Introduction to Mechanobiology
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Week - 01
Lecture - 04
ECM proteins: Collagen

Hello and welcome to our 4th NPTEL lecture on Introduction to Mechanobiology. So, in the last lecture, I started discussing about the Extracellular Matrix right the form ground, which is required for stabilizing cell shape.

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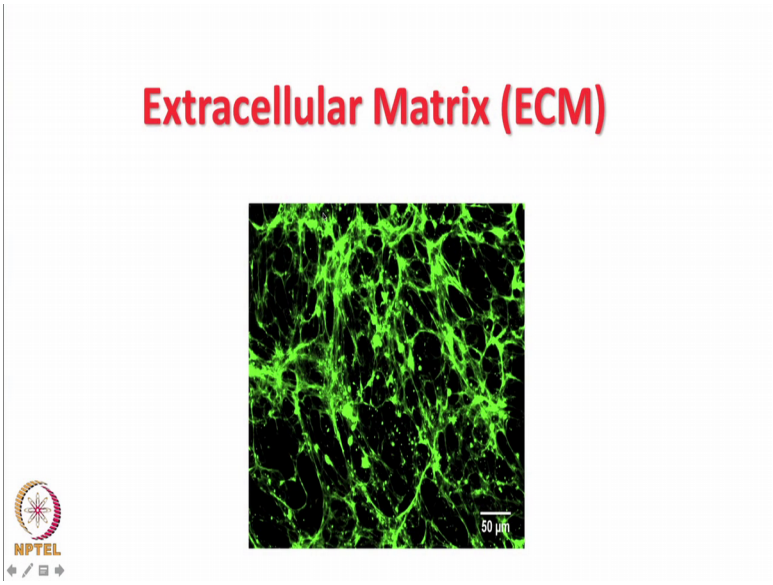

So, this shows the Matrix, which is secreted by 5 blasts. And what you find is so this of course has multiple different ECM proteins. But what you find is it has multiple features in it. It can have zones where, which are devoid of this any Matrix. So, this would amount to having a pore and this pore size is not uniform. But at the same time what you also have is these matrix fibers have different orientations.

So, what you can do is quantitatively determine: what is the average angle of orientation of these matrices. And not just this depending on the concentration of these proteins, which is the amount of this green signal in this whole area, that would be equate that can be equated to the average density of this matrix; so all of this are supposed to influence cell behavior.

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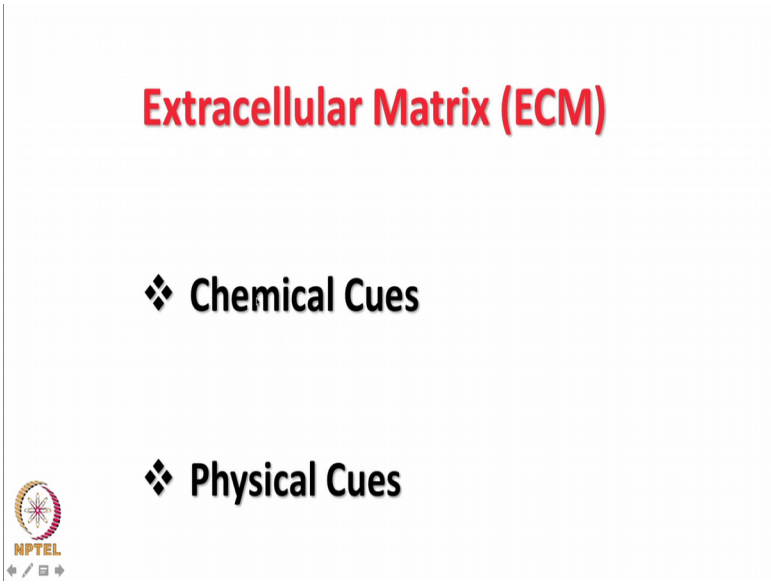

You can divide the entire gamut of cues that the ECM provides to sell in divide into Chemical Cues or Physical Cues.

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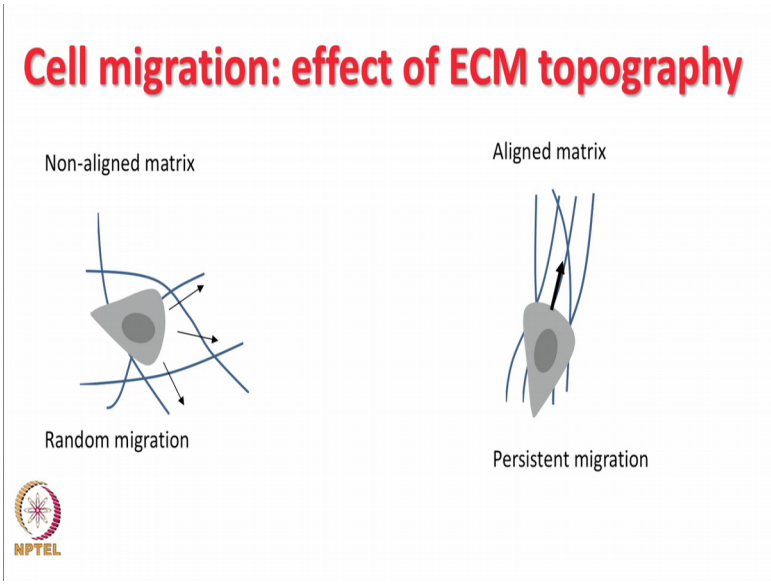

In last class, we had started discussing about effect of topography. What I mean by topography is the relative orientation of these ECM fibers. And what you see here is a cell migrating on a Non-aligned Matrix in which it is likely that it has in the absence of any other directional cue, the cell is going to exhibit Random migration. Versus in this

matrix these fibers provide some directional cue as a consequence of which the cell tends to migrate along one particular line.

