Introduction to Mechanobiology
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Week - 01
Lecture - 02
Cell as a Tent, individual Components

Hello, and welcome to our second class on our NPTEL course Introduction to Mechanobiology. So, in the last lecture I gave you a brief overview as what is mechanobiology and some motivation for studying mechanobiology. So, let me first recap our definition of mechanobiology.

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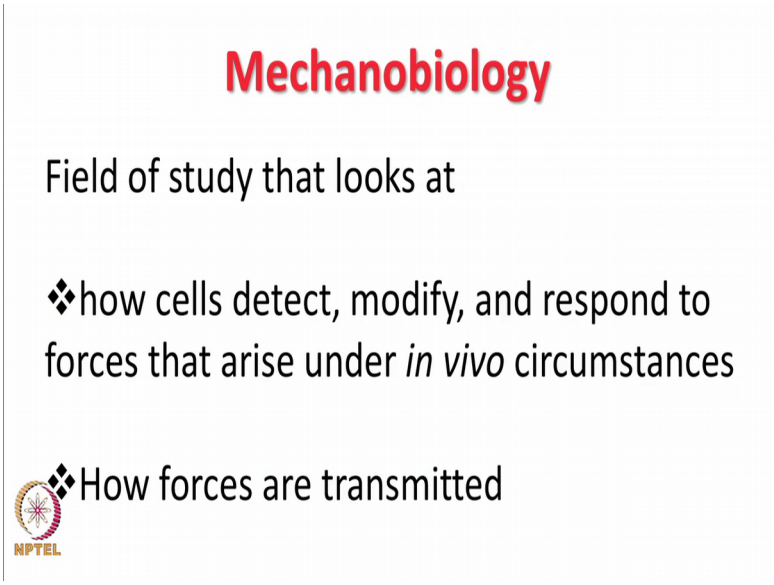

So, field of study where we try to understand how cells detect modify and respond to forces that arise under in vivo circumstances.

The last class I gave an example of the astronauts, who are more susceptible to suffering from bone defects because this stay under zero gravity conditions for long periods. So, in this field of mechanobiology we are interested in understanding how forces get transmitted and how they are integrated for range of cellular processes, and how in the context of various diseases the functioning and the properties of cells get altered ok.

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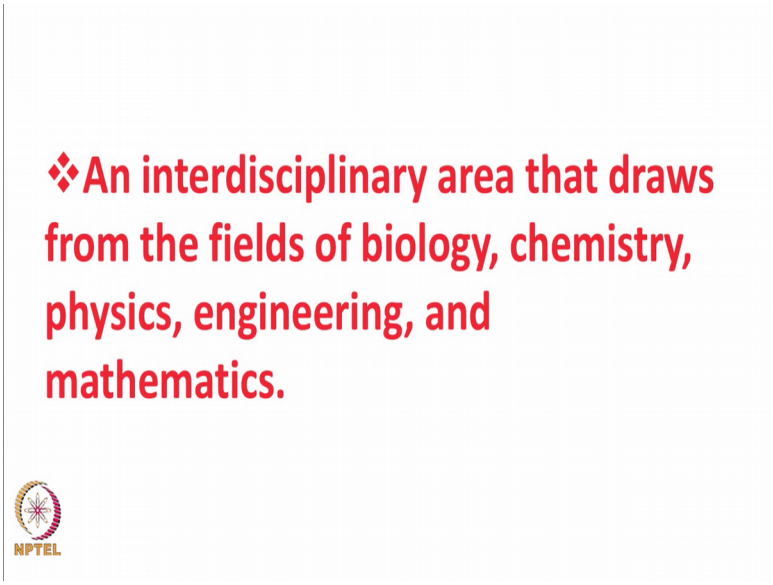

So, mechanobiology it is a interdisciplinary field. So, you have concepts and ideas from biology core biology that of chemistry physics engineering and mathematics which are integrated. So, it is a very rich field. So, no matter from what is a background you can address the same problem of biology from a different perspective depending on what is your core background ok.

So, I had touched upon solve the things about the different cell types which exist within our body and just to reemphasis the fact, the cells comes in different sizes and different shapes. So, of these RBCs are mature or mature RBCs are non-nucleated for the simple reason that they need to transit through small pores in order to supply oxygen ok.

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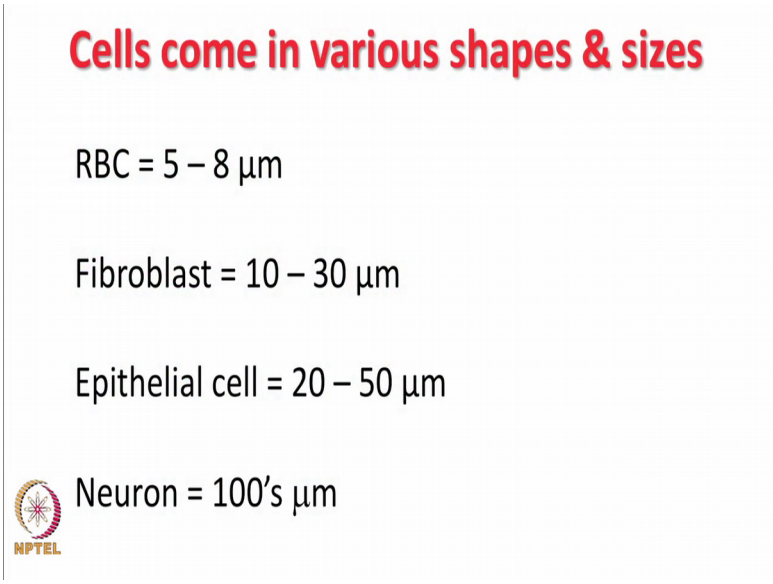

So, having a nucleus the nucleus is get fill like a stone inside itself because it has all the DNA which is forced inside a 5 micron diameter 5 micron size nucleus. So, it is super stiff, and that is the reason why RBCs do not have the nucleus for their function they are continuously transiting through the bloodstream and are exposed to high shear spaces at the same time they must have a mechanism whereby they can deal with the high shear stresses they are subjected to among the other cell types neurons are the longest in length and of course, their job is to transmit or sense information from outside, ok.

