Introduction to Mechanobiology
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Week - 01
Lecture - 03
Cell-ECM crosstalk

Hello, and welcome to our third lecture of the NPTEL course Introduction to Mechanobiology. So, in the last two classes I have broadly introduces to what constitutes mechanobiology and what are the different aspects of it, how multiple fields can contribute to our understanding of mechanobiology, and the importance of physics or physical forces in dictating biological processes. So, in the last class we introduced this abstraction of imagining the cell as a tent.

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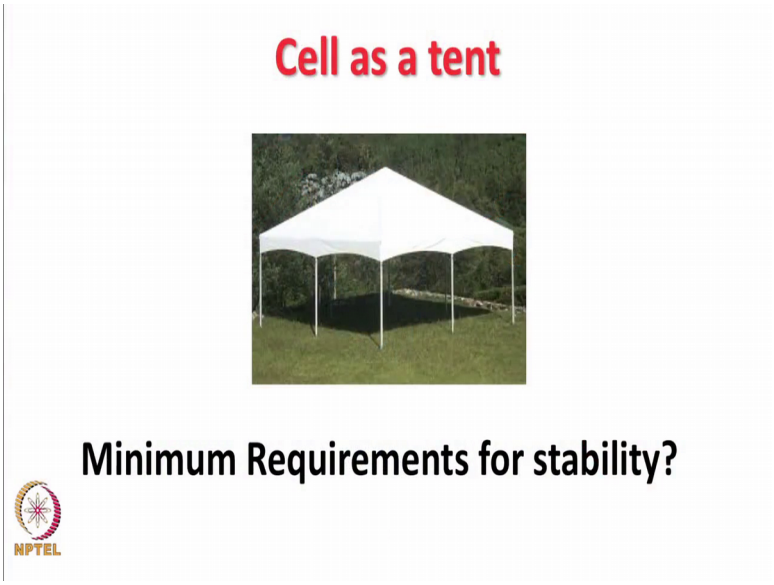

So, and I asked the question that what would be the minimum requirements for maintaining the stability of such a tent. So, I would like to state here once that this analogy applies to adherent cells that are which stay put which adhere to their substrate. This analogy would not work for cells like red blood cells which continuously circulate within the bloodstream and are not really adherent.

So, we said that there are four things which are essential for maintaining the cell structure; one of course, is the canopy. So, which is the biological the biological

equivalent being the plasma membrane, it separates the inside from the outside it acts as a selectively permeable barrier; however, it transports the essential nutrients also excretes whatever needs to be thrown out of the cell, and most importantly it participates in signal transduction.

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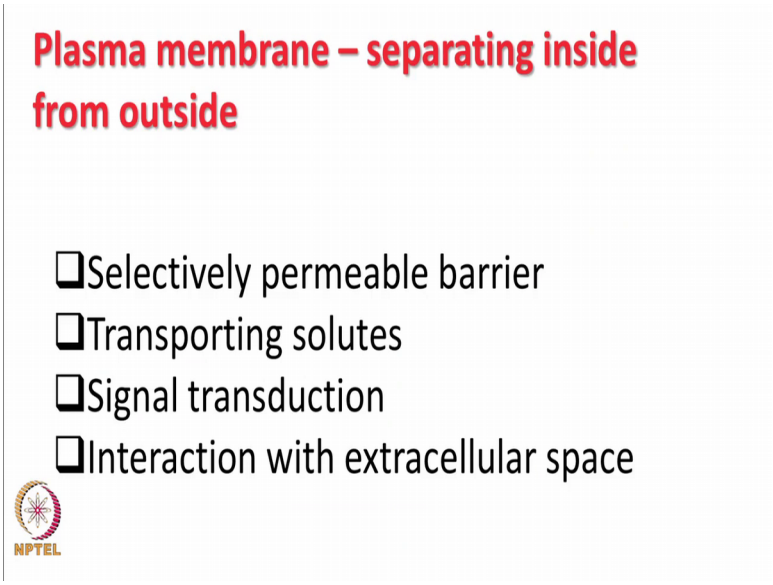

So, this signal transduction is mediated by proteins which are present on the plasma membrane, there are multiple proteins some of which are transmembrane proteins and we briefly raised talked about integrins which is of the most important transmembrane proteins, which link the inside of the cell to the outside of the cell. But there are other proteins which are either on the outer leaflet of the membrane or in the inner leaflet the membrane. So, proteins which are in the outer leaflet of the membrane would presumably be interacting with extracellular molecules, while proteins in the inner leaflet of the medium would interact with proteins within the cell.