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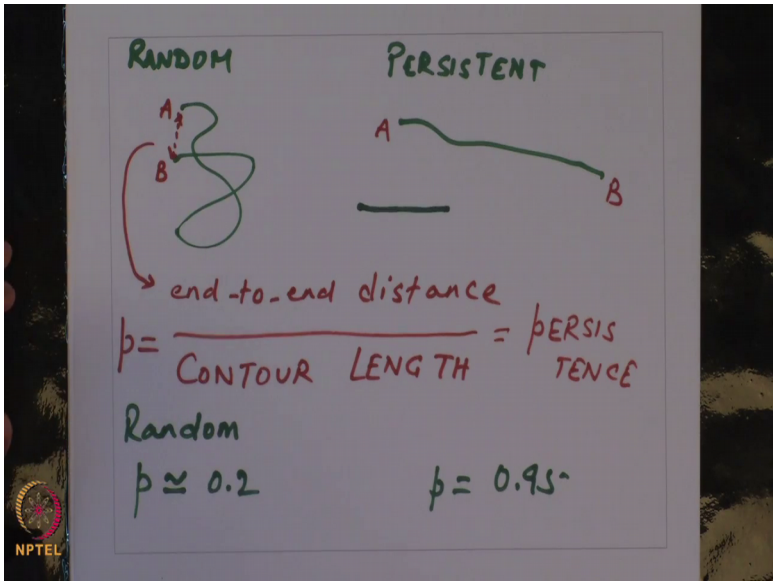

So, how do we quantify this mathematically? So, you can think about it as this trajectory being random this is a random trajectory versus this being more persistent.

How can you mathematically quantify this? One easy way to do is to take the endpoints. Let us say this is the starting point A and this is the ending point B. Similarly this is the starting point A this is the ending point B for this trajectory. You divide this distance A B. So, this is called the end to end distance. End to end distance and what you divide is by the contour length. You can find out this ratio of end to end distance by contour length. And in this case the total distance or the contour length is significantly higher compared to the end to end distance. So, your ratio this if I call this P we defined as some metric of persistence.

What you will have is for random you will get P let us say approximate let us say equal to 0. 2 versus in this case P will be 0. 95. We have a significantly greater value of P in case of persistent migration compared to P in case of random migration. And this is obvious so the highest possible value of P can be 1, because maximum you can have is a complete straight line in this case the contour length and the end to end distance are exactly the same.

You have a ratio you have defined the ratio, which is bounded between 0 and 1. This is one of the simplest ways to quantify persistence. But what you can also do is; so if I were to look at this particular trajectory I can break it into these individual segments. Let we redraw it. So, let us imagine that this is.

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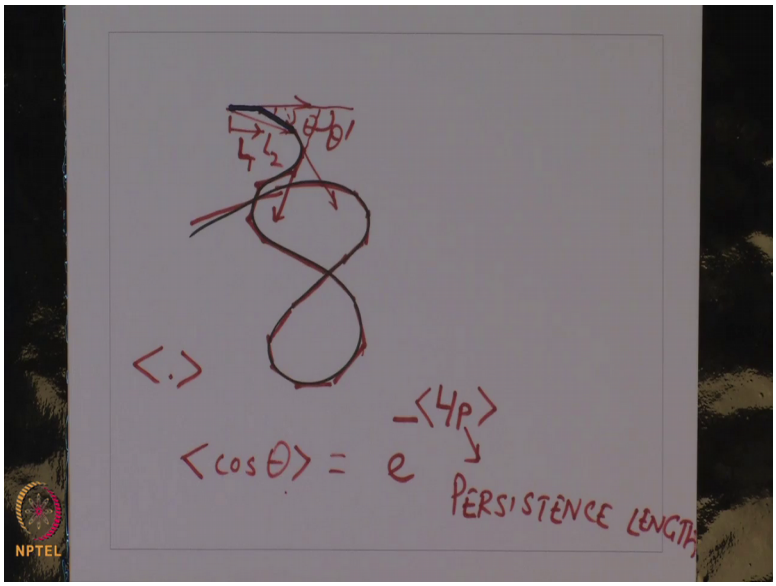

This is a trajectory of a cell. What I can do is I can break it into short segments, which are straight maybe it is better to show it with red I can bring it into short segments which are red.

What you saw is I have broken this entire trajectory to short straight segments. So, essentially what I can find out is between any 2 lines let say this and a distance d apart and some distance apart I can find out what is this angle theta. I can find out similarly between this I can find out the angle between this trajectory here, I will have some theta prime. So, you can arrive at an equation call average of cos of theta is equal to e to the power minus L by P, average of cos of theta is equal to minus e to the power L of P.