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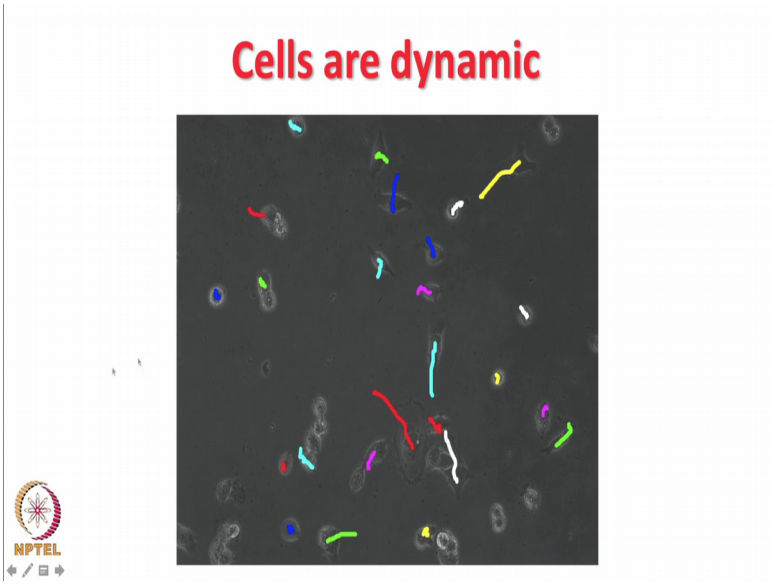

So, I had played this video briefly to tell you that cells are dynamic and this is what you see in a culture dish, so each of these cells. So, in this particular movie that I played the

lines of different colors depict the trajectories of individual cells within the culture dish. So, while you have some points some cells like this blue cell here which did not move much, you had saw these cells like the yellow trajectory here or the red trajectory here, but the cells really moved a lot ok.

So, this is purely under in vitro conditions there is no particular directional queue that has been provided for the cells to move, but it does convey the picture that these cells are intently heterogeneous or maybe it is are there because there are different stages of cell cycle, their intention or in that the tendency to migrate is not the same. So, I also like to emphasize that cells are you know the spread shape of a cell is actively determined by the surroundings in which the cell lies.

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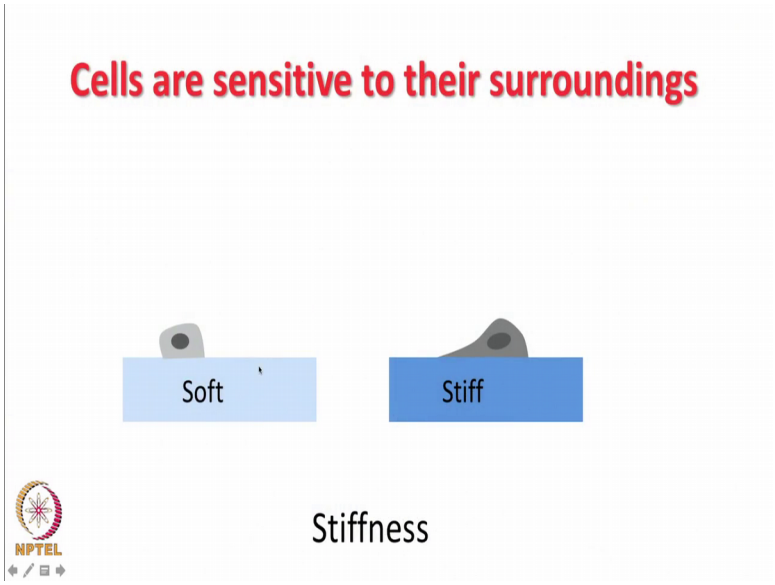

Just an example I have drawn this schematic in which you have the same cell whatever be it placed on a soft substrate versus on a stiff substrate.

So, what do I mean by soft versus stiff. So, each tissue in our body has a distinct mechanical property. So, bones are of course, the stiffest tissues in our body and neuronal tissue or the brain is among the softest tissue in our body. So, what this picture shows is when you plate straight a cell on something very soft or more brain like environment, these cell types tend to have exhibited more rounded or less spread morphology compared to these on a stiff surface, we existed much more spread morphology.

Also the way I drew this schematic it seems that this cell is kind of migrating versus this cell static which is true for a lot of different types of cells, but this ability to differentiate between a soft and a stiff substrate also depends on the nature of cell that you are talking about. So, for example, within our body if you think of cardiomyocytes, whose job is to exert a lot of forces they might be able to spread much more on a stiff surface compared to a cell which does not have cannot sense this difference.

So, that also brings us to the question that how does the cell know what is the property of it is surroundings, and what has been shown long back from this seminal paper where fibroblasts were plated on silicon substrates.

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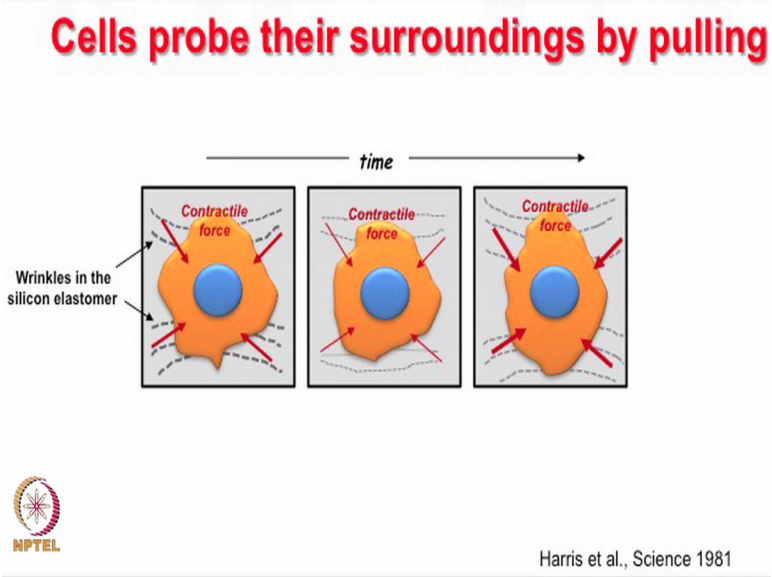

So, what you see as a function of time this fibroblast is sensing the substrate by actually pulling on it, you can understand it pulls on it because it induces wrinkles on the substrate.