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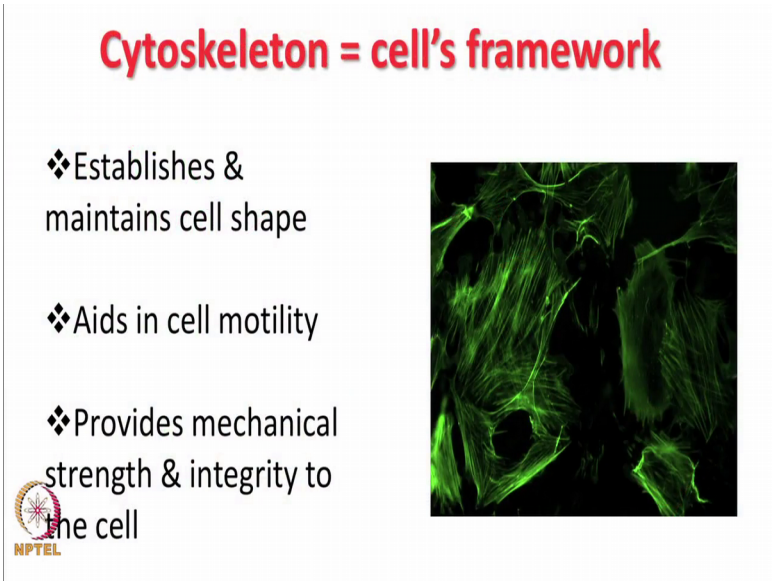

So, and after plasma membrane comes the cytoskeleton, this is the main element which maintains the structure of the cell. And this is just some images of mouse embryonic fiberglass which have been probed for filamentous actin and you see nice. So, these filaments are called stress fibers which span the entire cell body, and actually contribute or actively regulate the shape of the cell. Now one thing I would like to again harp on is that this is a static picture. So, it does not take into account the dynamics which is continuously going in the cells, as we observed in a movie that I had played in a cell transfected with life act. So, cytoskeletal is essential not only for maintaining the shape of the cell, but also for maintaining or aiding in motility which would mean that you need to continuously change shape or in other words this is mediated by assembly and disassembly of the cytoskelet.

So, there are three protein networks which constitute the cytoskeleton, the actin filaments the microtubule organized microtubules and intermediate filaments. So, we will briefly touch upon these cytoskeletal filaments and their dynamics later in the course.

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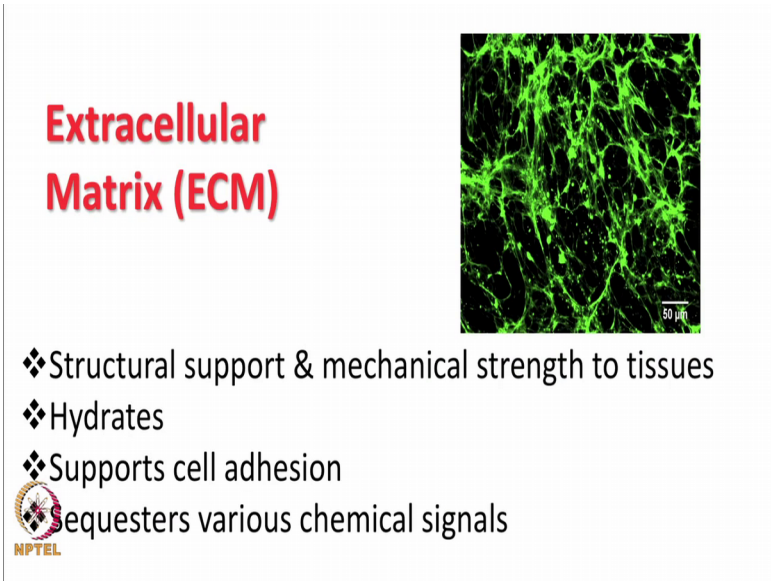

So, the outside of the cell are the strong base on which the cell rest is the extracellular matrix, it not only provides structural support for tissues, it acts as the scaffold on which cells sit and then it and then it the ECM integrates these cells into functional organs. But it supports adhesion and its sequesters various chemical molecules and growth factors, that can either be cross linked within the network or just adsorbed on to the matrix and lastly we spoke about focal adhesions.

So, I call them dynamic welds because they are not static so typical time scale of adhesions might be in the order of minutes.

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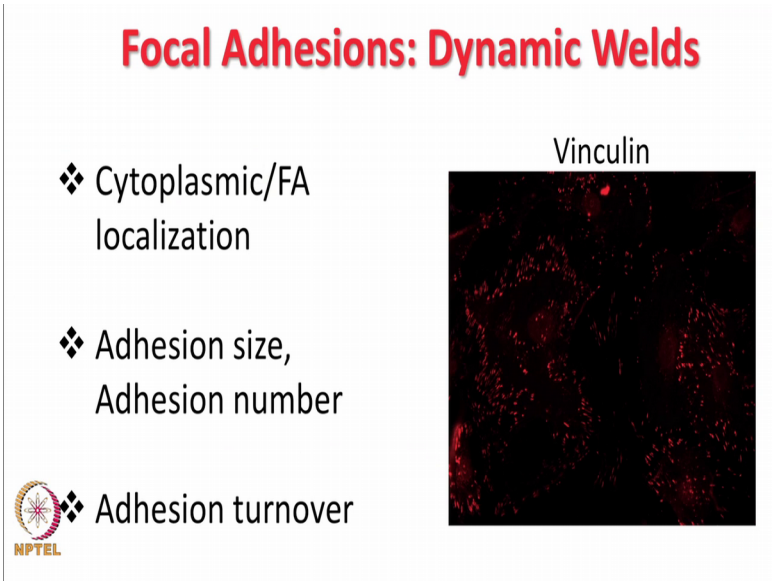

So, it depends on where what type of cell you are looking at. So, here I would like to mention one point is even for proteins which do localize at focal adhesions, it is not that hundred percent of the protein content is localizing at focal adhesions. So, the staining that you observe here, the staining of focal adhesions which is so crispier, is achieved by actually treating the cells with light did you know permeabalizing the cell lightly to remove all the cytoplasmic pool of the cells the cytoplasmic pool of proteins focal adhesion proteins and then what it remains is these proteins which are in which constitute the insoluble fraction and which are actively recruited at focal adhesions within these cells.