So, this bracket is called an average operator. So, you do this for let us say these 2 trajectories you can find out the angle. So, here the distance between these 2 trajectories is just this t L. So, this is L1 between these 2 this is L2 so on and so forth you can do. You can average it out and find out this P which is the persistence length. So, this would be a very nice way of characterizing the persistence of the directionality of individual

fibers, what is also known is this persistence length is dictated by the stiffness of the spring.

In other words if you think of this pen here this is completely straight, which means it is reasonably stiff. If you take a single strand of spaghetti uncooked spaghetti it is completely straight, but the same spaghetti when cooked would kind of have this kind of morphology. So, this persistent length is directly is dictated by the amount of bending rigidity how easy is it to bend. It is possible to obtain the bending rigidity from these persistent and measurements.

So, what would happen? If you plate a cell as I said that if you plate these cells on these aligned metrics it is there is greater chance of the cell will migrate along this.

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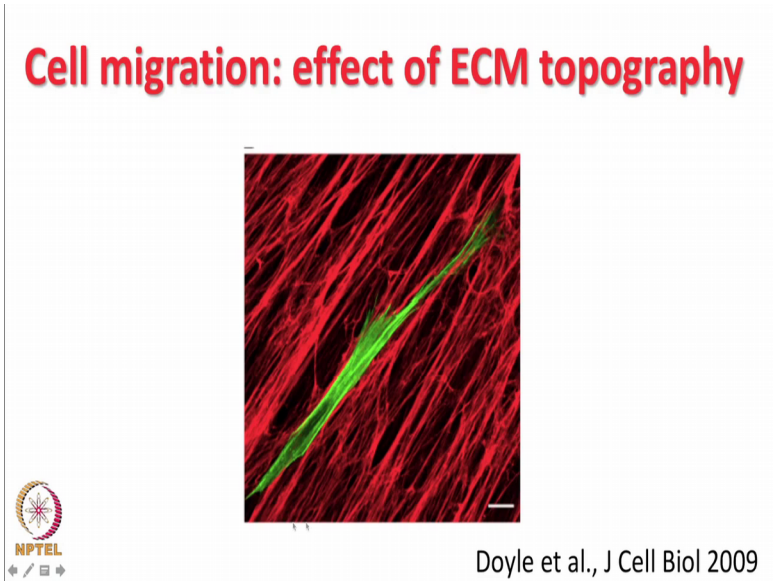

And this is made clear by this picture where you have the red depicts the matrix which you see is broadly aligned in one particular direction, and this in green shows the cell. So, what you see is the cell almost longest along the long axis of the fiber taking the fiber as a topography it can sense the underlying topography provided by the matrix it aligns along these and walks along the fiber.

So, it is the same reason that when you take a chunk of wood it is easy to cut along the grain as opposed to perpendicular to the grain. When all the fibers are aligned you can easily cut through the fibers. So, this is one case where the ECM directly influences cell

migration, but you can also have situations where cells can degrade the matrix underneath.

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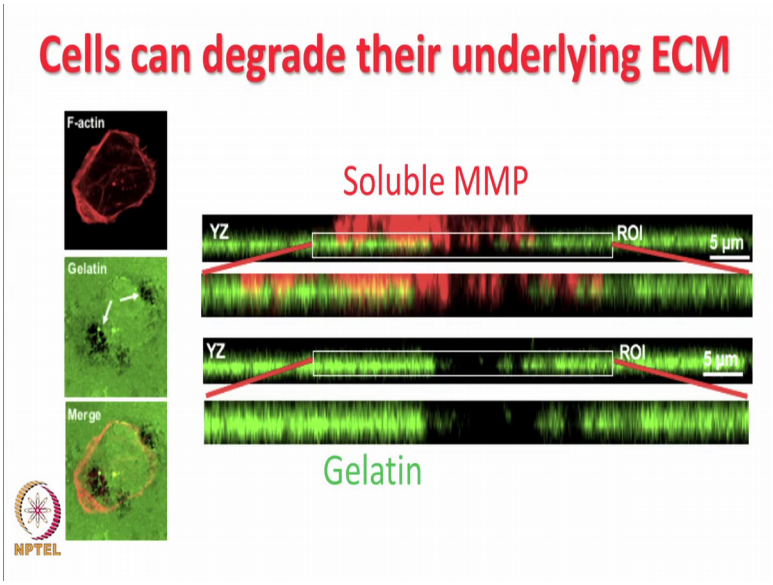

So, this is an experiment in which cells have been plated these are cancer cells, which have been plated on gelatin coated dishes. So, gelatinous fluorescently labeled.

What you see is. So, this is a 2 d. So, the gelatin is only like a layer it is a coating you can it has been fluorescently labeled and what you have done is we have plated cells this is stained for f actin on top of these matrices. What you see is underneath the cells there are these zones, which has is present as black patches, which are absent and which are present right underneath the cell. So, these are zones which have been locally degraded by the cell this amount of degradation is very clear from confocal z stack images.

What you see here in green is the layer of gelatin and what you see in red are actually parts of MMP's which are secreted by cells. So, MMP's stands for matrix metalloproteinase, these MMP's and actually colocalizing with the empty green space. So, where the green space is lacking the green signal is lacking you see that there are these red dots.