So, these come and go in this case these wrinkles are again vanished nearly vanished and completely vanished, but again they reappear. Showing that the cell is actively probing it is surroundings by pulling and this is what enables the cells to sense what is it in surroundings. Now this is a bright field or a phase contrast image how do we know what is what are the molecular players that participate in this sensing process or what are any kind of dynamics within the cell.

For that you have to use techniques like fluorescence microscopy in which your protein of interest might be tagged with a fluorophore, which you can watch under an inverted microscope.

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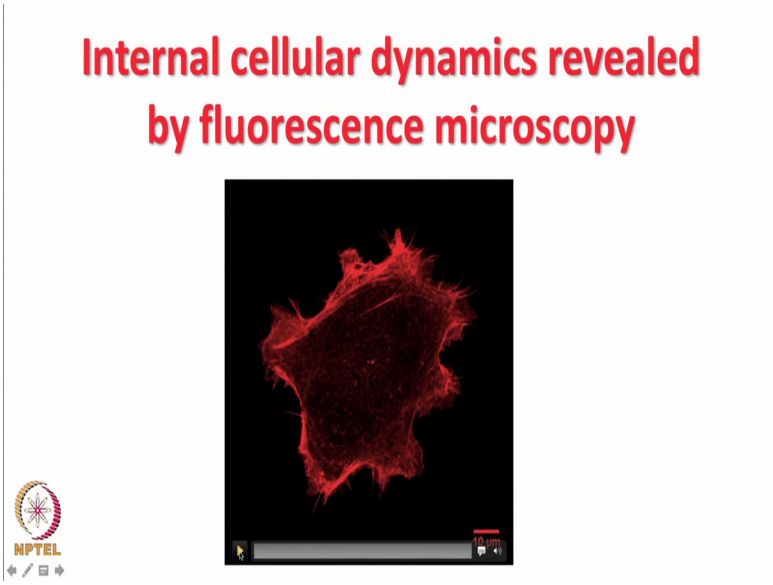

So, this is a sample picture this is a sample video again let me play it. So, this is the sample picture movie of a cell transfected with gfb life act. Life act is life act is a probe which probes actin dynamics it binds to actin and they are put traps actin dynamics let me replay this movie ok.

What is particularly focus on this this zone you see the cell. So, there is lot of intensity here and then eventually this portion of the cell is collapsing. So, this shows you that the cells were perhaps exerting some contractile forces here, let me play ones this movie once more there is lot of intensity of life act here suggesting some polymerization event and then some retraction this is a retraction event which you see.

So, overall if you look think of the cells, the centroid of the cell does not change much, but that still does not make the cell completely static, but the cell is continuously doing. So, dynamics is continuously going on. Even here you see some activity happening, but at least for this movie apart from some of these points most of the cell center remains in place ok.

So, now, I would like to make an analogy as to if from a mechanical standpoint how do I represent a cell. So, I would like to imagine the cell as a tent.

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So imagine this tent which is firmly secured in this ground. If I want to imagine the cell as a tent what mechanisms are: what are the requirements for ensuring that the cell is stable or it is stabling positioned in the ground. So, what are my minimum requirements so, what you see?

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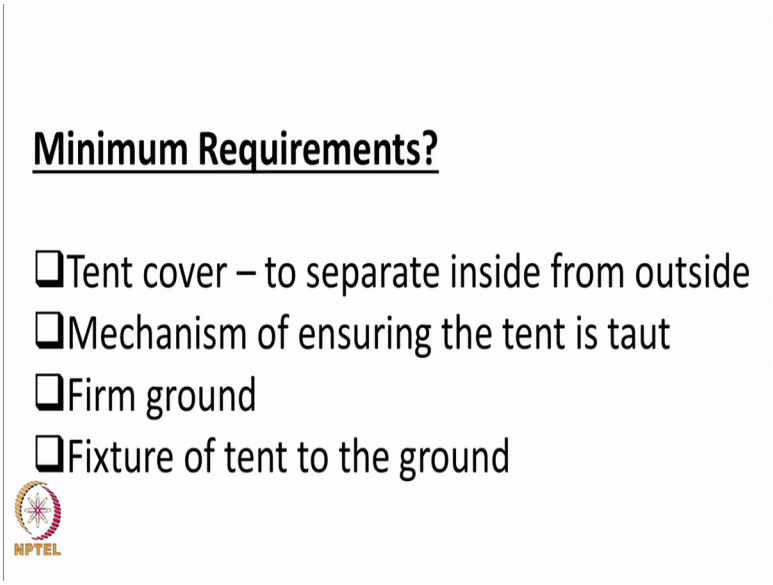

So, you need a tent cover this this tent cover or the canopy the biological equivalent. So, you want a tent cover to separate the inside from the outside, you have to have a mechanism of ensuring that the tent is stopped. If the tent is not firmly secured to the ground then any wind if there is more amount of wind than the tent will collapse and of course, this has to be firmed ground ok.

So, you cannot put this tent on water or something where this tension required for making sure the tent is taut cannot be sustained and of course, you need to fix the tent to the ground. So, what are the biological equivalents of these four entities that we start from the plasma membrane?