So, in other words you have a cytoplasmic pool and a focal adhesion pool, and you can probe of the relative fraction of the protein which is localizing at focal adhesion versus in the is in the cytoplasm by doing this experiment, in which you either treat them or treat the cells with a permeableizing agent to remove the cytoplasmic pool and then collect the remaining contents which will give you the focal adhesion pool of the focal adhesions. The other thing I want to you know state briefly is that the size of the adhesions and the average number of adhesions per cell is are two variables which would dictate, how stable the cells or how firmly attached the cells. One would expect in circumstances where the cell is supposed to be strongly adherent as in this particular case, you not only have prominent focal adhesions all over the cell periphery, but you also have these adhesions which are pretty big in size. So, in cells which are we cleared here in for

example, the in immune cells which are continuously migrating, these adhesions are much less well developed and are much smaller in size.

So, later in the course we will touch upon what are the how dynamics of focal adhesion is achieved and what are the players which participate in this dynamics. Last thing is adhesion turnover and that is what brings me to this term of dynamic welds in order to migrate, the cells keep to form adhesions and break adhesions are the rear. It is like a man walking you put your first foot forward you stabilize yourself which is equivalent of forming a focal adhesion and you dislodge your back foot, so that you can again bring it forward.

So, this is called adhesion turnover and in order to track adhesion turnover you would preferentially do lifestyle experiments, where you would transfer cells with some fluorescent focal adhesion protein, and track its localization at the focal adhesion. So, in essence that would provide information as to how the adhesion size is growing and then shrinking and from there you can find out the turnover rate. So, these are the four primary entities which regulate cell shape are required for cell to be stabilized.

We also touched upon in last class how this you know the what is the benefit of mechanical signals versus a chemical signal, and the time scale of a signal reaching the nucleus mechanically that is friar the cytoskeleton can beat three orders of magnitude faster than chemical signal propagation, where the molecules have to diffuse should the cytoplasm to reach the nucleus.

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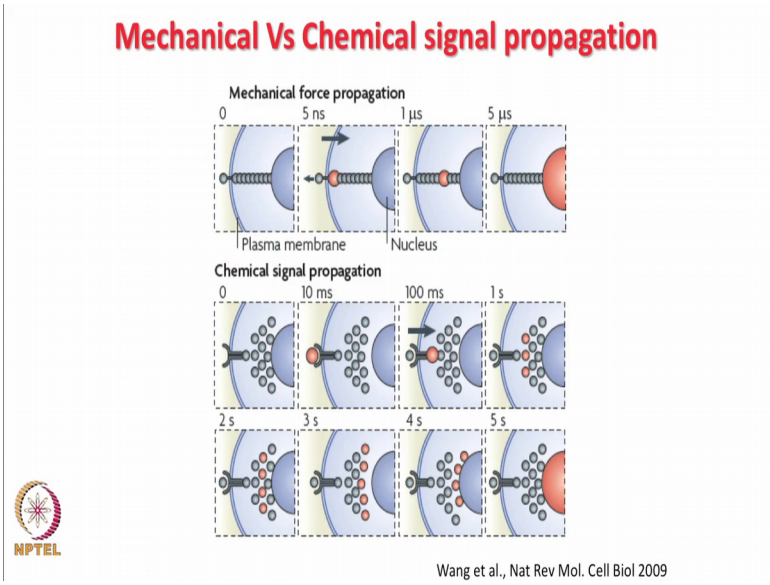

And this fast propagation in case of mechanical signal propagation is mediated by the cytoskeletal network, which on one end is attached to molecules like integrins which link the outside of the cells to the inside.

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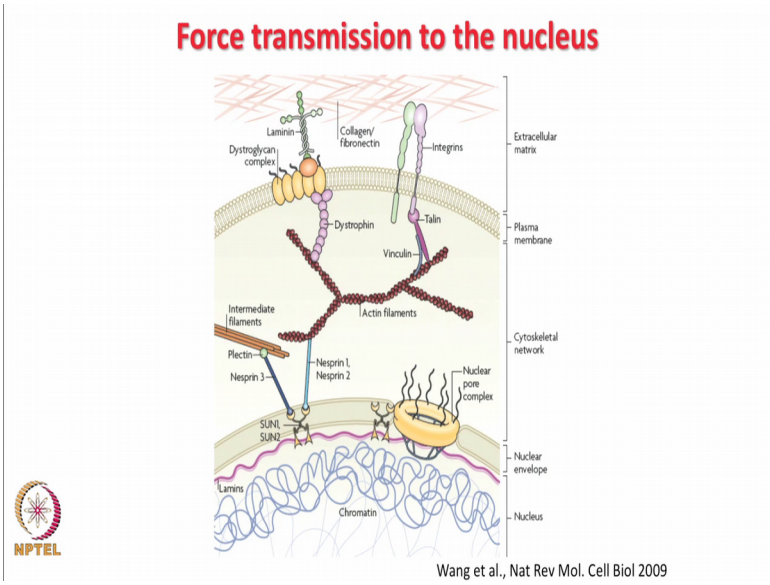

