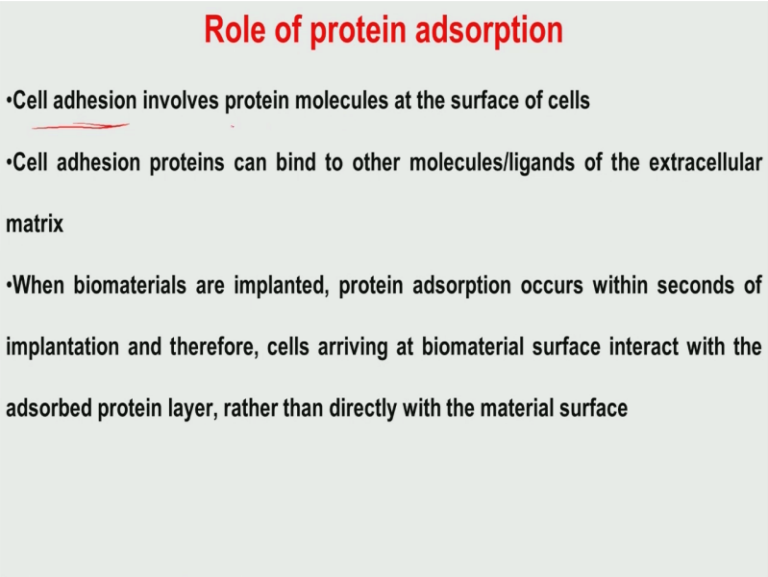
Biomaterials for Bone Tissue Engineering Applications
Prof. Bikramjit Basu
Materials Research Centre
Indian Institute of Science, Bangalore
Week- 03
Lecture- 12

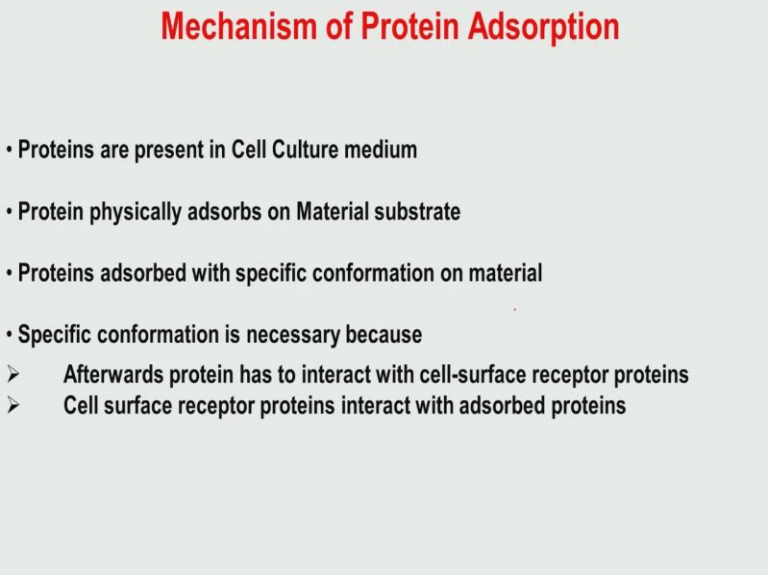
Ya, coming back to the discussion on the cell material interaction, let me (re) recapitulate what I have covered in the last module.

(Refer Slide Time: 0:31)



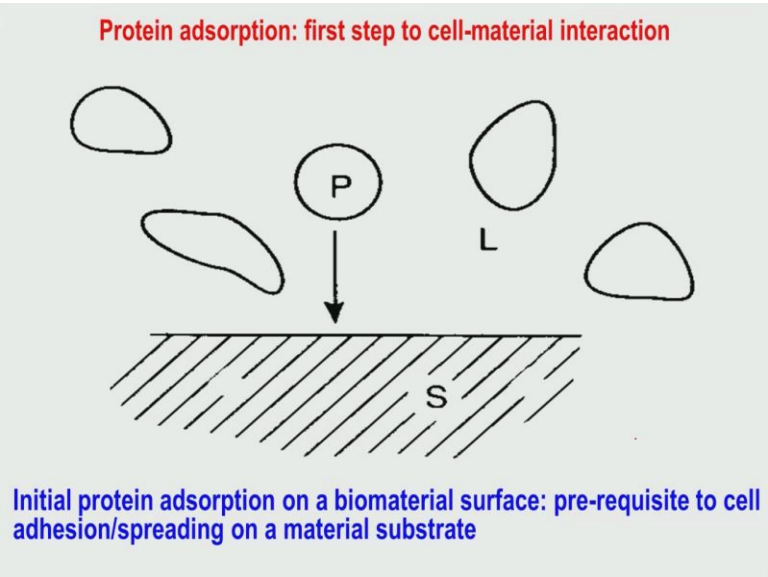
So cell adhesion, it involves the, first it involves the [pro] adsorption of the protein molecules. And the cell adhesion proteins can bind to other molecules or ligands in the extracellular matrix and this protein adsorption takes place, at the beginning at the, within first few minutes of the cell culture experiments.

(Refer Slide Time: 0:55)

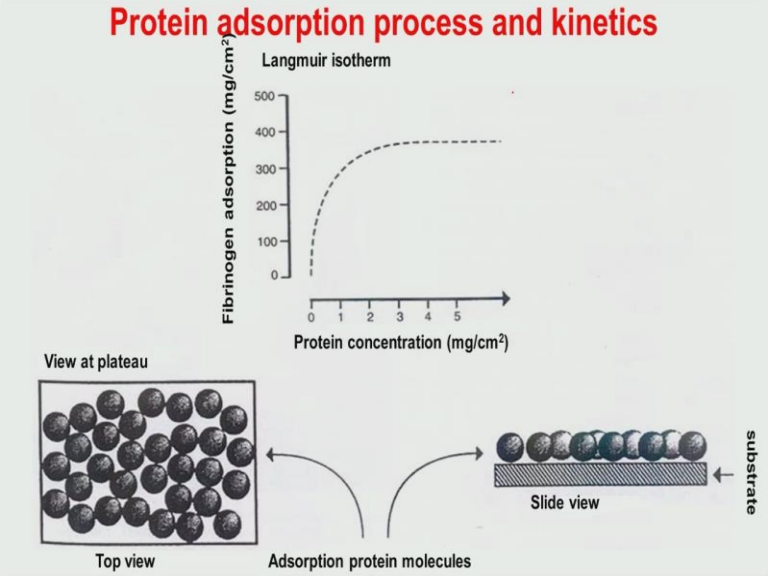


Now after proteins adsorbed with specific conformation of the (mat) on material, that specific conformation I have emphasised also in the last module. That is necessary because afterwards protein has to interact with the cell surface receptor proteins and cell surface receptor proteins interact also with these adsorbed proteins.

