretion rate is given by the ratio of two time constants; one is the time constant for diffusion, this is the tau and producing signals and another one is the secretion time constant, that is the tau secretion, that is K d R. Now, concentration at signaling cell surface or that is the source cell surface, that concentration is the C surface is equal to R F T by this, and then, you can simply do some arithmetic.

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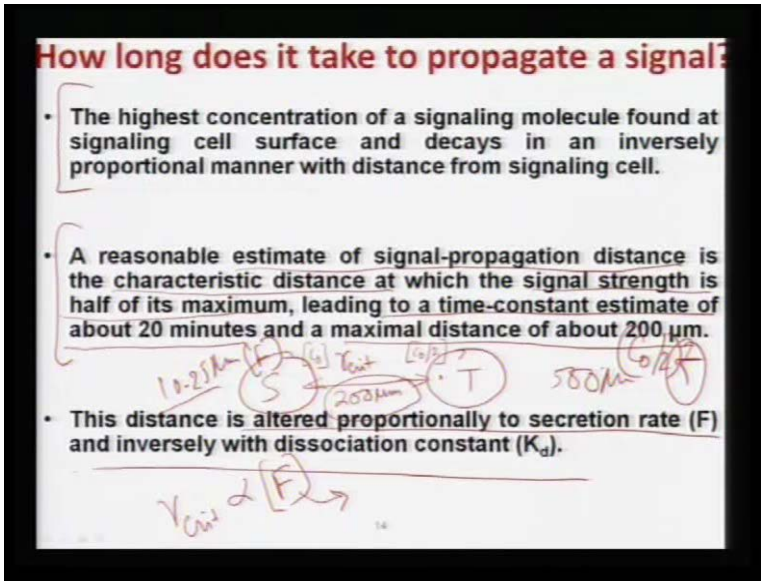

And what what is the physical meaning of all this expression? Physical meaning is that, the highest concentration of the signal molecule is found at the signal cell surface and that it decays in an inversely proportional manner, with distance from the signaling cell.

So, this is the physical implication that you have to bear in mind; that means, the signal molecule concentration will decrease as you go away from the one source cell towards the another target cell.

A reasonable estimate of the signal propagation distance with the characteristic distance at which, the signal strength is half of its maximum, leading to a time constant estimate of about 20 minutes and a maximal distance of about 200 micron.

So, what it means, let me just explain to you here. So, this is your source cell; this is you target cell. So, this particular expression, what it says, suppose your source cell here, the concentration is C naught, signal molecule concentration.

Now, your target cell minimum concentration should be C naught by 2, for these signal molecules to be effective for this target cell. If you do some calculations, considering that typically it is 10-25 micron is the cell diameter and so on, and so forth, then, what you find that, the critical distance through which the cells will be able to cells will be able to communicate to another cells, is typically 200 micron.

So, therefore, when you seed the cells in a typical culture plate, when you seed 10 to the 4-10 to the power 5 number of cells, instead of seeding 10 to the 4-10 to the 5 number cells, you just seed 10 cells, what will happen? It will happen like cell to cell distance would be much much higher than 200 micron.

So 10 cells, they will not be able to communicate to each other, just because their distance between two neighboring cells is too far. So, there will be absolutely no process of cell diffusion, cell growth and cell proliferation. It is clear? So, this distance is altered proportionately to the secretion rate and inversely, with the dissociation constant. So, this critical distance, whatever you can say, that is, R critical which is around 200 micron. So, this R critical is typically proportion to the secretion rate F.

So, secretion rate F means, the more the source cell will secrete large number of molecules, then it is possible that, even if the target cell is farther from the source cell by more than 200 micron, it is a 500 micron or so, then also, target cell will receive a critical concentration of C naught by 2 number of molecules at the target cell.

Let me repeat what I said. What I said is that, suppose your secretion rate is too high, secretion rate at the source cell, this is your source cell here. The secretion rate increases; that means, the F cell will secrete more number of molecules, signal molecules.

Now, I said that in the normal circumstances, the target cell should be preferably at a distance of 200 micron or less. Now, your F will increase; that means, your signal cell secretion would increase. Then, what will happen? Then, even if the target cell is here, let say around 500 microns, then also in the target cell, your signal concentration may be C naught by 2, so that, that signal may be effective for the target cell, understand. So, the critical distance would increase in that case, if the secretion rate will increase.