So, as this picture depicts the integrins are binding to the extracellular matrix on the outside and they are connected via focal adhesions. So, the this part of integrins also part of the focal adhesions the linked to the active network, and eventually through

intermediate cytoskeletal intermediate filament networks they are connected to the nucleus nuclear membrane and that will eventually link it to the DNA.

So, the force transmission to the nucleus is very fast and that is why mechanical signals can have a drastic effect in dictating how cells are responding or sensing. So, this also brings us to ask to the question that what would happen, if this signal proposition or if one or more elements of this network which connect the outside to the inside is perturbed.

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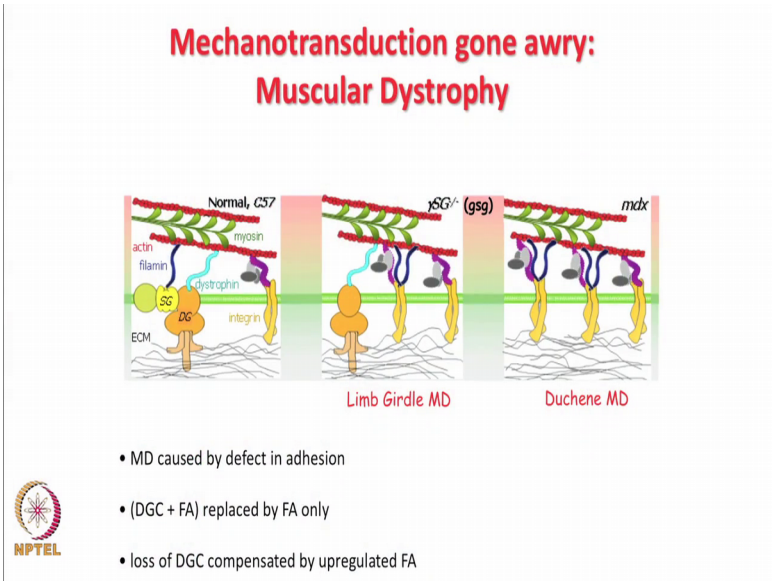

So, I would like to take a simple example of where of a disease where mechanotransduction is perturbed. So, this this disease is called muscular dystrophy. So, in skeletal muscle cells you have in adhesion to integrins which link the inside. So, this portion is the top of the inside of the cell, and this portion is the outside of the cell. So, in adhesion to integrins which link the inside of the cell to the outside, you have this other system called the dystroglycan complex which also links the outside of the cell to the inside of the cell. And through the dystroglycan complex does this protein called dystrophin which is the biggest protein in humans, which links the distroph glycan complex to the optimizing cytoskeleton. Now this is the structure of normal muscle or wide type muscle. There are various types of muscular dystrophies one of which is all of which involve perturbation of one or more components of this network. So, you can have

a mild form of muscular dystrophy which is called limb girdle muscular dystrophy, in which multiple components of the dystroglycan complex are missing.

So, particularly this molecule called SG is short form for sarcoglycan there is gamma sarcoglycan that is missing as a consequence of which there is still some links to the cytoskeletal through the dystrophin molecule, but the entire protein network is not there. You have another form of muscular dystrophy called duchene muscular dystrophy where the entire. So, because the dystrophin gene is not produced the entire dystroglycan complex is missing and this in this case the linkage is only through the integrin sets integrins ok.

So, if you see. So, this is in terms of the disease condition becoming worse and worse mdx or duchene muscular dystrophy is the most disruptive, and it causes eventually leads to death by age of 15 or 20. So, this is a much more milder form of dystrophy, but if you have if you look at the number of integrins integrin connections to the cytoskeleton. So, you would appreciate that the way the schematic has been drawn; there are more of these integrin connections which link the outside to the inside in this case and maximally in this in this mdx case. Suggesting the possibility that this is may be compensatory response you know employed by the cell. So, this is equivalent of saying that you have two networks a dystroglycan complex and a focal adhesion integrating, focal adhesion complex which together collectively regulate the first transmission from the outside to the inside.