(Refer Slide Time: 1:10)

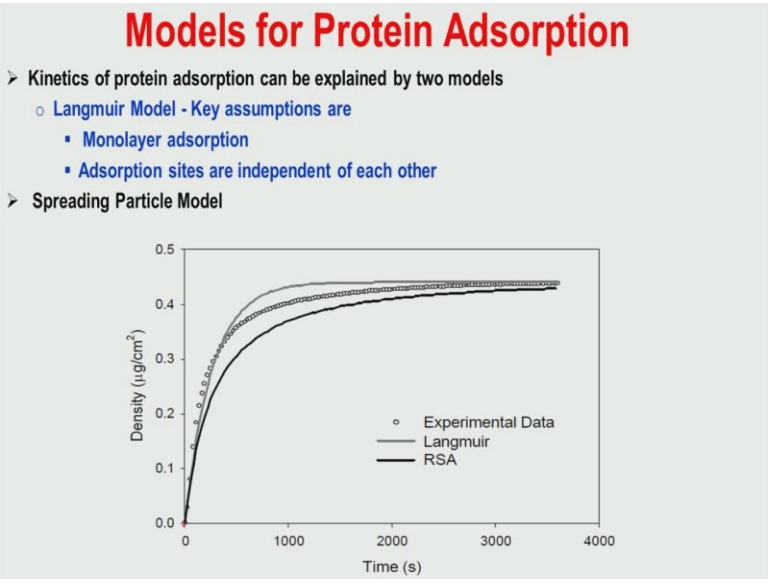


(Refer Slide Time: 1:25)



Now this protein adsorption as I mentioned in the last module, that protein adsorption, proteins are of different types, which which gets adsorbed in the biomaterial substrate, and this protein adsorption typically follows Langmuir kind of isotherm. If you plot protein adsorption as a function of the initial protein concentration you will get this kind of curve which reaches the steady state behaviour.

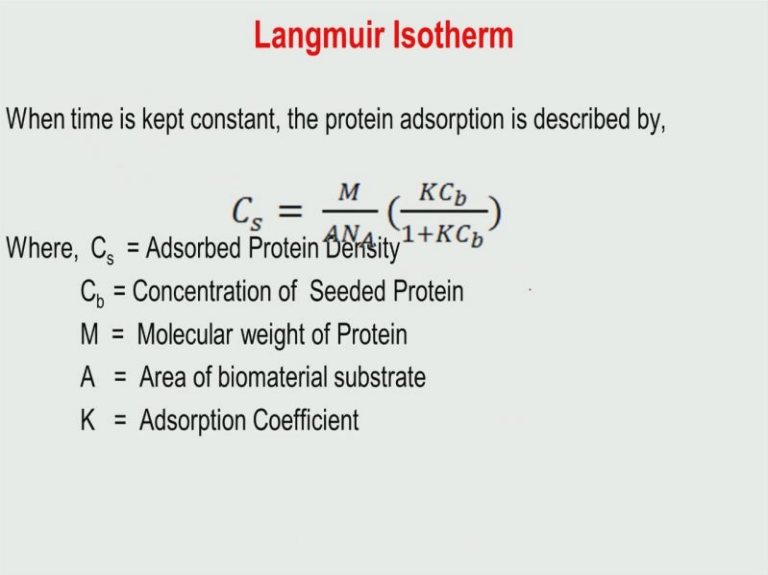
(Refer Slide Time: 2:00)



So this is on this Langmuir isotherms behaviour and similar protein adsorption kinetics you can also explain in terms of the kinetic dependence of the adsorption, if you plot the protein

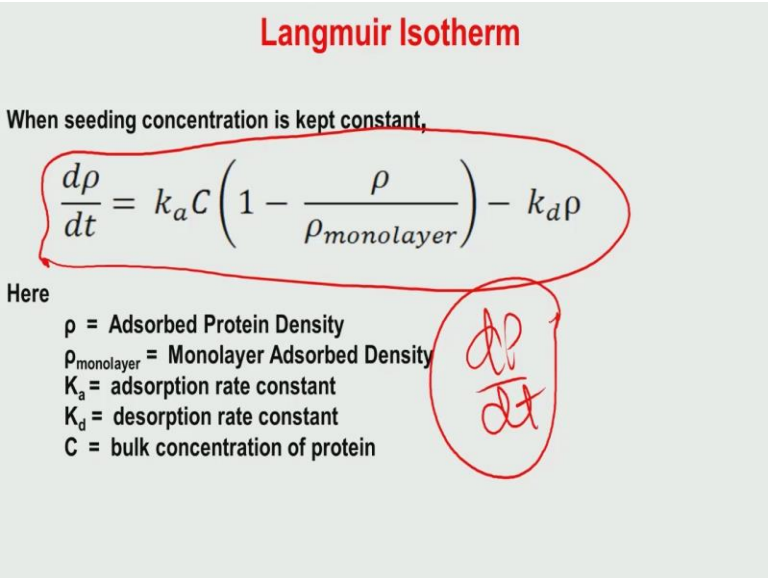
adsorption as a function of time. So initially the mono layer of proteins get adsorbed and then after that protein molecules. So here it goes. So this is the spreading particle model, what you see the density of the proteins and then as a function of time of adsorption if you plot then it also reaches a steady state behaviour.

(Refer Slide Time: 2:10)



So mathematically Langmuir isotherm can be explained by this particular equation, where CS is the adsorbed protein density, CB is your bulk protein density, K is your adsorption co efficient, M is the molecular weight of the specific protein of of that you are concerned and capital A is your surface area of the biomaterial substrate to which (protein) protein is exposed to. So this is not a very linear type of equation, as you can see CS depends on the factor that KCB by 1, (by) divided by 1 plus KCB.

