Introduction to Mechanobiology
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Week - 06
Lecture – 27
Mechanobiology of Diseases: Cancer I

Hello and welcome to our today's lecture of Introduction to Mechanobiology. So, in the last two classes, we had started discussing about mechanobiology of stem cells or how physical factors can influence stem cell differentiation.

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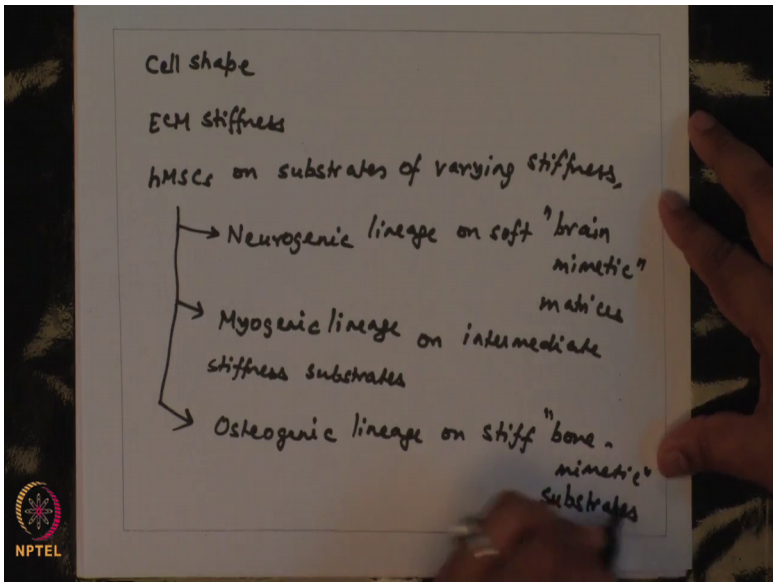

So, in that regard, I discussed two papers; one was regulation through cell shape and the other was through ECM stiffness. So, in the case of ECM stiffness, it shown that if you plate hMSC on is you know substrates of varying stiffness. So, these hMSCs differentiated into a neurogenic lineage on soft brain mimetic matrices. A myogenic lineage on intermediate stiffness surfaces, stiffness substrates, which mean that of muscle and on stiff surfaces and osteogenic lineages on stiff bone mimetic substrates.

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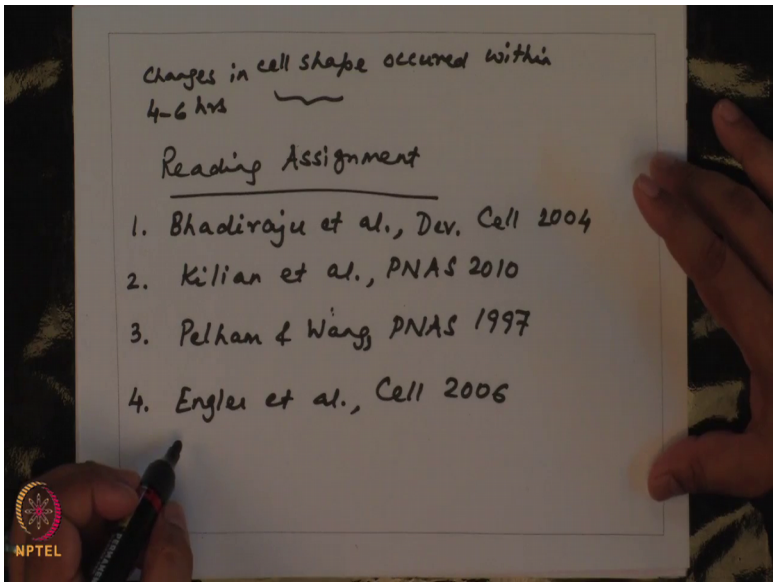

So, in all these cases you would see if you recall that the change in cell shape occurred within 4 to 6 hours. So, this is also even though stiffness is the signal, but this is happening these differentiation is happening through changes in cell shape; at earlier time points you have cell shape being programmed and this cell shape imprints on the overall state of the cell and dictates either a neurogenic or myogenic or osteogenic cell. So, as reading assignment, I would recommend you to read four papers. This was the developmental cell paper where cell density influenced stem cell fate via regulation of cell shape. This was the other paper where they used of same size, but change is in aspect ratio. So, the third paper was the first study to show effect of substrate stiffness in regulating cell spreading, and the final study was about stem cell fate, on substrates of different stiffness.