Actually these are proteases which degrade the matrix. So, cells can why is degradation necessary; degradation is necessary not just in the normal case where cells continuously remodel or remake them reorganize the matrix, but this degradation is essential for

cancer cells in which cells spread from one position in the body to another position in the body.

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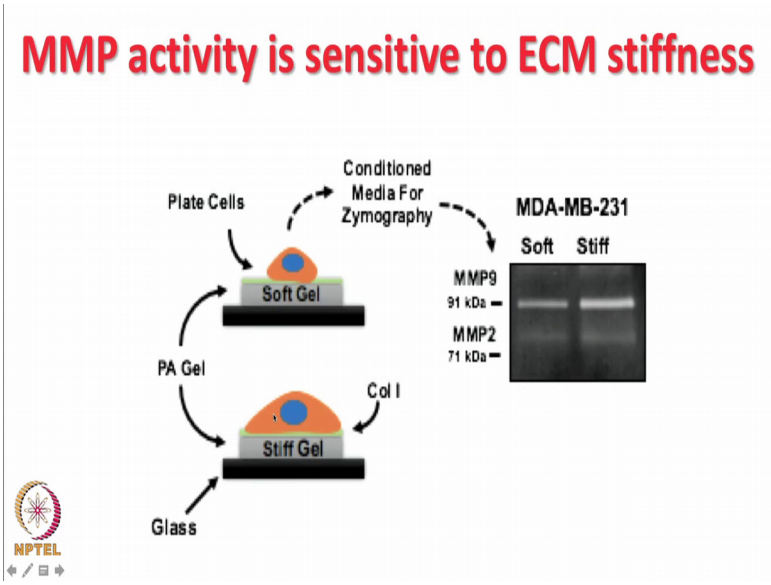

What is also interesting is that this secretion; this secretion of MMP is dictated by the passive stiffness of the substrate. This is an experiment in which these cancer cells have been plated on soft polyacrylamide hydrogels or on stiff polyacrylamide hydrogels. In both these cases the gels are coated with collagen one which this is a one ECM protein which is recognized by the cells. And what you can do is in order to sample the amount of degradation or the amount of ability to degrade, you can process the conditioned media and do an experiment called gelatin zymography in which you take the conditioned media you lie flies it you collect the essential you know you make it highly concentrated and you run it on a western blot on a page where gelatin is there.

These proteins within the conditioned media will actually migrate on this gel and then when you activate the gel they actually we degrade the local gelatin. So, as a consequence you will get these white bands. So, what you see in this particular case there are 2 bands corresponding to 91 kilo daltons and a much weaker band at 71 kilo daltons. These correspond to MMP 9 of 91 kilo daltons and MMP 2 of 71 kilo 91 kilo daltons

What you see is in both of these cases the amount, there is a stiffness dependent modulation of this MMP activity. Suggest that the cells not only sense the matrix properties. In this case the stiffness and as accordingly modulated the extent of MMP

secretion that you detect by this activity as it. The other term I used is remodel. So, one way of remodeling can be degrading the fiber as is has been the case in this in these experiments, but you can also have remodeling by actively pulling or deforming the fibrils without degrading them.

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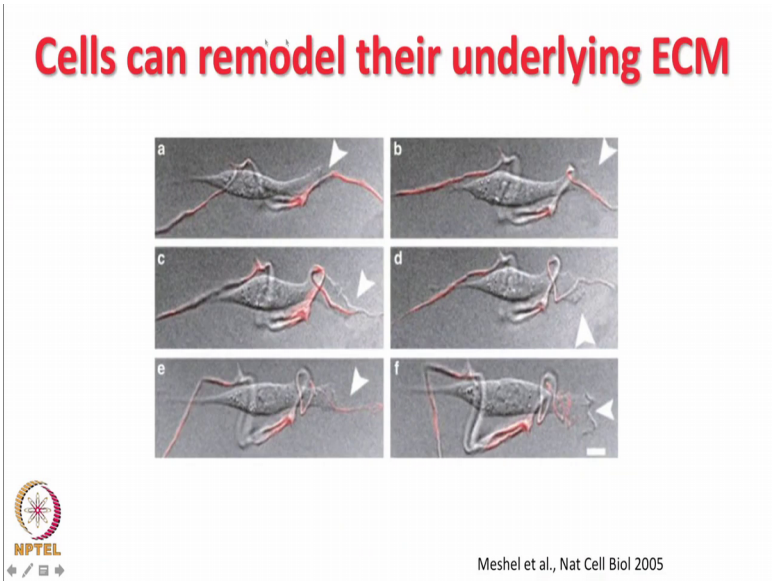

This is an example where a cell is interacting with a single fibril and almost pulling it along it is length. So, this is what you see the fibril is pulled along it is length and it gets deformed. So, cells can remodel by degrading or by physically changing the fibrils. What these examples show is that there is a dynamic crosstalk between cells and extracellular matrix: the cells secrete the matrix they also remodel the matrix.

So, it is not that all types of cells can secrete the matrix. So, cells like fibroblasts are the ones or smooth muscle cells they secrete lots of ECM proteins. At the same time cancer cells in particular can degrade or remodel the ECM.