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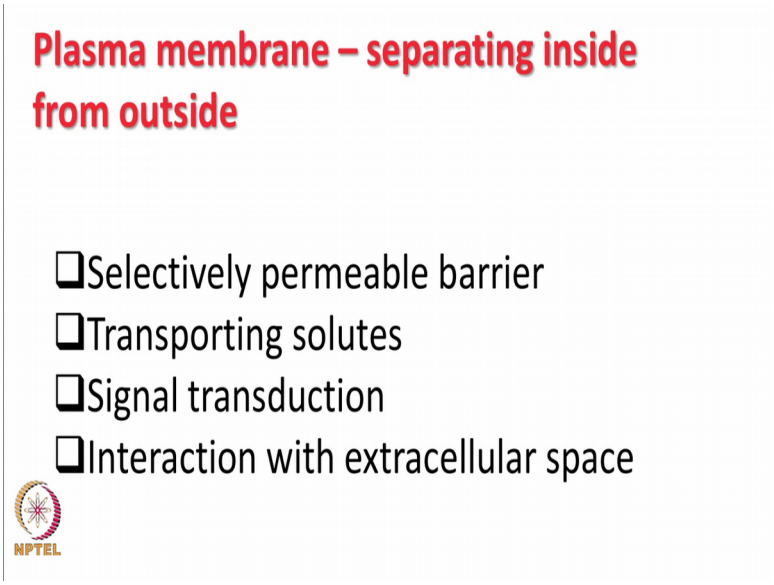

So, the plasma membrane is that which prevents or separates the inside of the cell from the outside.

So, it acts as a selectively permeable barrier. So, it can transport solutes and within the plaso if I want to draw a plasma membrane you have lipid bilayers and within this lipid bilayers there are some proteins which are sequestered you have these proteins. So, this is a protein here what I have drawn and this is my lipid bilayer ok

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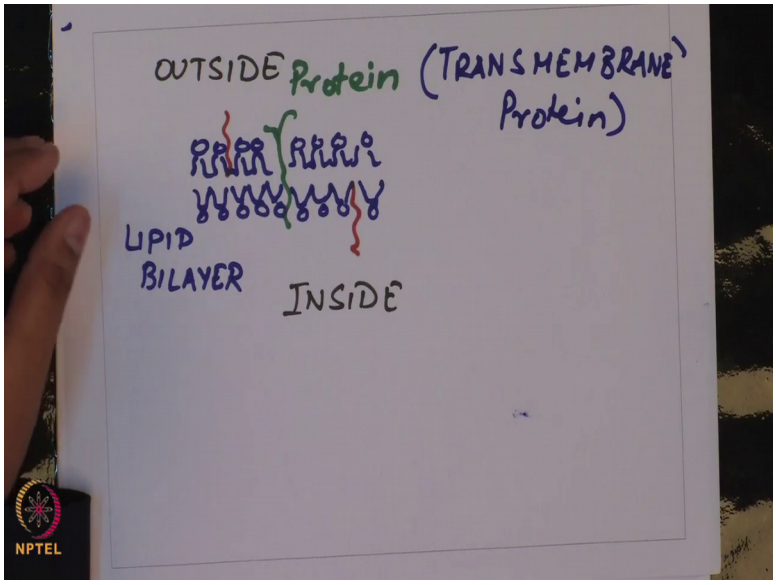

So, there might be some proteins. So, what you see I have drawn three different protein one green protein which spans the entire lipid bilayer. So, this is my outside and this is the inside. So, there are some proteins which span the entire lipid membrane and these are called transmembrane proteins. So, this is firm of a transmembrane protein, but you can have proteins which are either sequestered to the inner leaf of the lipid bilayer or the outer leaf of the bilayer ok.

So, these transmembrane proteins are the once which actually talk or interact with the extracellular space. So, the extracellular spaces have might have other proteins or molecules which are secreted proteins which are secreted by other cells, and they are freely diffusing in the extracellular space. The second entity is the cytoskeleton the cytoskeleton is essentially the cells framework and this is what make sure that the cell has a concrete shape and it is not like some silly putty, ok.

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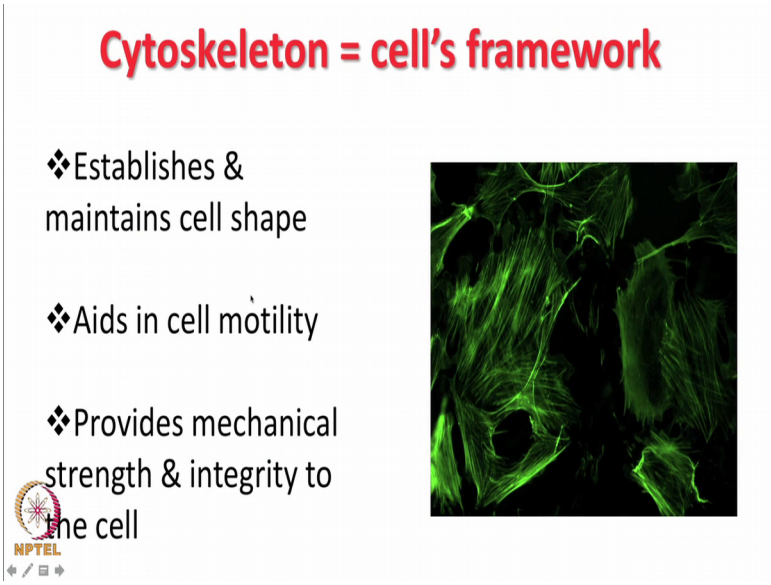

So, the cytoskeleton establishes and maintains the cell shape it aids in cell motility. So, in the case of phagocytosis the forward motion which is driven by forward motion of the macrophage or the neutrophil is driven by actin based polymerization forces which pushes that in the membrane, it also provides mechanical strength and integrity to the cell.

So, in the case of any external force on the cell the cell can resist those forces. So, you can clearly see. So, this is an image of mouse embryonic fibroblast which have been stained with phalloidin, phalloidin is an agent which binds to f actin or filamentous actin. So, these green lines what you see are filamentous actin. So, you can see the fact that these lines are all straight suggest that these filaments are under tension ok.