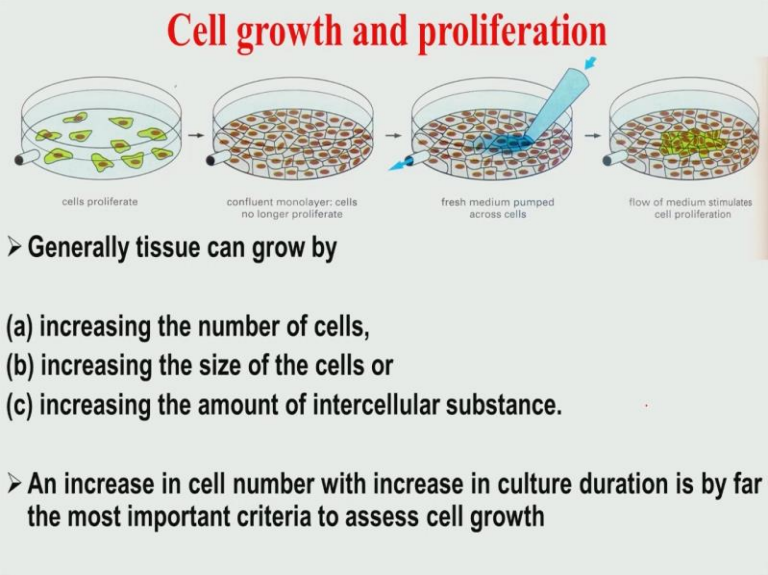
(Refer Slide Time: 3:10)



This is the second expression which is also equally important in describing the Langmuir isotherm that is D rho by DT, that is the change in the protein adsorption density with respect to the time of incubation, is equal to 2 terms, the first term is related to the adsorption, the second term related to the desorption. So adsorption terms, as you can see here, that KA term that is the adsorption co efficient, Capital C is your bulk concentration of protein which I have explained in the last equation as CB term. So here you can see this is the bulk concentration, CB.

The same thing I have mentioned here C, or I can explain it as CB term, into 1 minus rho up on rho mono layer. So rho monolayer is the monolayer protein concentration, rho is the concentration of protein at any time T, KD is the protein desorption co efficient and rho is again adsorbed protein density. So this is, this expression is valid only when your initial seeding concentration of the protein escaped constant and these expression is valid at any given time T, when T is constant and protein adsorption follows this Langmuir isotherm.

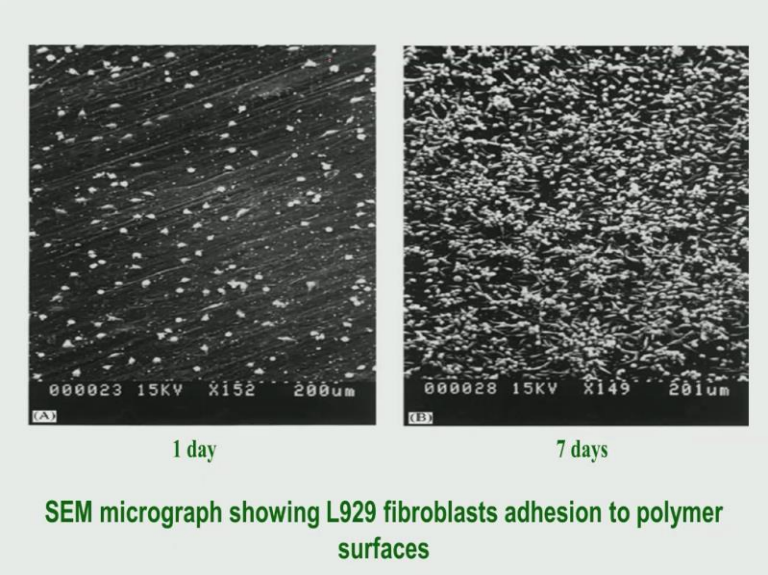
(Refer Slide Time: 4:25)



Ok, this slide is essentially recapitulation of what I have mentioned to you in the last module; the cell growth and proliferation. So this is the typical experiments that has been shown in it without any biomaterial substrate on a cell culture, tissue culture plastic which is used as a reference or control sample in all the cell culture experiments. Now what you see here, that initially cells stop proliferating then it forms a confluent monolayer and when the fresh medium is pumped in across the cells and then flow, flow of the medium also simulate cell proliferation. So if you give some flow, media flow, so it is not a static culture.

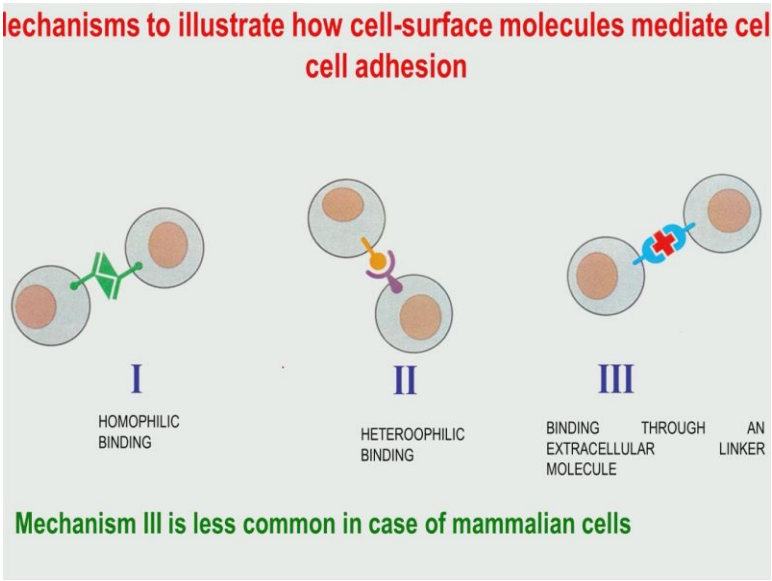
It is little bit more dynamic culture, so if you give some flow that means you are essentially giving some sheer to the suspension culture and therefore cells numbers also would increase. So tissue can grow by increasing the number of cells or size of the cells or inter cellular substance. An increase in the cell number with increase in the culture duration is by far the most important criteria to quantify or to establish cell growth which I have sufficiently explained in the last module.

(Refer Slide Time: 5:40)

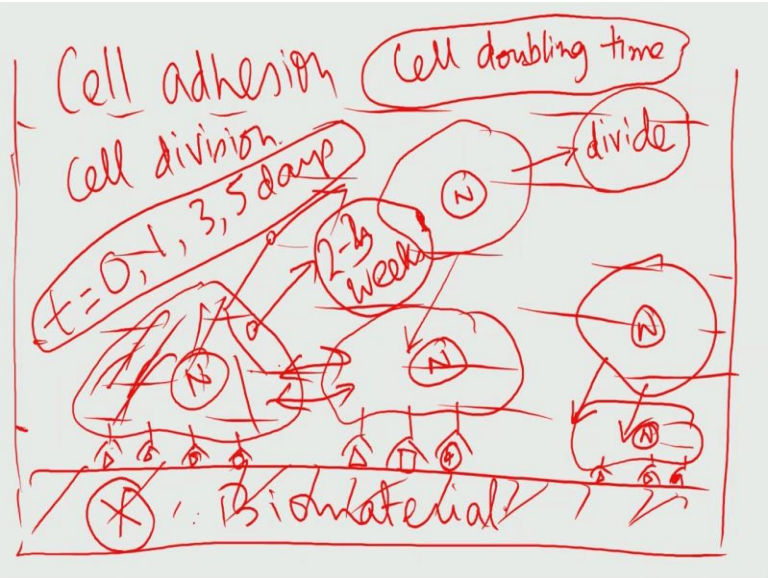


So, observation of cell, morphological observation or quantification of the cells adhered to a biomaterial substrate, needs to be done from a microscopic study. Here you see this is a 1day, after 1 day this is your number of cells and this is your 7 days. The total number of cells, it is spreading all through all along the surface. So this is the one of the very slowest growing cells. That is L929 mouse fibroblast cells. So if you now plot, if you have some observations at day 3 or day 5, and if you now plot these cell numbers, certainly you can see that the cell growth is kind of showing, shows the very linear pattern.