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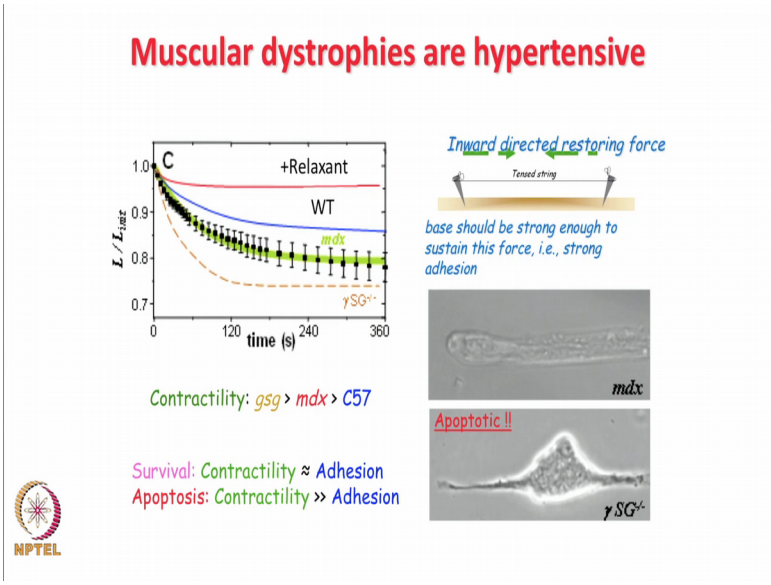

So, what has been known is as the consequence of integrins being over expressed. So, this would be that integraine expression would be higher up, in these disease context and. So, what has been seen is when you look at these cells in culture. So, these are mild tubes formed from mdx cells or gamma sarcoglycan deficient cells. So, what is what was particularly interesting is these cells even when the cultured in vitro within the tissue culture, they often have this kind of morphology indicative of much higher apoptosis. Why would this cell kind of apoptosis and if there is some change in the biophysical properties. So, one possibility was that the cell.

So, we have earlier seen that cells exert contractile forces on the substrate to fill the bulk properties. So, you can estimate that contractile forces by doing this (Refer Time: 13:38). So, imagine the cell as a tends string which is anchored to the substrate via multiple points. So, these are this is the equivalent of focal adhesions ok.

So, if this system is to be sustainable and if you are increasing the tension in this string then the substrate has be has to be strong enough or the adhesions which the cell engages with the substrate has to be strong enough. So, that this tension this tensile forces are sustained by the substrate so, in order to estimate this what you can do is imagine if you take a reagent which cleaves the string at one of these adhesions. If this was in the tension and this is relaxed and this is what this particular quantification shows what it tracks is the lid. So, you imagine you come down with a pipette and peel the cell from

one end. So, by peeling what you are doing is you are disrupting the adhesions which are holding the cell at one end. So, you are causing this localized imbalance of forces because of which if there was tension in the system, this system should relax. So, if I track this length as a function of time, and then normalized to its initial length, you have the cell which starts from a value of one which is from the initial length and it slowly drops. So, after some time you see that there is no more change in distance suggesting that this relaxation is because of the tension which was inbuilt in the system.

So, this should mean that if something should relax to a greater extent, than there was more amount of tension in the system. So, you call this the ability to exert forces then pooling on the substrate is contractility and what you see is compared to wild type, mdx becomes more contractile or hypertensive. Gamma circle I can deficient cells they are way more hypertensive. So, contractility is highest for gsg compared to mdx, and both of them are more contractile than C57 cells. So, what this experiment suggests is survival. So, in mdx though the disease is very lethal there is no survival issues. So, eventually patients die of organ failure and this is the case where contractility or the forces which the cells are exerting are reasonably balanced by the adhesions which the cell is able to form with the substrate.

In contrast if contractility is way higher then what will happen is the cell will pool on its substrate the substrate will be in unable to reduce those forces leading to the cell rounding. As you as you see in this phenotype here suggesting that in case of apoptosis you have these imbalance that the effect of contractility is so high, that adhesions are not enough as not strong enough to sustain that pool. So, to summarize broadly what I have discussed so far you see how cellular responses to mechanical forces are crucial and involved in numerous diseases.

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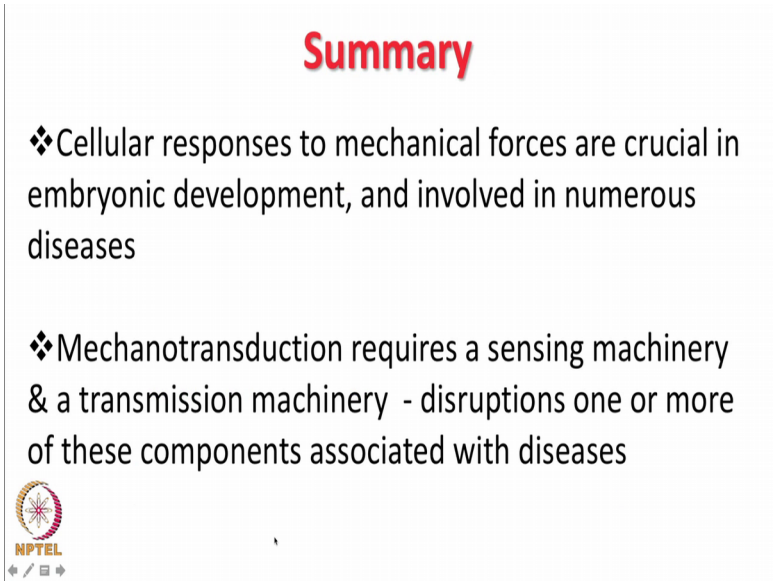

So, and for mechanotransduction to occur you have a sensing module and a transmission module. So, either disruption in one or more of these modules will lead to disease context.