But these filaments; so this is a snapshot suggesting that these are all very fixed in space, but in order to move these filaments, but continuously assemble and disassemble. So, there is dynamics to it. So, actin is only one of the cytoskeletal proteins you also have microtubules, one of the most important things which.

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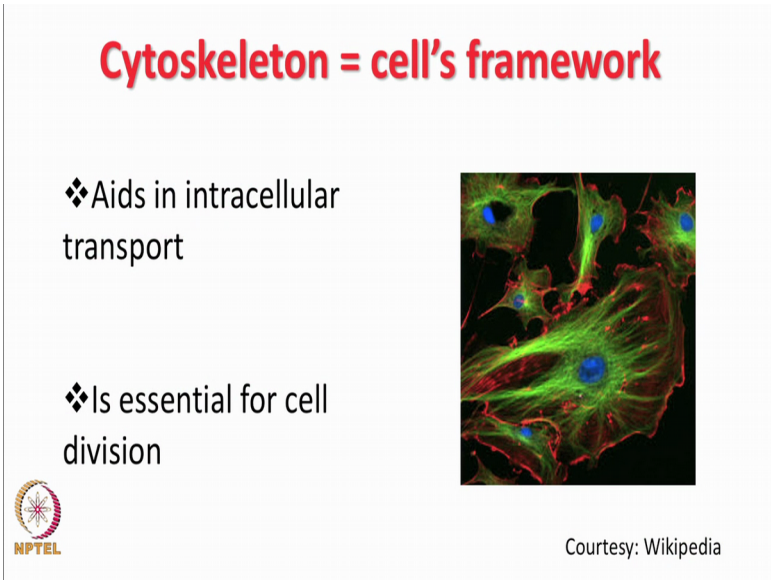

So, in this picture you have the microtubules which are spanning from the nucleus outside the nucleus and spanning till the periphery of the cell. So, the microtubule is the one which aids in intra cellular transport and is a key protein for cell division. Most of anti cancer drugs target cancer cells by perturbing or playing with the microtubule dynamics within the cell. So, cytoskeleton is the cells framework which may make sure is the mechanism which ensures that the cell is taut.

Now, you need a farm ground and what is that firm ground this is called the extracellular matrix. So, it is the structural support which is necessary for cells to be scaffolded to form tissues and organs it hydrates. So, it acts as a storage for water it supports cell addition and it is sequestered various chemical engines.

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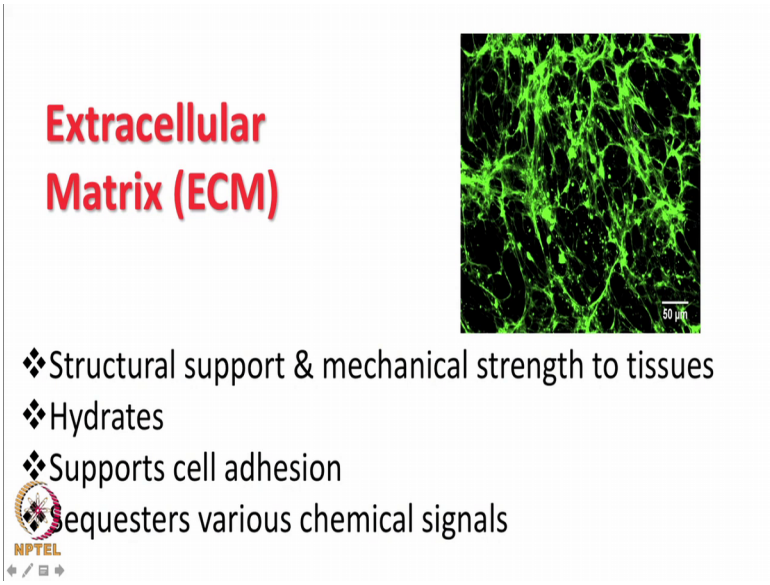

So, this is an example this this is an example of the extracellular matrix which is secreted by mouse embryonic fibroblast, you see the fibrillar nature of this protein.

So, this has been stained with a dye called ester which will bind to all proteins which are secreted. So, there are more than multiple extracellular matrix proteins which are presumably present within this matrix that is secreted, but this matrix is actually integrated to form a network, which supports the tissues structure and finally, you have focal additions or dynamic wells.

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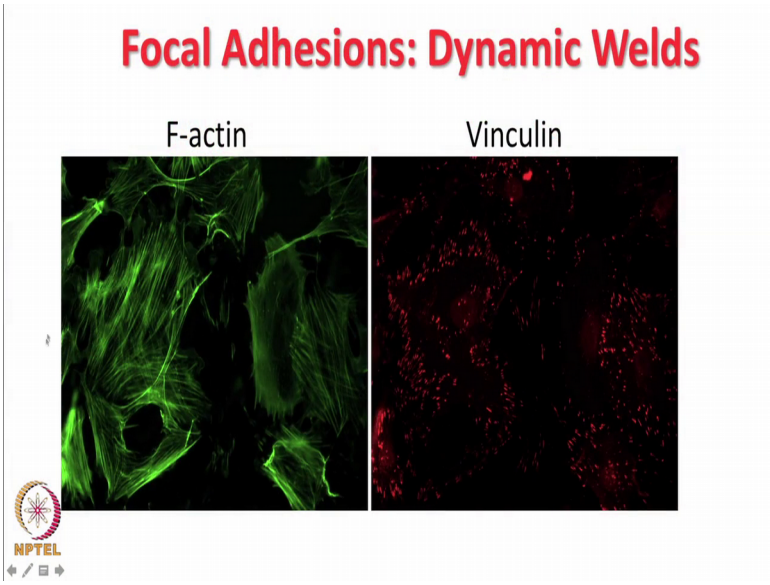

So, this is a picture I had earlier shown of cells which stained for a F actin. So, underneath each cell you have these red dots. So, these are the adhesions or the positions at which the cell is physically connected to the substrate or the extracellular matrix. So, again once again for the cell to be dynamic these adhesions are also dynamic in a way that they constantly turn over.