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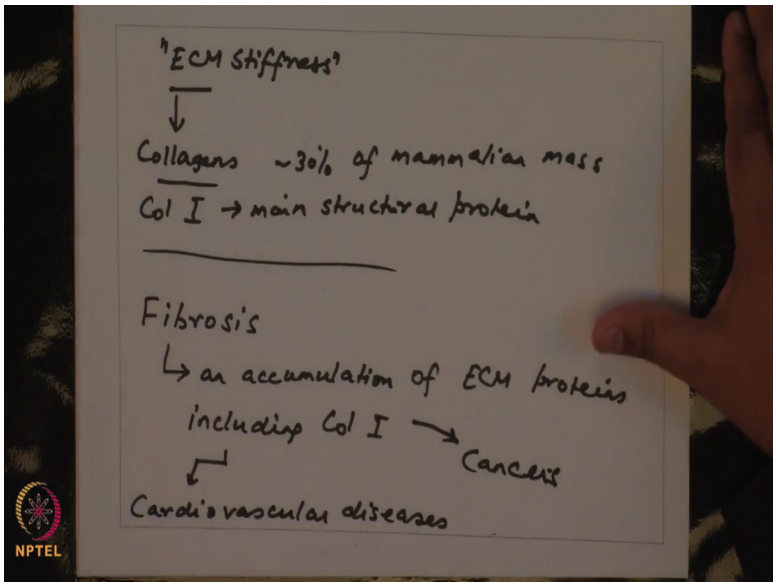

So, these studies demonstrate that your stiffness ECM stiffness is a major regulator of stem cell fate. Now, there are various conditions. So, if first of all again just to recall in terms of ECM stiffness, the major constituent of ECM is collagen, and these account for roughly 30 percent of mammalian mass. And col type 1 is the main structure protein and so fibril protein. So, collagens are the major proteins which contribute to the overall ECM properties. So, if ECM stiffness is changed, one would intuitively guess that it has to do with changes in collagen levels and that is indeed true. So, you have a host of different diseases which broadly come under the umbrella of fibrosis. So, what is fibrosis? It is an accumulation of ECM proteins including collagen 1. So, you have it in diseases in cardiovascular diseases and in cancers.

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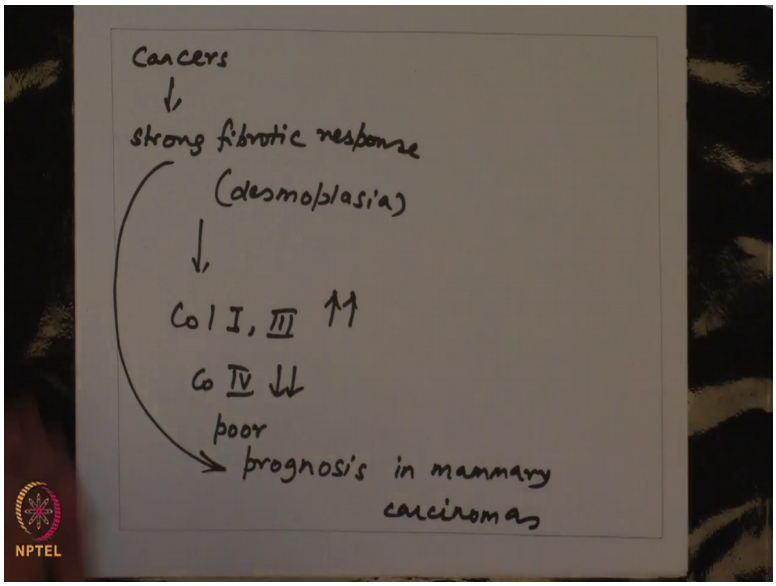

So, what has been observed? So, what is observed is that there is a strong fiber in cancer. So, in cancers you have a strong fibrotic response which is also known as desmoplasia and this involves accumulation of col 1 and 3, these are up unregulated and you have degradation of col 4 this is degraded. And what has been observed is there is a strong correlation between fibrosis and prognosis. So, generally what has been observed is called poor prognosis fibrosis is correlated with poor prognosis in case of mammary carcinomas. So, you have this link between increase in ECM deposition and its cross-linking.