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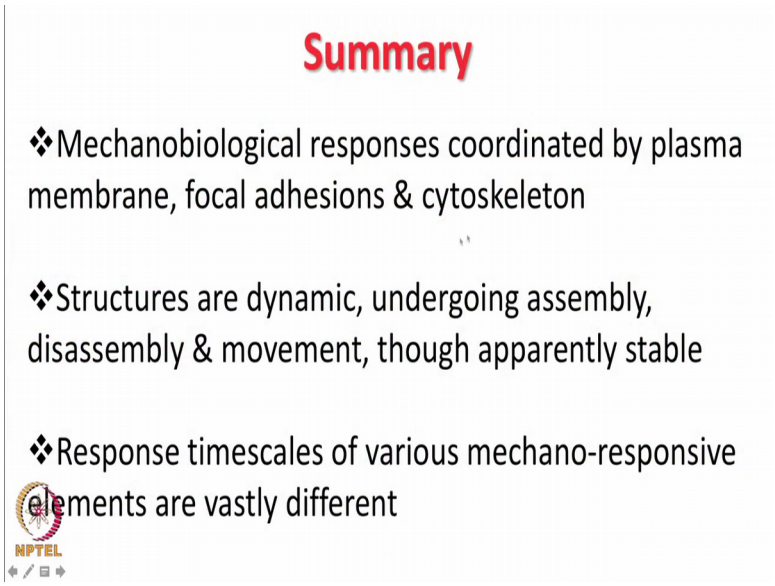

So, mechanbiological responses are coordinated by plasma membrane adhesions and the cytoskeleton the structures the cytoskeleton or focal adhesions they are dynamic that is how the enable movement. However, the time scale the response timescales of different elements can vastly differ. So, that brings us to the end of a broad introduction to what is

mechanobiology, now I will switch gets and I again gets started by looking at each of these structures that is particularly the extracellular matrix, the cytoskeleton the focal adhesion in greater detail ok.

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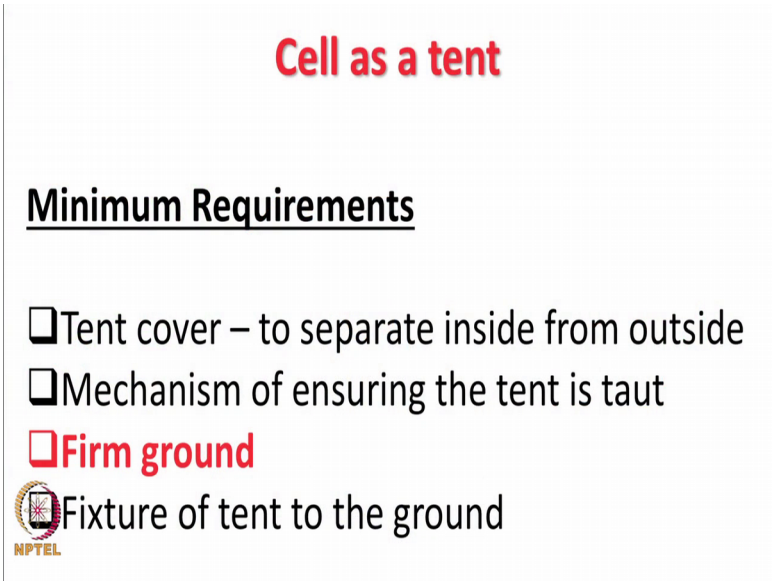

So, coming back to the requirement or to our abstraction of cell as a tent, today I will start with focusing on the firm ground and what is this firm ground? Firm ground is this extracellular matrix like.

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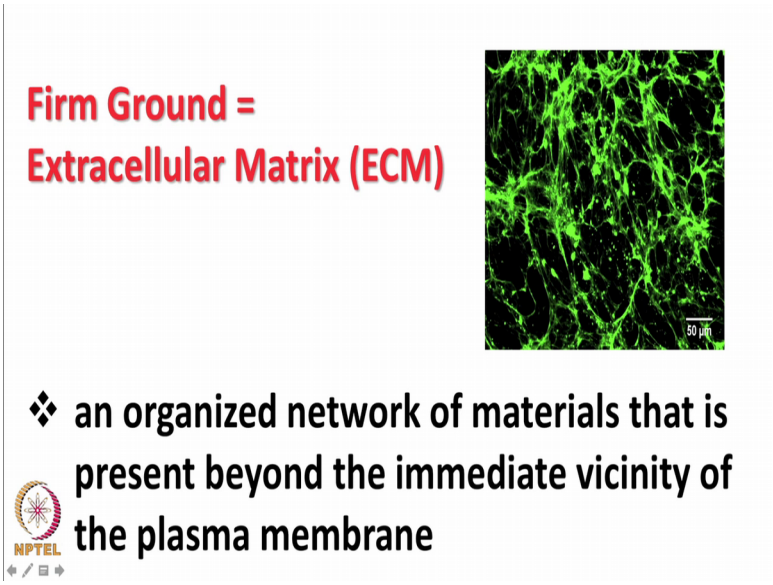

Similar to the experiments for gamma circuit like on deficient cells you can very well imagine, if I have a cell sitting on this matrix which is exerting lot of forces which this matrix is unable to support, then that will lead to collapse of the cell or detachment of the cell. So, properties of the extracellular matrix when I see properties not only the chemical properties also its physical properties are equally important. So, the ECM is an organized network of materials that is present beyond the immediate vicinity of the plasma membrane. So, outside the cell it is also on what. So, truly speaking inside or what is cells exist in a completely three d environment, but there are also cases for example, if you look at the endothelium where it is more like a flat surface.