So, after the initial introduction we will start discussing details of the properties of the accessory matrix properties of this vocalization so on and so forth. So, if I want to ask the question. So, for us at the whole organismic level we have so many different sensory organs we can touch, we can feel, we can see, we can we can breathe so on and so forth.

What is it that the how does the cell react to events in it is surrounding in vivo and the answer to that lies in this process of mechanotransduction where it converts mechanical stimuli to biochemical signals.

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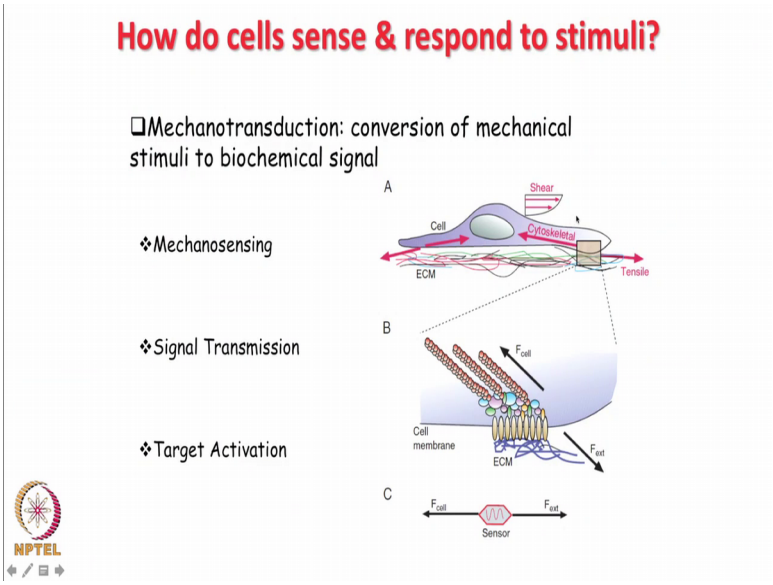

So, there are three parts of this one is the sensing part, one is the transmission part and the target activation part. So, what this schematic shows is this extracellular matrix on which the cell is sitting you have at these proteins where there are the physically linked to the cell is physically linked to the outside.

Cell actually exert forces which are contractile in nature, the same forces which are fibroblast was exerting on a silicon substrate, and this is how this signals get transmitted

all the way to the nucleus and that dictates transcription and so on and so forth. So, all this process could also have happened not just to this mechanical route, but through passive diffusion of molecules which bind to membrane proteins at the periphery and then come in and reach the nucleus to direct cell signaling pathways.

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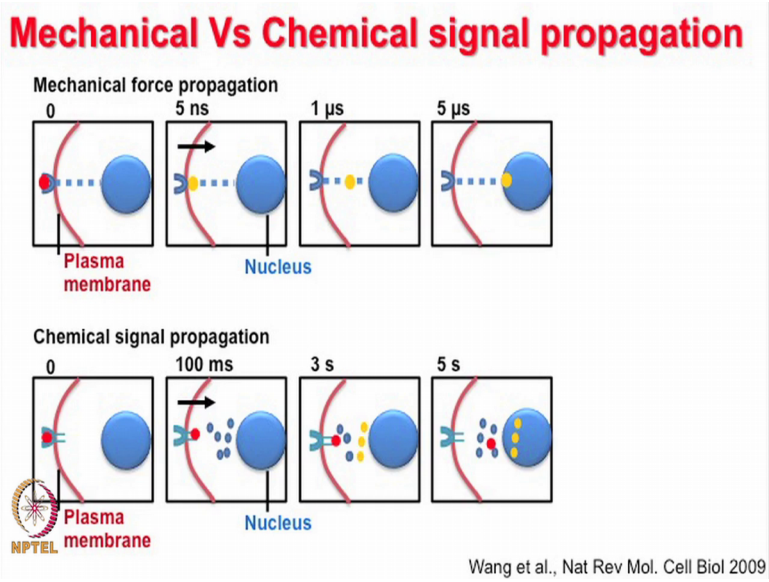

That is possible of course but what it turns out that compared to the mechanical force propagation time scales which in the order of microseconds. So, this is a picture shows schematic shows that if you had a physical linkage between the outside of the cell to the inside of the cell, then any signal from the outside can get propagated all the way to the nucleus in as little as 5 seconds 5 microseconds. In contrast to the chemical signal propagation where something binds is internalized and it diffuses all the way to the nucleus which can take several seconds.

So, this is three orders mechanical force propagation is three orders of magnitude faster than chemical signal propagation ok.

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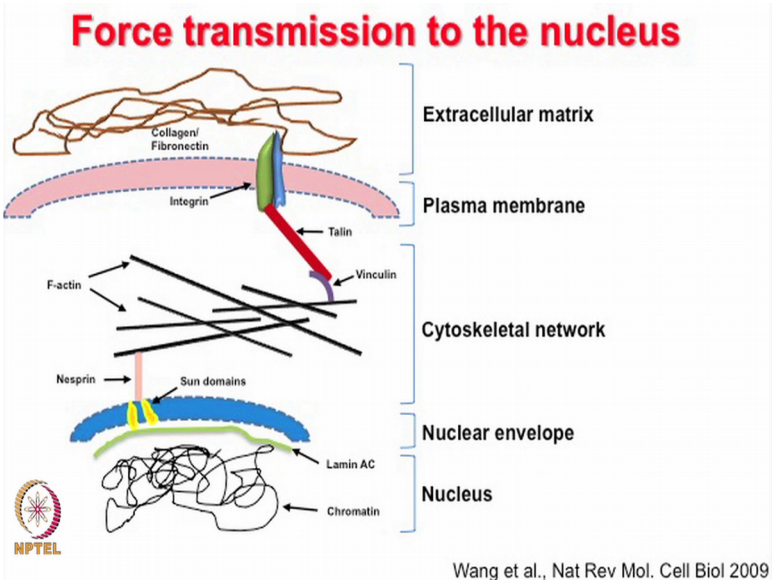

And this is made possible by physical linkages between the outside and the inside. So, this is the nucleus and you have linkages through the actin network through intermediate filaments so on and so forth, which links signals to transmembrane proteins like integrins there are other proteins as well like dystroglycan complexes through which signal is relayed all the way to the nucleus this is a system of structures, which are dynamic which relay information from the outside to the inside ok.