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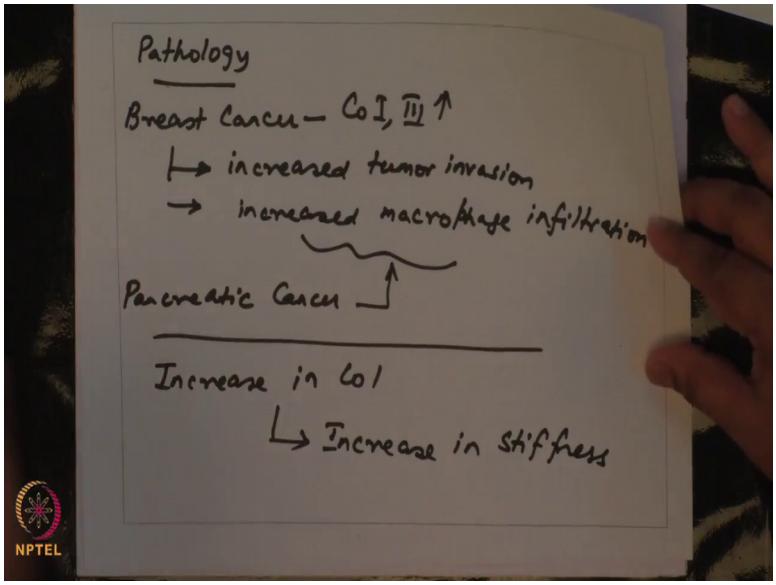

So, if you think of pathological tissues associated with fibrosis, you have breast cancer where you have col 1 and 3 up and this has been associated with increased tumor invasion and increased macrophage infiltration. Similarly, was the case of pancreatic cancer. Same two points are true for pancreatic cancer also. So, what has been so all of these kind of correlate that as you have increase in, so increase in collagen would mean it to increase in stiffness.

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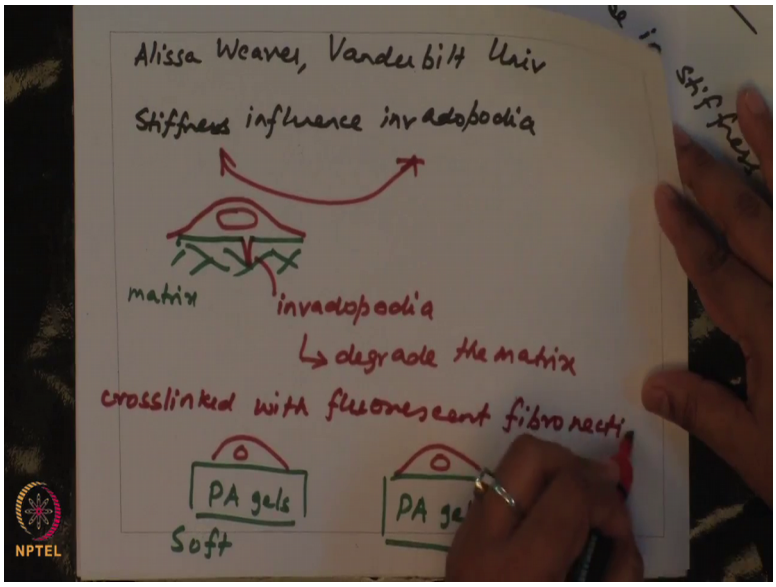

So, to study the effect of this increased stiffness, so people have done, so Alissa Weaver at Vanderbilt University. So, she probed how does stiffness influence invadopodia. So, just to recall what is invadopodia. So, if you have matrix, so inside view if you have matrix invadopodia were these protrusions, your cells. So, these are these protrusions which protrude into the matrix. So, this is your matrix and they degrade the matrix. So, what she showed what the experiment that Alissa Weaver did was teach she took two substrates these are PA gels - polyacrylamide gels; one was soft and the other ones was stiff. And she plated cells on these substrates and asked that is there any correlation between stiffness and invadopodia. So, these substrates were cross linked with fluorescent fibronectin.