So, what are some of the functions of extracellular matrix? The ECM actively regulates shape and activities of the cells it is sequesters various growth factors.

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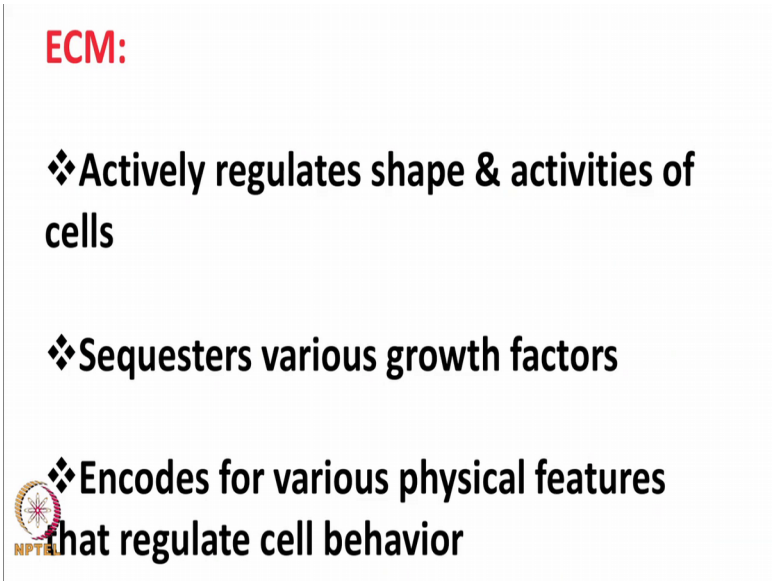

So, as I said that growth facts some of these growth factors are actually cross linked into the matrix. So, you need signals by other cells to actually cleave the growth factors and release them and then activate them. And ECM encodes for various physical features that regulate cell behavior. So, that brings us to the question how does the ECM come into play to begin with, and it is perhaps not surprisingly that cell secrete their own ECM fibroblast for example, in connective tissue are one of the main cells which secrete lot of collagen ok.

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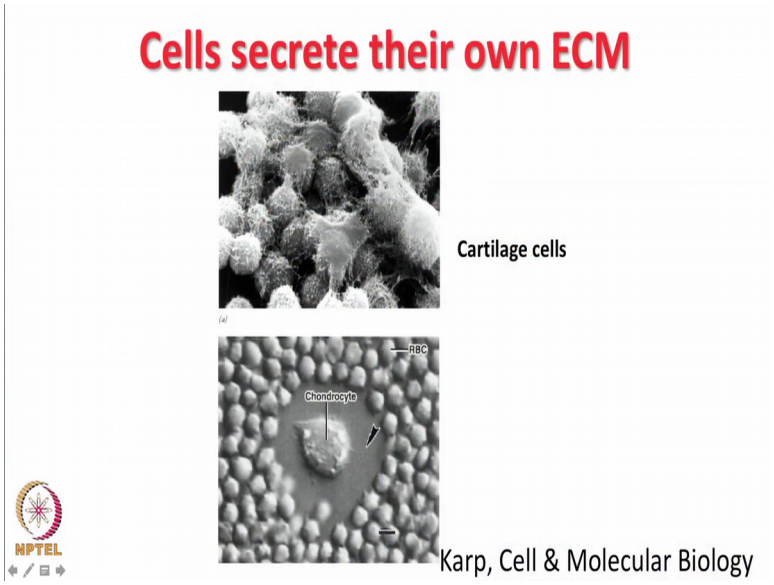

So, these are just two schematics of the matrix secreted by the cells. So, in this case this is a matrix recruited by the cartilage cells and it binds all the cells together into this through this mat which is woven by the cells themselves.

This is another example for chondrocyte and the matrix secreted by it. What you see around it are red blood cells which cannot get inside the matrix, which is secreted by the chondrocyte it suggests that the matrix is so dense, that the pore size does not limit or can restrict entry of other cells. So, such a matrix might also provide resistance for cells to migrate through as you will see later. So, if you look at this particular picture of the extracellular matrix, what you find is there are. So, if I were to take each fiber and look how it is aligned, what I would find is a distribution which is uniform, which means that there is likelihood of fibers existing in multiple different alignments without any preferential direction ok.