So, let us discuss some examples of what happens when you expose cells to some kind of forces and the simplest case I have the case of osmotic swelling.

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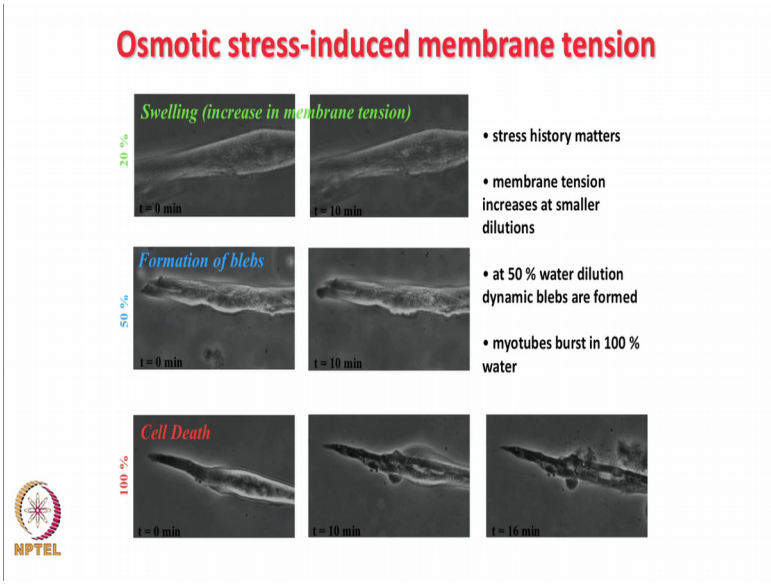

So, what you are looking at here is a muscle cell actually of myo tube it is a in vitro myo tube, which is exposed to water. So, on when you have 20 percent water in this whole thing, what you can see is the membrane of the cell does not exhibit any obvious changes in it is phenotype or in it is morphology; however, when the osmotic stress keeps on increasing you see the presence of these structures at the corner these are called blebs and eventually if you put the cell in 100 percent water, it cannot balance osmotic forces eventually the cell will burst ok.

So, these are examples of how an osmotic shock leads to alterations in the phenotype of the cells.

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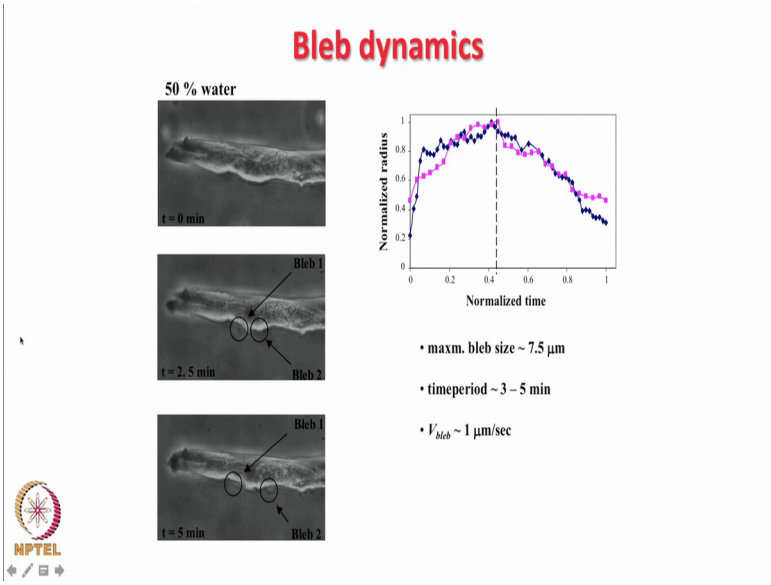

So for the case of 50 percent water as I said so, you can look at these individual structures or blebs, what you see is each of these blebs can actually traverse on the surface and they have a characteristic time scale in which the first they grow bigger and bigger and eventually they are again pulled back into the cell.

So, you have blebs size in this particular example is of the order of 7 to 10 microns and they have a time period of 3 to 5 minutes over which they exist and of with a velocity of the bleb order one micron per second. So, this shows you now. So, there must be a way in which for 50 percent case in which these blebs are getting formed and the blebs. So, they are growing in size and then they are again reducing in size suggesting that there are some self-regulations.

So, what is regulating the self; that means, there must be some sensor which detects this increase in membrane tension and accumulates there and if you see this is an example of a protein called gamma sarcoglycan what you see is wherever there is a bleb.

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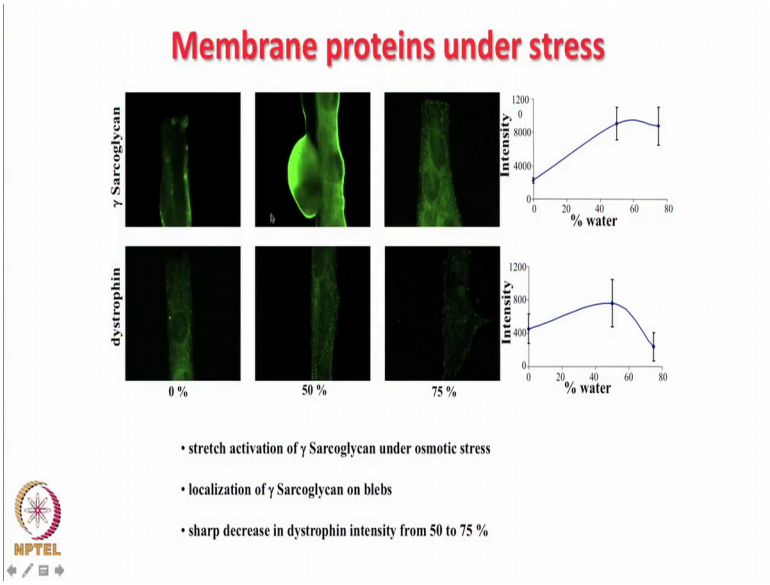

There is an increased localization of this particular protein called gamma sarcoglycan. So, you can track this dynamics first of all you can also track how in response to increase in membrane tension, this protein accumulates at the location site of the bleb and this intensity figure so, you can track the average intensity of this protein at the bleb this average fluorescence intensity as a function of the percentage of water, and what you see is intensity increases and saturates, suggesting that this is one such mechano sensor molecule. In contrast we have this other protein called dystrophin which kind of drugs when there is increased amount of water added ok.