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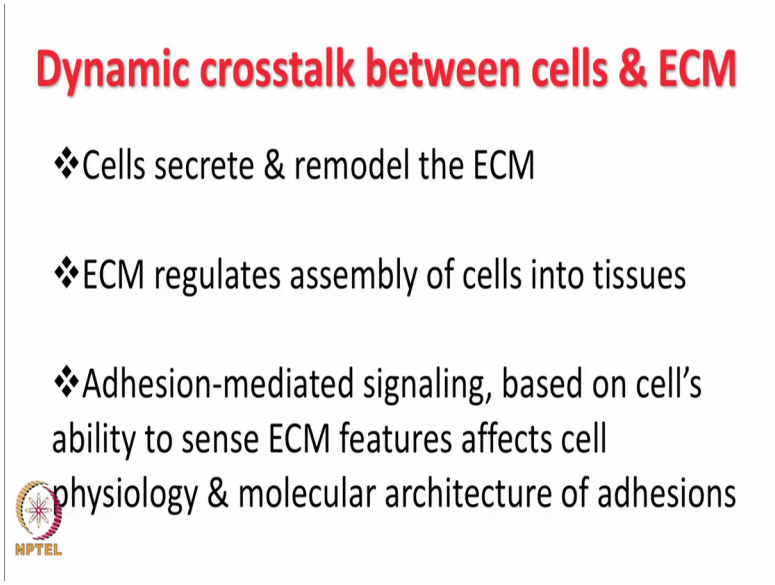

So, you have this dynamic crosstalk in which one makes the matrix and reforms deforms it. I will then now go to Tissue organization in skin in particular. So, if I were to draw how skin tissue is organized. So, you know skin is a regenerating tissue.

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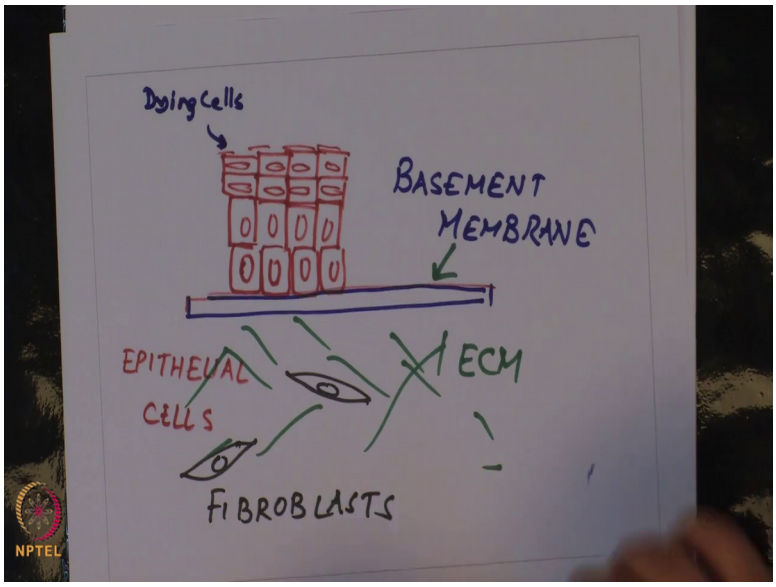

What you have are a layer of cells these are called epithelial cells. What you will find is the way I have drawn this particular epithelial cell. So, you have multiple layers of epithelial cells like this. The way I have drawn the epithelial cells you see the orientation of the nucleus is vertical. So, they lie on this membrane on this membrane and on the

other side you have ECM matrices and also the presence of fibroblasts. So, these are fibroblasts, this is the ECM, and what this entity is in particularly interesting this is called the basement membrane.

So, on outside you have your epithelial cells these continuously divide, and as they move out from the basement layer the orientation of the nuclei go from vertical to horizontal, and eventually at the outside layer these cells are dying. This process continues all the time, where these cells divide push the layer up and at some level the orientation of the nuclei change and then the outside the cells keep dying and this is how we have regeneration. But what separates the extracellular matrix containing the fibroblasts from the epithelial layer is this membrane called basement membrane.

The basement membrane or the basal lamina, it is hardly 50 to 200 nanometers in thickness. And this basement membrane surrounds most cell types or muscle cells, nerve cells, fat cells in lining of blood vessels in epithelial tissues and also in lining of digestion respiratory tracts.

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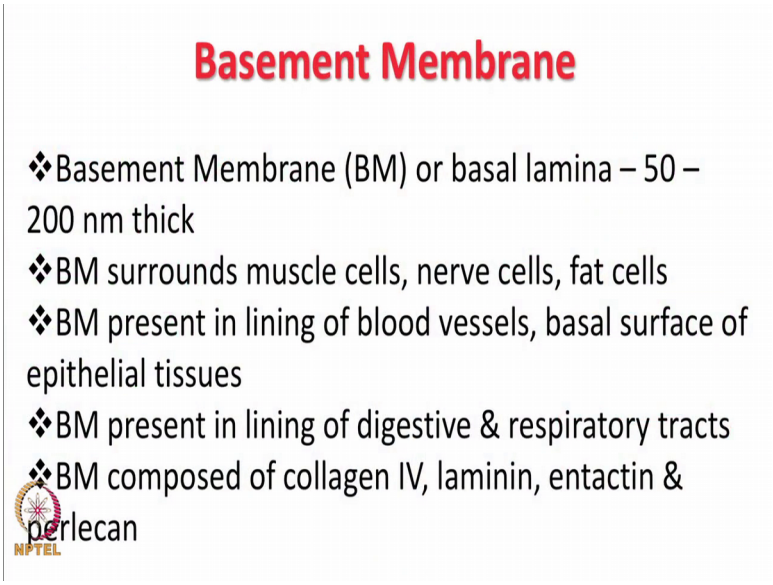

It is composed of multiple ECM proteins and what the basement membrane does it actually separates adjacent tissues within organ. It kind of defines what an organ is. It generates signals for survival, it provides mechanical support for the attach cells, also serves as a substrate for cell migration. In other words cells might be able to migrate on

this basement migration laterally, but most importantly it acts as a barrier to passage of macromolecules and to cell invasion.