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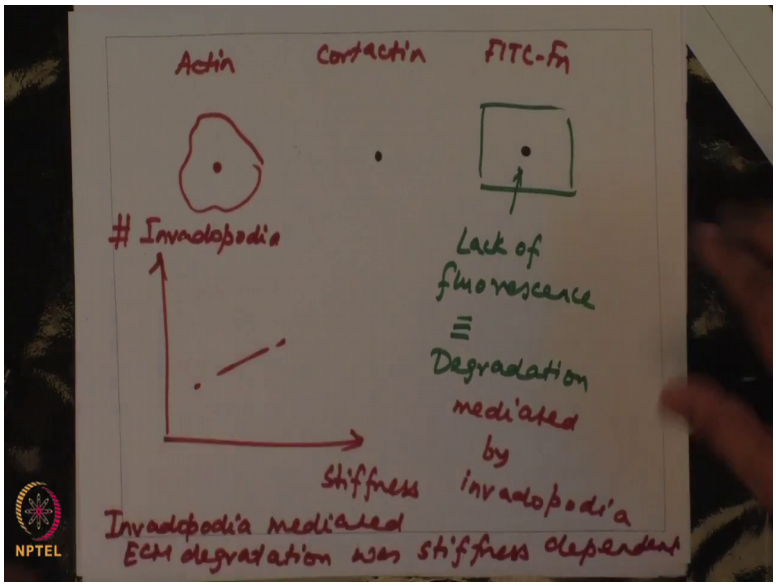

So, in top view what you would observe is if this was, so you take three images one of actin one of caught actin or any other invadopodia marker and then one of the fibronectin. Let us say fitc fibronectin. So, what she found was on soft substrates let say this is this top view, this is a picture of a cell and here the matrix. So, you saw that wherever there was so this is an example of an invadopodia, you have a parallel colocalization with caught actin. And at this point if this is my matrix, at this point, there was no fluorescence. So, this is lack of fluorescence. So, this is equivalent of degradation. So, suggesting the formation of invadopodia. So, this degradation is mediated by invadopodia. So, what she showed was that as you increase stiffness, the number of invadopodia or the degradation scales with stiffness. So, invadopodia mediated degradation was stiffness dependent. So, this study suggested that your increase in stiffness might be leading to this phenotype.

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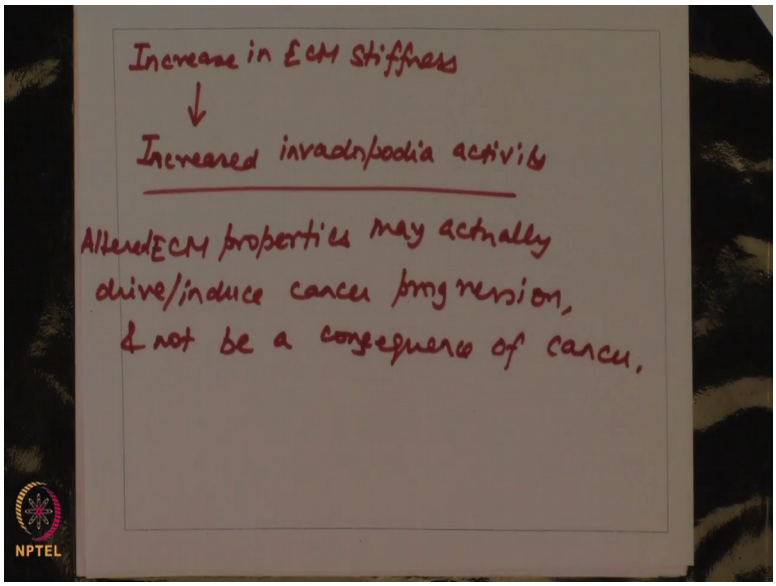

So, it remains unclear whether this increase in stiffness, you have increase in ECM stiffness, and this is correlated with increased in invadopodia activity. So, these kind of point to the possibility that ECM stiffness or increase altered ECM properties may actually drive slash induce cancer progression and not be a consequence of cancer.