(Refer Slide Time: 6:25)



(Refer Slide Time: 6:35)

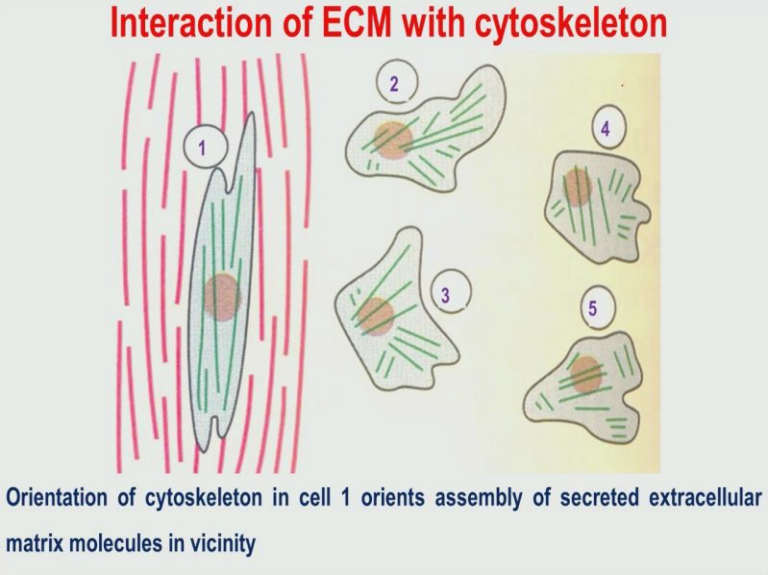


Now one of the things in the last module I have mentioned that once a biological cell adheres to a material substrate its sends soluble signal in proteins to neighbouring cells and (af) cells after receiving the soluble signalling protein signalling molecule, they decide whether to go towards the biomaterial substrate or not or to they decide whether to adhere to a biomaterial substrate or not. Once these cells, once these 2 (neighbour) once these cells will adds up on a biomaterial substrate then there is an opportunity for the 2 neighbouring cells also to interact with each other. In other words these 2 neighbouring cells, their cell surface proteins which can be exposed to each other they also get an opportunity to interact with each other.

So therefore that cell to cell interaction is being discussed in this slide, now what you see here, this is called homophilic binding. Homophilic binding means, that, homophilic binding means, so this is the cell surface receptors from cell A and cell B which are of the same cell type and is a similar kind of cell surface receptors they come, so they bind very easily with each other.

Now heterophilic binding means that there is 2 different types of cell surface receptors, which is coming from 2 different cells, C and D let us say, and then this is called heterophilic binding. Now if this direct binding of 2 cell surface 2 neighbouring cell surface receptors is not possible and it has to be mediated by extracellular linker molecules, that is also possible so this is called type 3.So this type 3 mechanism is by far is less only observed mechanism which can explain the cell cell interaction.

(Refer Slide Time: 8:35)



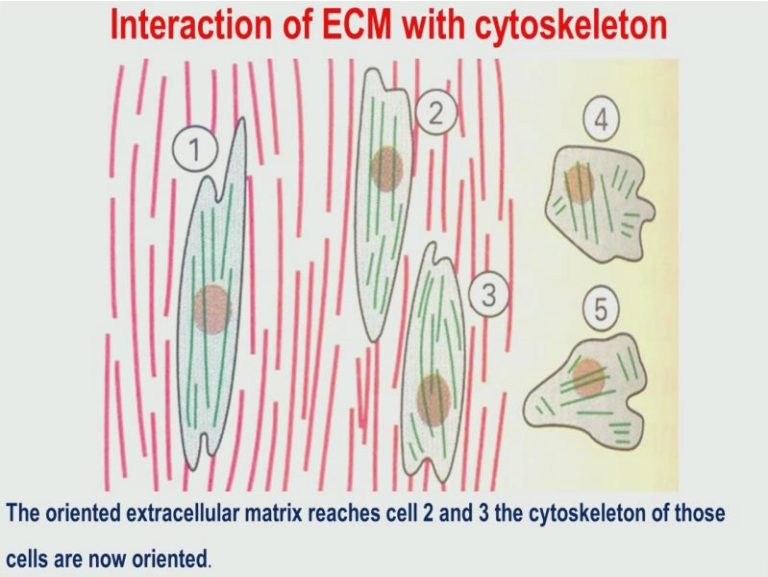
The other things which is important for you to realise that once a cell adheres to a biomaterial substrate cells also secrete some of the proteins molecules and some of the macro [bio] biological macro molecules which gets out of the cell to the exocytosis process to form an extra cellular matrix which as, I have explained in the last to last module as well. Once this extracellular matrix is formed which essentially composed of the elastin fibres as well as this fibrous collagen protein.

So this extracellular matrix has been shown here the way I am drawing here, or I am trying to sketch here. Now once this extracellular matrix forms the orientation of the collagen (fibre) collagen fibres and the orientation of the actin filaments as you can see, they are oriented almost parallel to each other. However in the neighbourhood of this cell number 1, if there are 4 other cells are there 2,3,4,5. If you notice their actin filament or the cytoskeleton fibres, they are oriented quite differently with respect to the ECM around cell number one. What it means that there is a clear mismatch in terms of their orientation between the intracellular cytoskeleton and extracellular matrix collagen here.

Now if the more and more [col] extracellular matrix is synthesised around the cells and therefore in order to provide sufficient support to these particular cells so we remember, one of the functions of the extracellular matrix is to provide mechanical support or structural support to the cells it contains, so therefore, these interaction or this mechanical support will be much more

effective if and only if that collagen fibres in the extracellular matrix are oriented almost (in the) parallel to that of the cell (in) number 1.