So, if this invasion was somehow perturbed then that would lead to disaster or any kind of disease. It basement membranes job is actually to segregate these tissues into separate entities.

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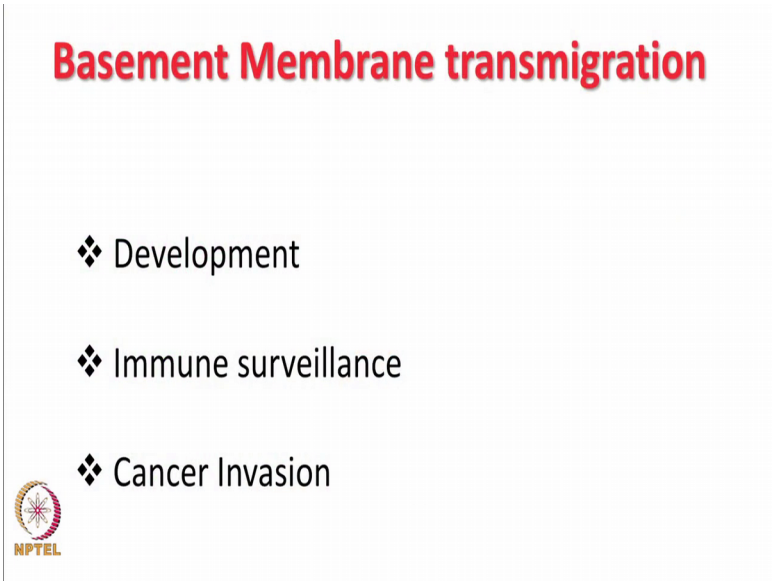

There are the basement membrane is breached only in 3 different context: one during development when the entire body is being generated in multiple organs and generated. For immune surveillance, so your immune cells need to reach out to all corners of the body they should have special mechanisms to be go through the basement membrane. The other one is cancer invasion, where and cancer is the case where these epithelial cells they lose the control of division and once they breach the basement membrane they can go anywhere within the body. You have seen many of us have benign tumors which are you know which might be on the surface.

These are cells in which the cells have divided indiscriminately, but are still unable to breach the basement membrane. So, that is a benign tumor, but once they breach the basement membrane then the disease becomes malignant and you have cancerous growth. So, cells cancer cells are those specialized cells, which can degrade through the basement membrane. Now even though the basement membrane segregate cells on 2

sides of it is not that cells on one side cannot get information about the other side. So, the basement membrane is very thin.

It is possible to see through the basement membrane, how let me give an example. So, think of doing an experiment in which you make a gel a hydrogen and you (Refer Time: 19:03) given stiffness and you tweak the height of thickness of the gel and you plate cells on top. So, and you track the average cell spreading area as a function of substrate stiffness.

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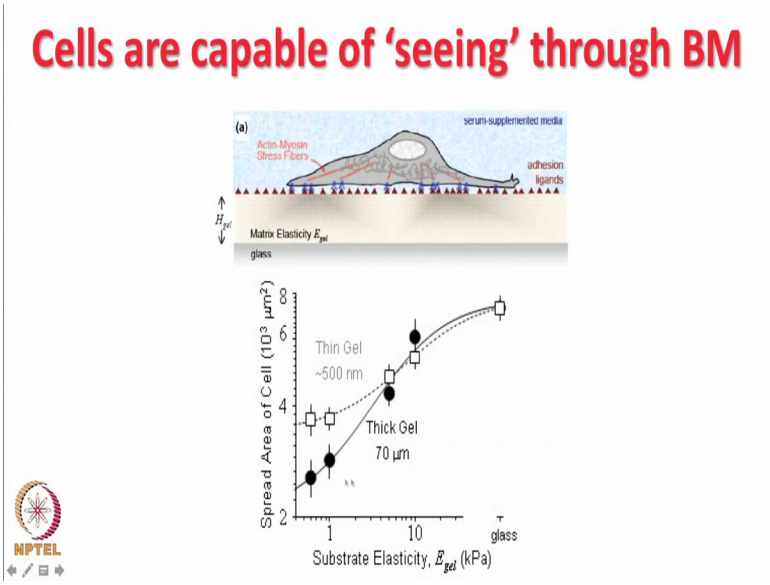

Now, what has been shown for multiple cell types is generally cell spreading increases and beyond that certain stiffness cell spreading saturates, this is the normal spreading profile on a stiff on thick gel; so thick gel this is 70 microns and what people have shown is 70 microns is reasonably thick for the cell that it cannot see the this gel is resting on something which is very stiff in this case class. However, if you keep making the gel thinner and thinner at one time what the cell is sensing is an aggregate stiffness of the substrate and of the gel on top.