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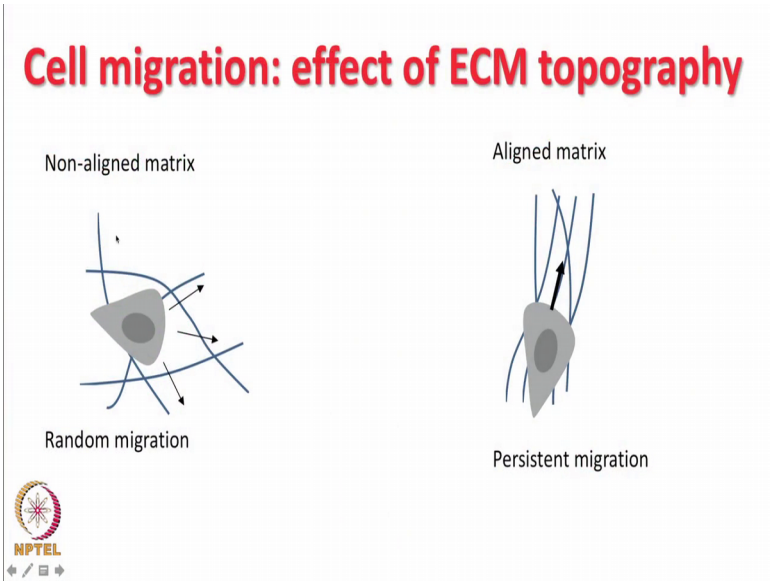

So, you can have a situation of. So, what I would call a nonaligned or a completely random matrix and it is possible to envision that if you have a cell sitting on this kind of a random matrix, what you would find is the cell has no directional cue from these fibers. So, it has equal propensity to migrate in any direction. So, given the way I have drawn the cell where this edge and the leading edge is towards this, the cell can choose any one of these three directions for migrating. So, there is no directional cue provided by the cell and this is what you would typically envision happen if you look at cells on tissue culture plastic.

So, there is some you know matrix secreted by the cells which gets deposited into the substrate, and the cells can migrate in any direction completely randomly depending on how it is polarized at that in state. However, if you have an if you envision another situation where the matrix fibers are very aligned, then you could see that these cells have a greater put greater tendency to migrate along the direction in which the fibers are aligned. So, what you would get is persistent migration versus random migration. So, how if I were to draw, what would constitute as random migration versus persistent migration?

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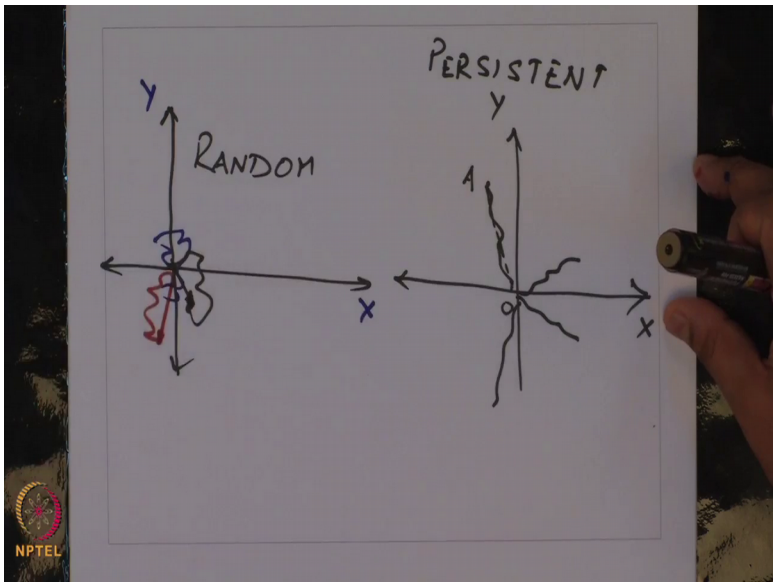

So, let me draw a trajectory. So, if I were to draw the trajectories of individual cells which are migrating randomly. So, this might be one particular trajectory this might be one particular trajectory this might be one particular trajectory. So, what you find. So, this is x this is y. So, what you find is between individual trajectories you might have a situation where up one particular cell has moved a greater distance from the center, while other cells might just be sitting in that position versus if you have for. So, this is random if you had cells where the trajectories looked more like this you would agree with me that this migration is more persistent. Now what might be a metric, for how to quantify whether a migration profile exhibited by a cell is random or persistent. So, they has there can be multiple formalisms think of just this trajectory here.

Let us say O A here and I draw the distance that it travels from starting point to the ending point over a point period of time and let us imagine. So, this is persistent. So, let us imagine that both these are representative trajectories of cells over taken for the same duration in other words let us say that we have tracked the migration of these cells for 12 hours and we got these profiles.

So, one way is to track the net distance that these cells travel over that duration. So, when I say net distance means from the center how far did the cell go. So, you would imagine that in this particular case the cell would migrate to a greater extent compared to this case. But this is not foolproof because even within here you have cases where in case

of this red trajectory this distance might be very good might be reasonably high compared to this case. So, there is lot of heterogeneity in this. So, there has to be some formalism, which has to be used in order to better quantify the type of persistent migration.

So, I will stop here for today, and in next class I will give some discussion as to how we can quantify persistent versus random migration.

Thank you.