So, this is an example showing that there are different proteins which either sends certain specific cues in response to the force that you exert.

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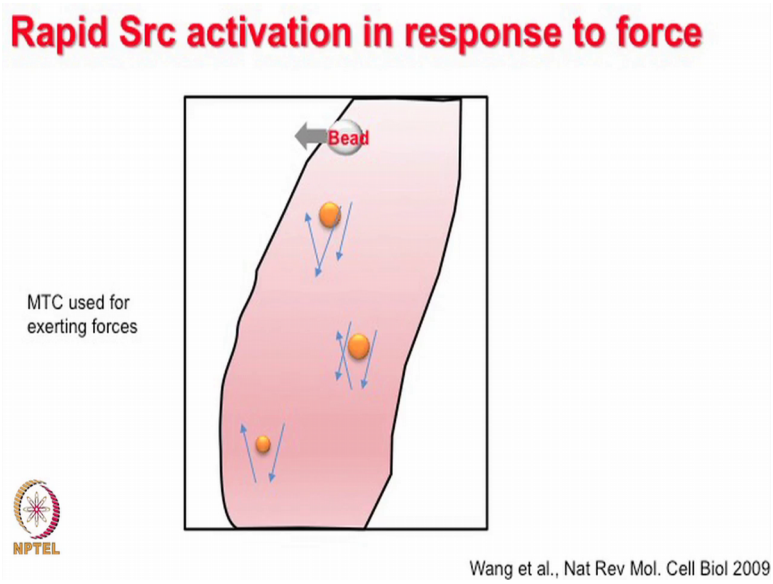

This is another example in which what has been done in this experimental setup is a bead has been physically linked on top of a surface of the cell by coating the bead with some extrasellar matrix protein and allowing it to settle on the cells, and when you exert forces on this bead what you see is this localization of this protein called SRC as specific positions which are far away from the location at which you have exerted the force ok.

So, this is just an example showing that this activation is very fast and it need not be at the side of the exhaustion point at which you exert in the force, it might get activated much further. So, this also brings us one point that there are different mechano responsive elements within the cell and there are diverse timescale associated with each of them.

So, I have just jotted down three different structures called focal adhesions podosomes and invadopodia. So, these are different structures they are not all present in all cells. So, adhesions are of course, present in all adherence cells again they are dynamic depending on the average motility of the cell, the focal adhesions might grow or the turnover focal adhesions might vary, but they are the ones which link the cell with the substrate.

But you have also these structures called podosomes which are present a lot in macrophage type of cells which are fast moving and what podosomes do that they actually degrade the vitrics. So, not only to the form adhesions focal adhesions can form adhesions, but with podosomes you can selectively degrade or remodel the matrix similar

function is also played by this structure called invadopodia. So, this shows you the different structures respond in different ways depending on what their function is within the in vitro context ok.

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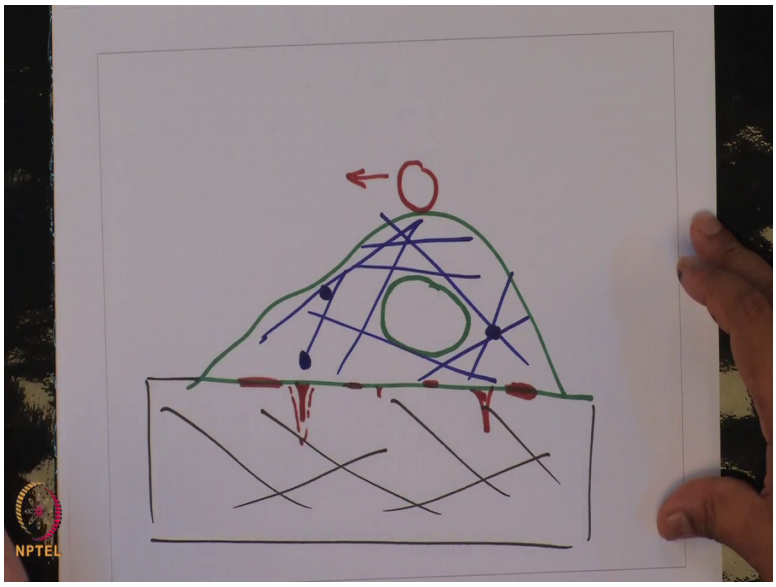

So, if I want to draw a cell on a substrate this is the nucleus and let me draw. So, I can depict adhesions like this and I can depict the actin network. So, what I have drawn here is the actin network which connects or which scaffolds the nucleus around it. So, when you exert a force here let us say the ball, you exert a force here then these the cytoskeletal actually links transmits the forces from this in outside to inside at different positions.

So, the localization or the selective enrichment of proteins at different sites as we saw earlier, might happen at different points which are far away from the site and these structures so, in contrast to focal adhesions if you look at invadopodia. So, they actually protrude into the matrix. So, I should probably draw the matrix this is your matrix.

So, these structures actually protrude into the matrix, invadopodia actually protrude into the matrix and degrade the environment locally. So, an invadopodia it is possible and for an invadopodia to grow in size or to disappear altogether. So, if you have small invadopodia they might form and disappear. So, there are different time scales associated with it depending on the rate at which the cell is migrating.

With that, I thank you for your attention I look forward to discussing things next class.