What you see is when the gel is thin this spreading on particularly on soft gels where on thick gel spreading was significantly less cells were mostly remaining rounded on as you increase the thickness after as you reduce the thickness of the gel, what you find is cells spend more and more. So, if I draw horizontal line here. So, on the soft 500 Pascal gels 500 Pascal thin gel the cell is spreading as if it was on a 3 kPa stiff gel.

Cell is sensing something stiff. So, advantage; what it conveys is that if you have something stiff. So, if I make a substrate like this.

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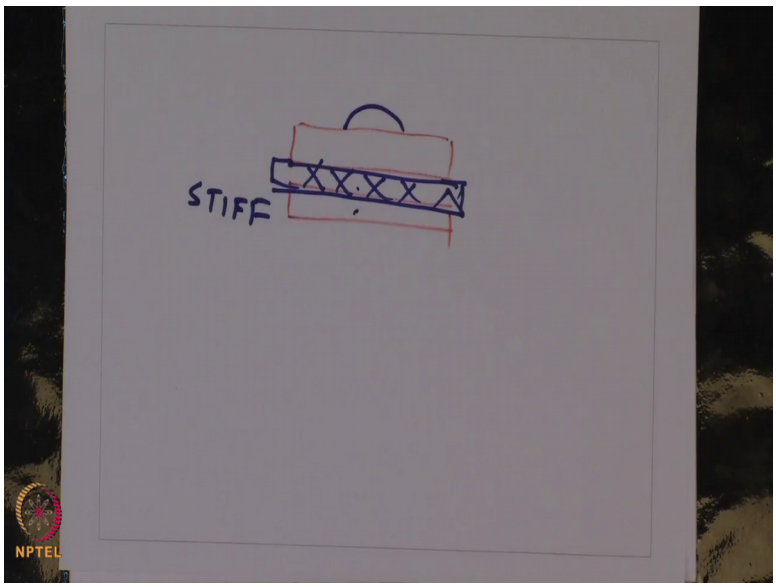

If I have a gel on 2 sides and I put something in between. So, if I put something in between which is very stiff. any the cell which is sitting here might be able to sense what is sitting here or down there. So, it is possible to see through the basement membrane because it is thin.

So, you can still by exerting forces cells these forces will get transmitted through the basement membrane to the other side. So, this again conveys to you why the thickness of the gel is paramount and how the basement membrane being very thin can be helpful for forces being transmitted across the basement membrane.

I will now go and discuss some of the ECM proteins which are of key essence in directing behavior of cells and the most important of them is collagen.

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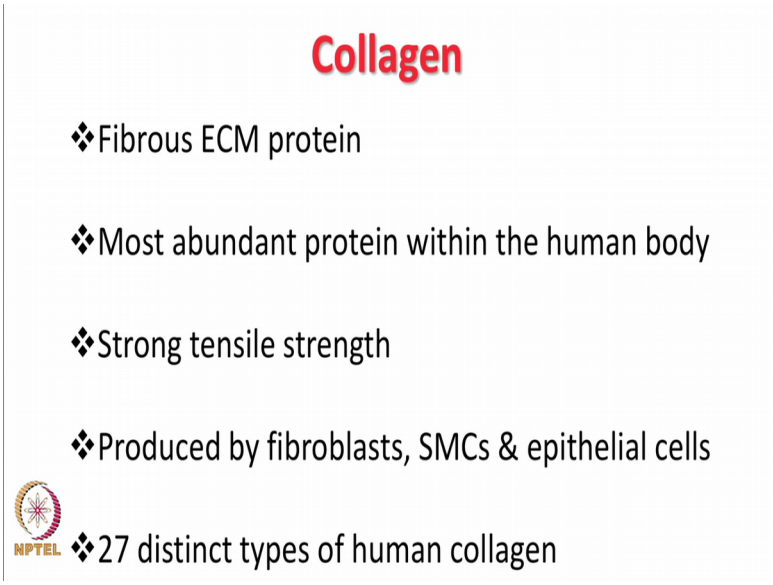

Collagen is the fibrous ECM protein it is the most abundant protein within the human body. It has strong tensile strength, it is produced by fibroblasts smooth muscle cells and epithelial cells. And you have 27 distinct types of human collagen.

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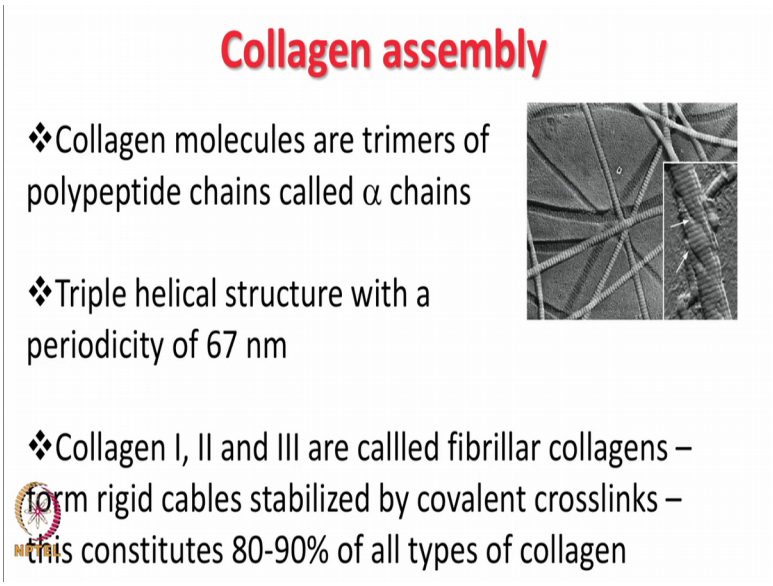