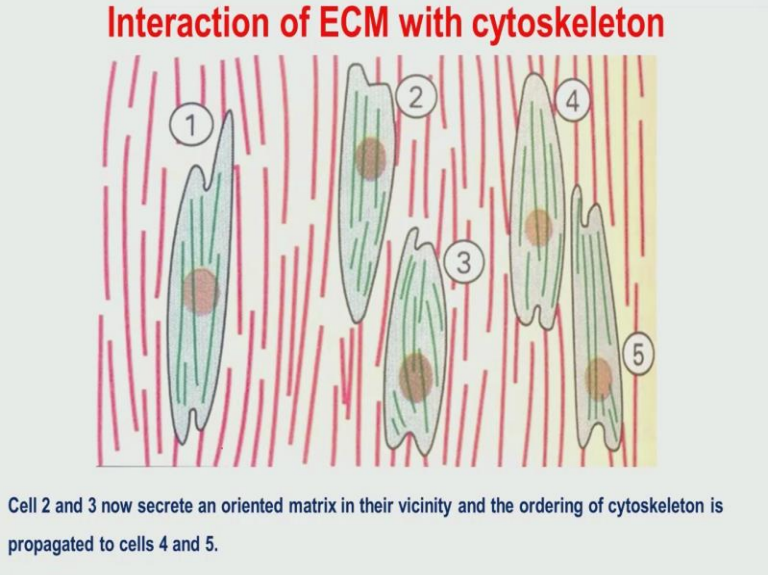
(Refer Slide Time: 10:48)



So in the next slide as you can see that as the extracellular matrix progresses formation progresses, then these cell number 2 here, and cell number 3, they are cytoskeleton reorganisation takes place and as I said, that this is one of the way that you know cell kind of adapts to itself by cell chips,(cell) cell chip changes and that is what exactly happens if you see the cell shape here in the marked as 2 and 3 and if you see the cell shape here 2 and 3, you would be able to realise a distinct differences, in terms of the cell shapes and cytoskeleton orientation.

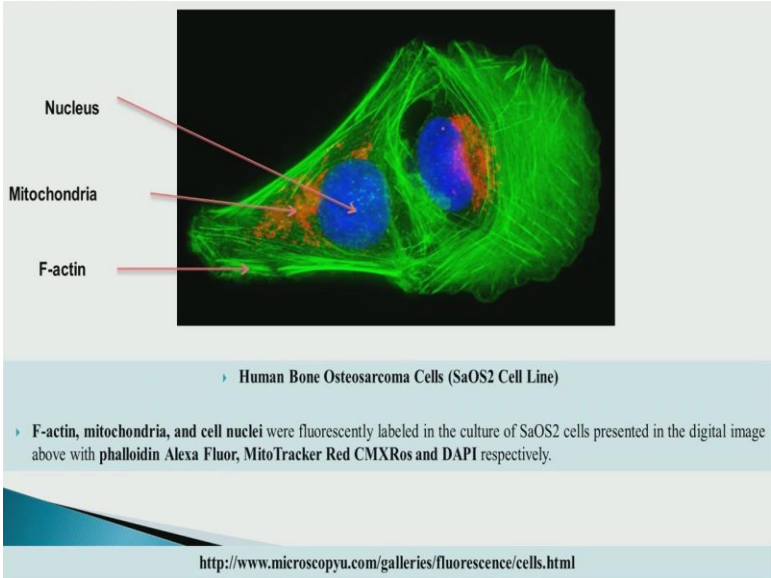
And this cytoskeleton reorganisation (is) has been helpful now to for easy, to provide more mechanicals or structural support to cell number 2 and cell number 3. However 2 other cells which are also shown here, cell number 4 and cell number 5, their cytoskeleton is still not oriented with respect to the extracellular matrix.

(Refer Slide Time: 11:55)



Now what happens therefore in the next stage, in the next stage as you can see here, that cell number 4 and cell number 5, their cytoskeleton is also oriented now with respect to the extracellular matrix here and now cell number 1,2,3,4,5 all 5 cells are oriented perfectly with respect to the extracellular matrix and that is what is mostly biologically desired or this is, this is the scenario that would help extracellular matrix, now, to (provide) extracellular matrix, now to provide sufficient structural support, to all five cells together.

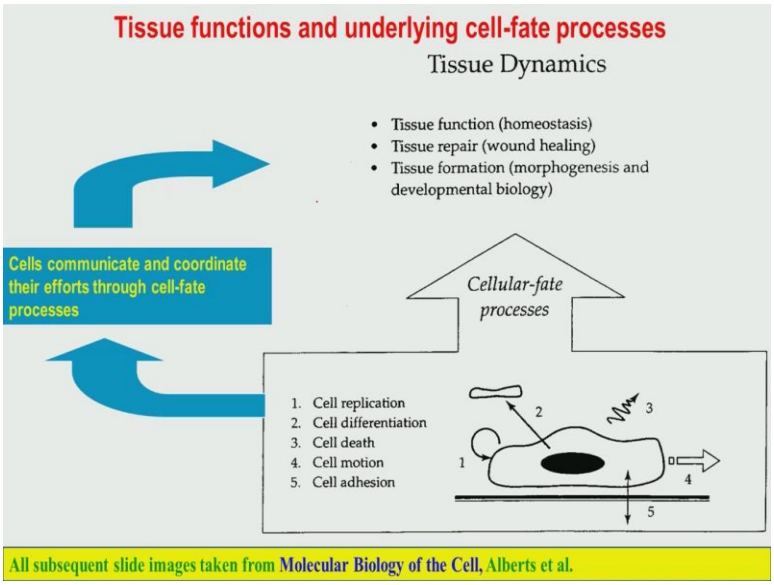
(Refer Slide Time: 12:35)



Now one of the techniques that I have mentioned to you earlier is that fluorescence microscope, this is one of the typical images of the fluorescence microscope cell cell images which you can grab using the fluorescence microscope.

Now what you see here, the cytoskeleton organisation within the cells, you can see in the green stained fibres, this is your actin filamentous fibres you also see the blue coloured region, this is your dapi, dapi stained nucleus, and nucleus means you have a DNA, so DNA intercalates with the dapi stained and therefore it keeps this blue colour. You also see somewhere these red coloured dotted lines, dotted points. So this is like small small organelles, and these organelles are essentially mitochondria, this mitochondria can be stained with microtracker and with this staining, that you can see here, very clearly at least 3 organelles, 3 (pa) structural parts, 1 is the cytoskeleton actin filament, 1 is nucleus and 1 is mitochondria.