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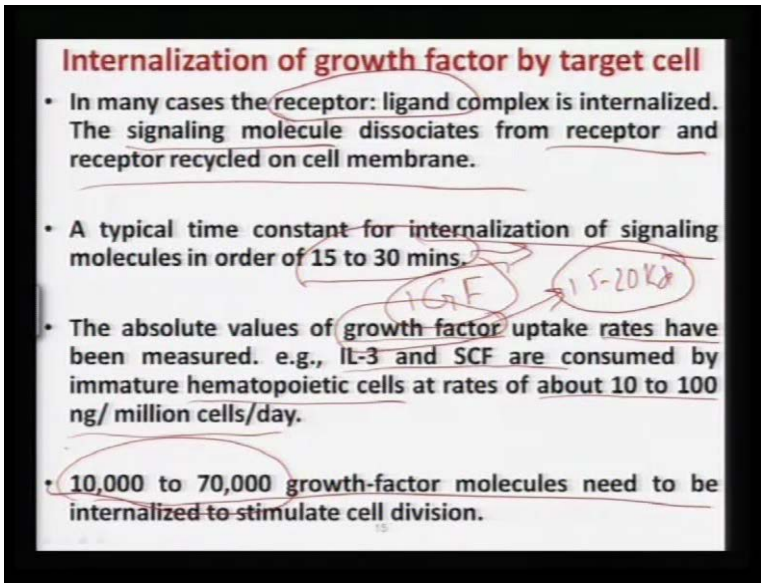

So, the another another factor is that internalization of the growth factor by target cell. In many cases, the receptor ligand complex, receptor means cells (( )), receptor ligand means that is the signal molecule is internalized in. That means, signaling molecule dissociates from receptor and receptor recycled on the cell membrane.

A typical time constant for internalization of signaling molecules in the order of 15 to 30 minutes. Now, why this time constant is important and this numbers are important. That means, so here is your cell surface receptor, this is your signal molecules. So, what I am trying to say here, signal molecules will come; they will interact with the cell surface

receptors here. Then, it may so happen, then once it binds, it can get dissociated from the cell surface receptor and it can be internalized, halts in this cell.

Now, typical time constant means, the time scale over which this association and dissociation that take place, that is of the order of 15-30 minutes. Now, this time scale is important because, this time scale, from this time scale you can understand that why your typical culture time is not 2 hours, but, it is 24 hours or 72 hours.

That means individual cells, if you want to activate by signaling, for individual cells it will take 15-30 minutes. Now, there are, let say 10 to the 4-10 to the 5 number of cells and it takes place in a very random manner. So, you can say that large and large number of time you require, several hours before all the cells will be activated.

So, that is the reason you may find that, after you culture on a same sub state, it is not that all the cells they have proliferated or they have expanded in the similar manner. Some cells they are expanded much significantly, some cells they have started expanding. Why there is a response difference? The response difference is means, that individual cell which has expanded very significantly they might have received large number of signals on the neighboring cells.

Whereas, the other cell which did not expand significant, which did not proliferate significant; that means, simply because that cell might not have received large number of signals like the other cells. So, because of the difference in the signaling processes or because of the difference in the amount of signal molecules that they have received, that signal molecules that they have received, the cells also proliferate or cannot grow.

So, therefore, all the cell-fate processes of the 10 to the power 5 cells that you are culturing on a substrate, they are different. Just because, that that individual cells, they have received different amount of signal molecules for their activation, for the cell fate processes.

So, the absolute value of the growth factor uptake rates have been measured and this is for I L 3 and S C F. Now, this is like insulin growth factor, you remember. So, growth factor means, so, this growth factor definition of growth factor is 15-20 kilo Dalton.

So, small protein molecules whose sizes are 15-20000 kilo Dalton, 1 Dalton means atomic mass of a 1 hydrogen atom. So, they are consumed by intermediate hematopoietic cells at a rates 10 to the 10-100 nanogram per millions cells per day and 10000-70000 growth factor molecules need to be internalized to simulate to stimulate the cell division.

That means, you can see now that 15-20 kilo Dalton, that was growth factors are there and this is like 10000-70000 growth factor molecules need to be internalized. Internalized means, need to be taken inside the cell, in order to promote the cell division like cytokinesis mitosis, etcetera.