So, if you look at collagen assembly you see that there are actually collagen is conserved trimmers of polypeptide chains. You have 3 chains, 3 ropes which are wrapped around each other this gives you a triple helical structure with the periodicity, you have these banded structure the spacing between these bands is 67 nanometers.

You can have multiple differences as you. So, that there are 27 distinct types of human collagen. Some of them are fibrillar. These are called fibrillar collagen specifically collagen I II III are fibril collagens, and these rigid cables that you can generate of individual collagen fibers can be further stabilized by covalent crosslinks.

So, fibrillar collagens are the majority of all collagens. So, they constitute nearly 80-90 percent of all collagens.

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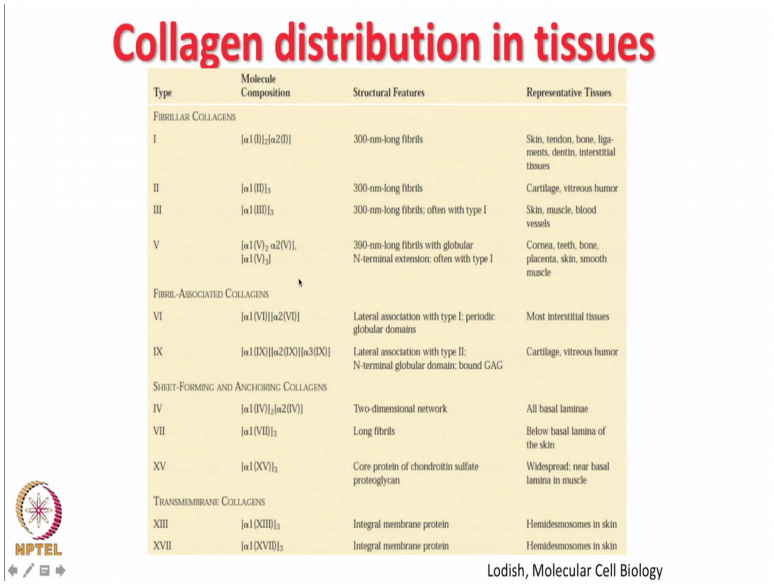

If you look at the collagen distribution in tissues, what you find. So, if you look at fibril collagen there in tissues like skin, tendon, bone or ligaments collagen to cartilage pictures humor skin muscle blood collagen 3. So, the fibrillar collagens are present in high content in those tissues where lots of forces get generated.

For example, the tendon or bone and which need to be study. So, they actually participate in providing tensile strength 2 ds structures 2 ds organs.

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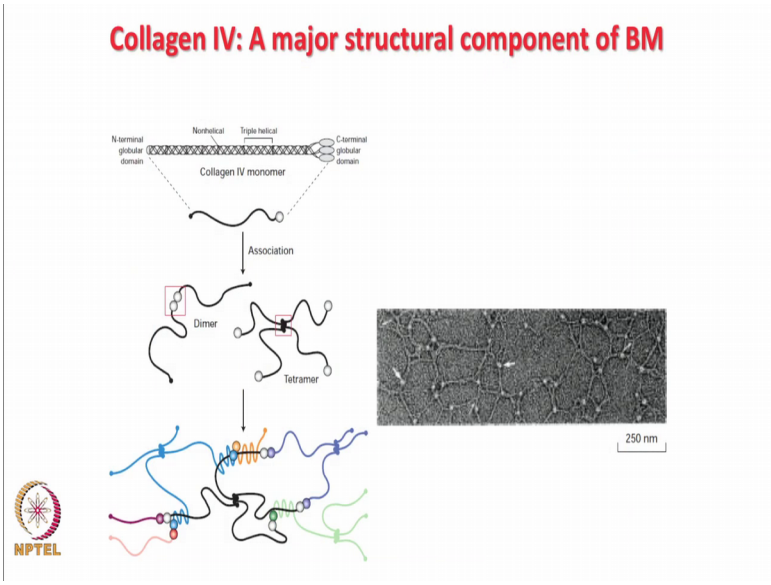

However, apart from collagen one you can have collagen 4 which is a main in a structural component of the basement membrane. So, what collagen 4 consists of it forms is tetramer units which then come together to form this mesh like structure which is further stabilized by other molecules inside.

So, with that I stop here for today. I have briefly given your gist of how extracellular proteins extracellular metrics can regulate cell behavior. In the next class we will look at how properties of matrices are dictated by the density of ECM proteins or their organization. And how dynamically depending on forces which are exerted on these structures; the properties of the matrix itself is not a constant, but it can keep changing. In other words these matrices exhibit non-linear elasticity. That is what I will discuss in next class.

Thank you for your attention.