(Refer Slide Time: 13:45)



Okay, now in reference to the significant discussion that I had in the last module that once the cell adheres on a material substrate through these focal adhesion complexes, so (foc) focal adhesion complexes and this focal adhesion complexes after that what will happen that whether there are couple of opportunities or there are couple of (c op op) options that the cell would have, 1 is that, whether the cell would replicate.

Replicate means, it will self-propagate like the cell would give rise to another daughter cells so 2 daughter cells or cell would differentiate to another cell type that is a different cell type and in

traditional biology, if you want to show that a mother cell or a parent cell is differentiated to another cell type you must show nucleus with different colour or different shape or different (shaded) shaded nucleus just to distinguish that, that this nucleus is different, [bet] these 2 cell nucleus are different 1 is the undifferentiated cell and another 1 is differentiated cell.

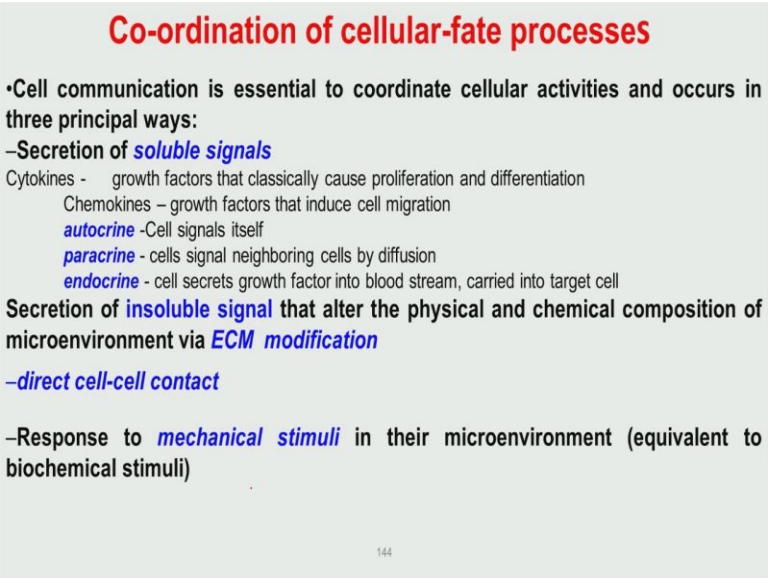
Number 3, if you remove all the cell signalling processes in the neighbourhood of (a) of this isolated cells, then cell can also activate its own suicidal mechanism to undergo cell death. I will tell you later that cell death normally takes place by 2 process; 1 is called cell apoptosis, that is called programmed cell death another 1 is called cell necrosis that is called accidental cell death, so which is essentially due to the physical damage to the tissue or cells.

Number 4 is cell motion or cell migration or cell motility. So this motility, cell motility cell migration, these are all synonymous terms ok? So cell migration means like a human being walks on the ground or the road, similarly the cell also can crawl on this particular biomaterial substrate. So the, the, these kind of transport or going from one place to another on another non biological synthetic surface, this process is known as the cell migration. And fifth one is cell adhesion that I have mentioned earlier.

Now all these processes what I say, all these processes, that cell replication, cell differentiation cell death,(is) they are grouped under one particular name called cell fate processes. So cell fate essentially means that when a cell has been cultured on a biomaterial substrate what would be its fate. Means whether the cell would grow or the cell would differentiate or the cell would not be able to survive or cell would adhere to the material substrate or cell would migrate or cell would become mobile on the biomaterial substrate.

Remember the cell migration or cell (motility) motility is kinetically extremely slow process. I will give you some numbers in the later on and then you will (realise) that typically cell migration takes place with a speed of several microns per hour. That means in a single hour a cell can move ahead by the process of migration only few microns. Few microns means 10 to the power minus 6 meter. So then you can realise how slow is the cell migration process. Now all these cell fate processes are essentially the result of a co-ordinated cell signalling process, so therefore one has to also define what is cell signalling process.

(Refer Slide Time: 13:45)

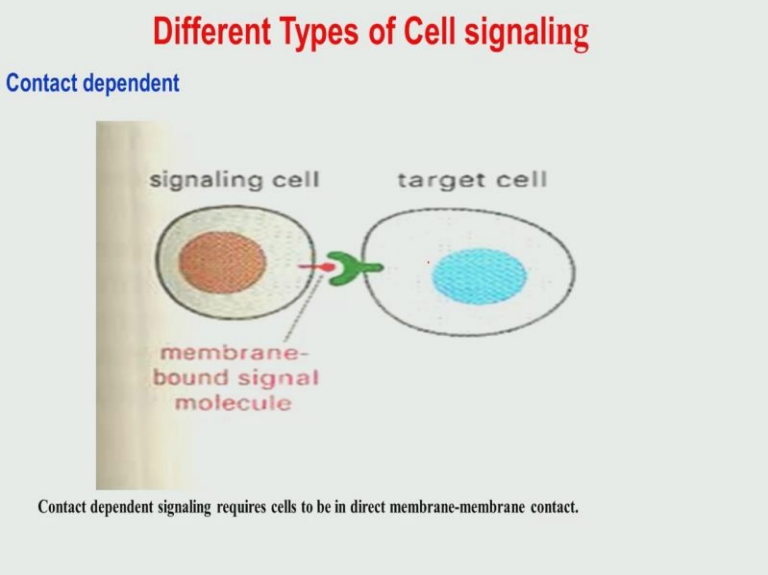


So cell signalling process is nothing but a part of the cell communication and the cell communication is essential to co-ordinate all the cellular activities and it occurs in 3 (process) 3 principal ways; 1 is the soluble signal, second 1 is the insoluble signal, third 1 is the direct cell to cell contact.

I will explain to you 1 by 1, but first you see that what is a soluble signal. So soluble signal means like cytokines like these are like growth factors, so therefore one has to explain what is growth factors also. So growth factors are extremely small protein molecules which can stimulate certain cell fate processes like proliferation and so on. So the small protein molecules mean their molecular weight can be few kilodaltons. So chemokine is 1 of the growth factor that induce cell migrations, cytokines is 1 of the growth factors that classically cause proliferation and differentiation.

But there are three types of growth factors also important soluble proteins. Autocrines, auto means similar type of cells. Paracrine means cell signals to neighbouring signals and endocrine means cell secrets growth factors which is transported through blood streams and carried into a target cell. So essentially cell signalling means you have a source cell and you have a target cell. So source cell and target cell here, this signalling molecules, so here signalling molecules can be transported to the from source cell to target cell and as a result the cell signalling processes, the cell communication takes place. And this cell signalling processes leads to the several cell signalling processes enables several cell fate processes to take place.

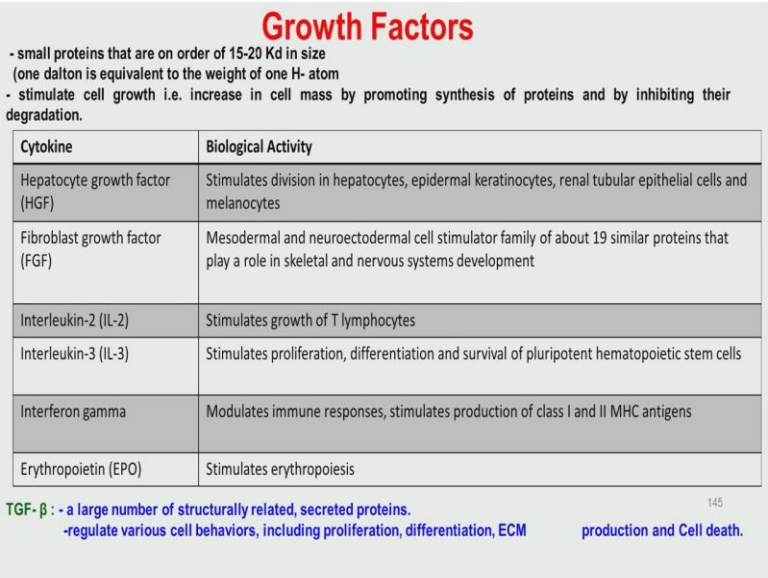
(Refer Slide Time: 20:10)



One of the cell signalling process is through the tide junctions or the gap junctions. This tide junctions or gap junctions are commonly seen in case of epithelial cell. Epithelial (means) epithelial means that is the skin the skin tissue cells. So (epthe) and and then also this is any closely spaced cells. There the cell signalling can take place very easily as you can see from here to here, the cell signalling process takes place. So this is 2 neighbouring cells signal and target cell, it is in the physical proximity. So this is the example of direct cell to cell cell to cell contact.

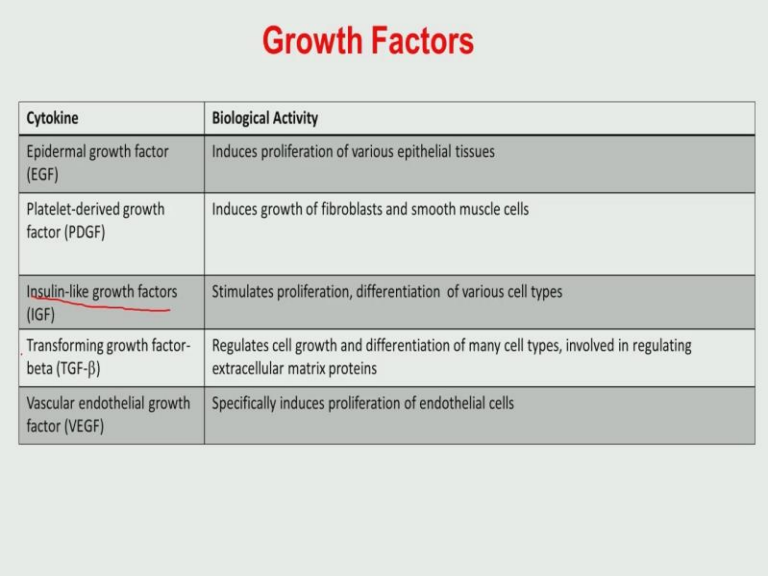
What is the other thing that I was trying to tell you is that, from source cells, from source cells to target cells these process can take place by diffusion or (is) if there is any some blood vessels (b) (b) blood vessels are there, so the signalling molecules can be transported through the blood vessels and can get carried to the target cell also. So this is the kind of third one that is the endocrine signalling process which I just explained. And if the signalling process goes by diffusion from one source cell to another target cell then it is called paracrine signalling process.

(Refer Slide Time: 21:45)



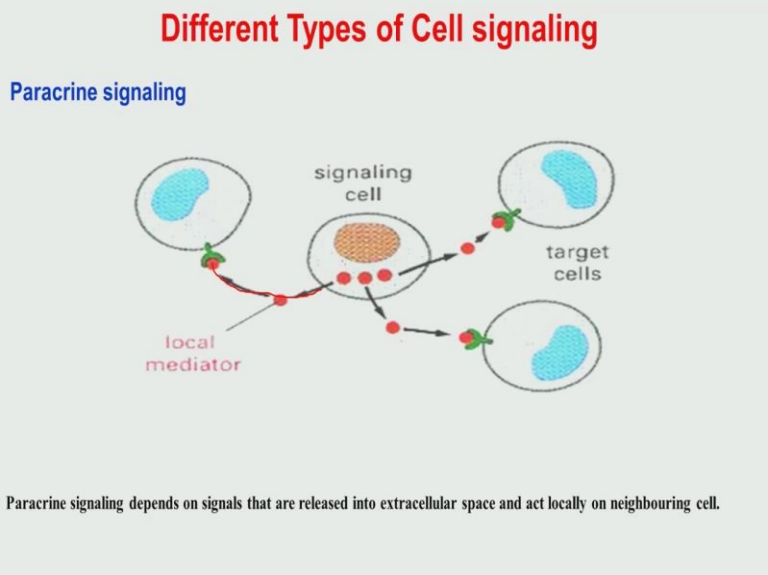
I mentioned about the growth factors. Here it is clearly mentioned it is a small protein of the order of size 15 to 20 kilodalton is size and one Dalton is equivalent to (1), weight of one hydrogen atom. So the functions of the growth factors that it can stimulate the cell growth that is increasing the cell numbers and so on there is the other growth factors are there, various types of growth factors which you may not remember all of them, but a few of them are being highlighted here. 1 is fibroblast growth factor, so that is responsible for skeletal and nervous system development.

(Refer Slide Time: 22:25)



You have insulin like growth factors, and another 1you have transforming growth factor beta. So that regulates cell growth and differentiation of many cell types. So these three growth factors perhaps it is useful to remember in the context of bone tissue engineering applications.

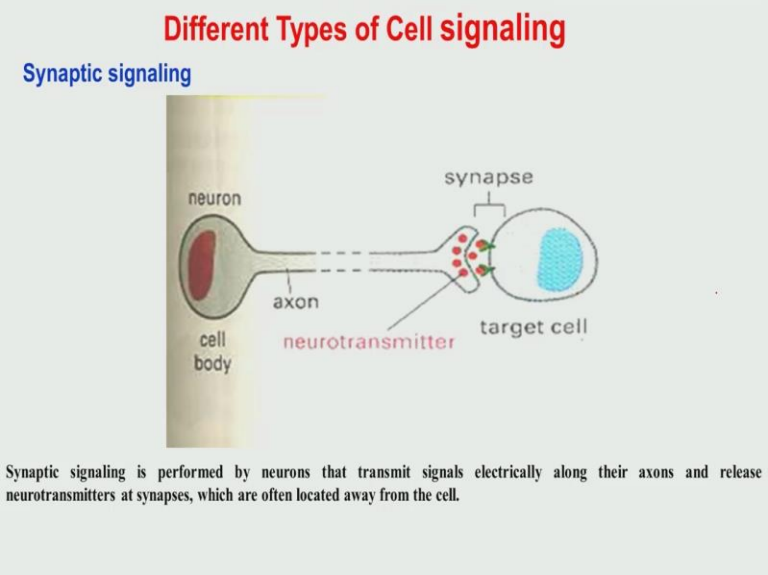
(Refer Slide Time: 22:25)



So this is what I was mentioning, paracrine means it goes to diffusion, this is your source cell or signalling cell and this is your target cell. So there are three target cells here, this is target cell number 2; this is target cell number 3. And from the source cell this small small signalling molecule is kind of transported through the target cells slowly and what you notice here, the biological interaction is actually takes place not directly but through cell surface receptor proteins. So this signalling molecules will go and then they will get, they will immediately being packed to the cell surface receptors. So here again cell surface receptors is kind of being packed to the signalling molecules.

And as a result there is some downstream signalling mechanisms get activated in this target cells and that downstream signalling mechanisms once it is activated they can help in the protein synthesis inside this target cells. They can also, if they are transported significantly and getting into the nucleus of the cell then they can alter the gene expression of the target cell also, therefore enables the differentiation process to take place. So I have explained these things in a very natural, very brief, that how signalling molecules when they are being transported they (are) they can activate the protein synthesis, they can activate the cell differentiation process and so on.

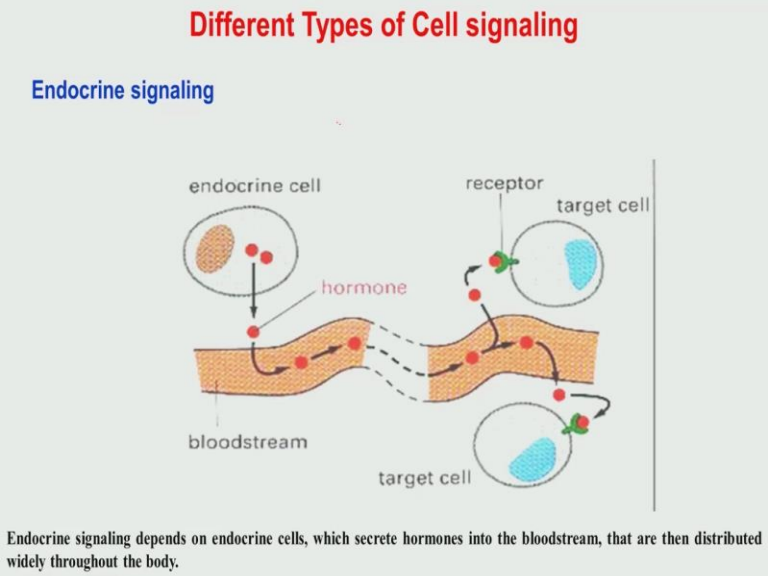
(Refer Slide Time: 24:25)



So this is another type of signalling process that is called synaptic signalling. So synaptic signalling essentially means you have a neuron, so it is a very long characteristic structure so you have a neuron, you have axon here. This is a cell body and nucleus, and you have a synapase and the synapse is here and you have a target cell T is there.

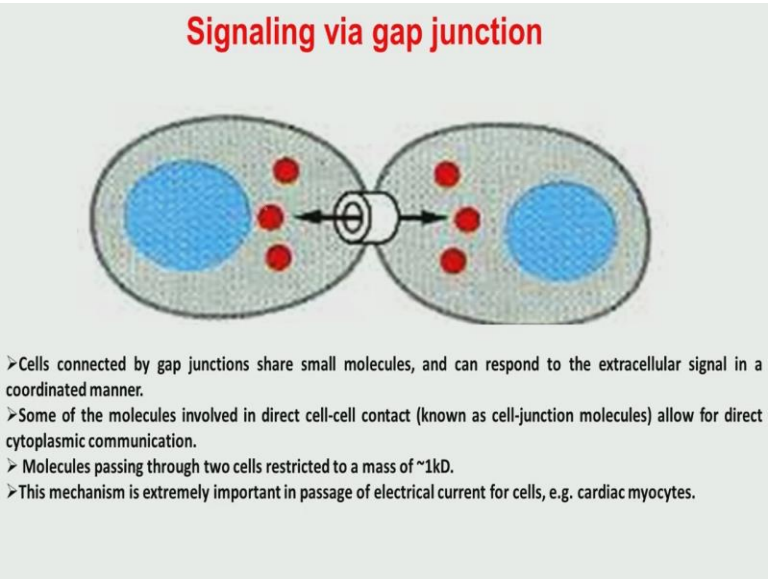
So here again signalling molecules or neurotransmitters once it is transported (through the) towards the target cells, then the cell surface receptors of the target cell will kind of be hooked to the signalling molecules and as a result certain downstream signalling processes gets activated and that will influence the way this target cell would otherwise function.

(Refer Slide Time: 25:15)



Paracrine signalling process, endocrine signalling process I have mentioned. So this is your source cell and this is your target cell, there is 2 target cells that has been shown here. So the signalling molecules being transported through the blood stream, through the blood vessels and they can transported towards the target cells. They get packed to the cell surface receptors in both the target cells and thereafter they will influence the cell functionality of the target cell.

(Refer Slide Time: 25:45)



And this is the gap junctions here like how the signalling molecules are being transported through the channels which is present at the cell membrane level itself in both the cell

membranes and they are so physically or tightly spaced these 2 cells so you cannot even distinguish that which one is source cell and which one is target cell.

For (unders) general understanding if you consider the left hand 1 is the source cell right hand 1 target cell then what happens we are trying to understand that once the signalling molecules get transported through this porous channel what would be the kinetics of this cell cell signalling molecules and how many number of cell signalling molecules can be (pass) can pass through this kind of gap junction that is what I will explain to you maybe in the next module.