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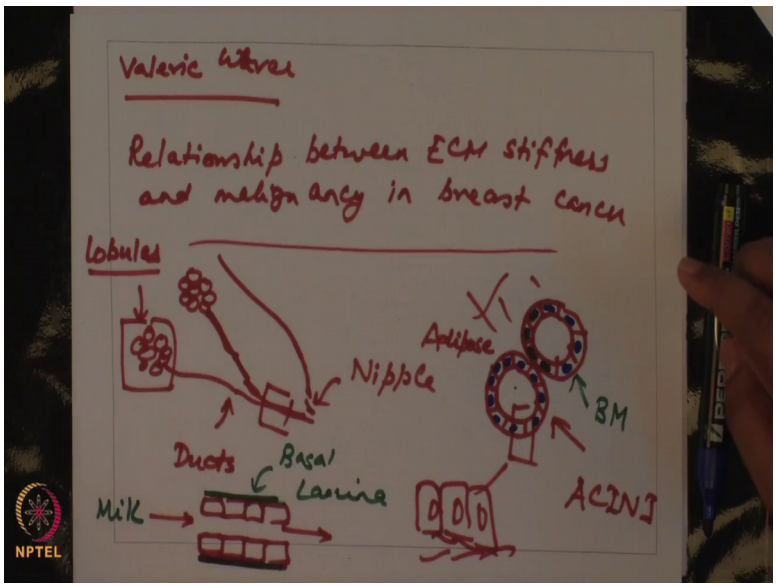

So, to answer this question Valerie Weaver, so decided a number of experiments on breast cancer invention and the relationship between extracellular matrix stiffness and malignancy. So, what she is probed was the relationship ECM stiffness and malignancy

in breast cancer. So, we will discuss this work, but before I get started let me briefly give the anatomy of the breast. So, in the breast, what you have, you have these lobules which secrete milk and these so you have this kind of structures lobules which get connected and eventually lead to the nipple.

So, milk is secreted and it is connected via these ducts. So, these are ducts and these are called lobules. So, if you zoom in onto a lobule, if you zoom in onto a lobule, what you will see and make other lobule these are your lobules and if you. So, these are surrounded by adipose, adipose tissue. So, if you zoom in further if so these are gaps inside which direct the milk secreted by these cells to the nipple. So, if you zoom in even closer, so then what you see is there some extracellular matrix on top of which you have these epithelial cells and these structures are called ACINI.

So, similarly if you look at the ducts, so what you will find is you have these epithelial cells. So, this is the long axis of the duct. And underneath this, you have the basal lamina. Even here, surrounded by this is the basement membrane. So, these are specially restricted. So, these cells allows milk, so this milk flows along the ducts around these things. So, in case of cancer, you cannot get two types of cancer.

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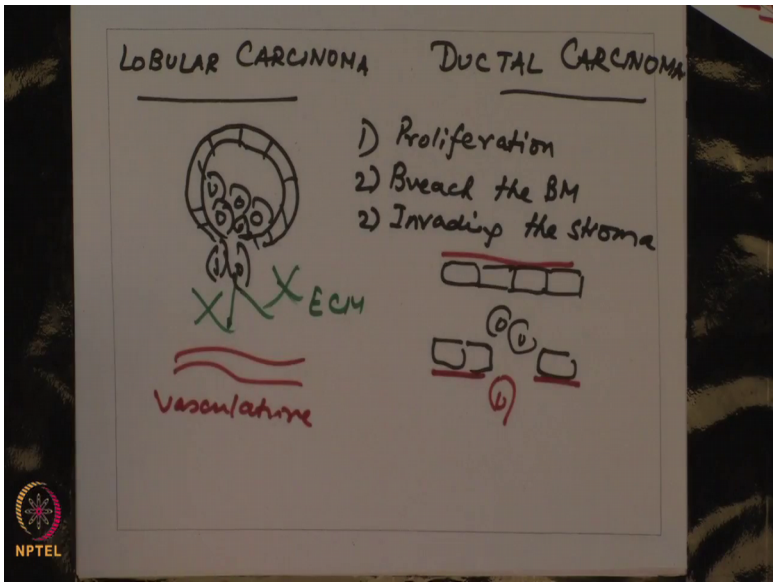

You might have two different types of cancer which are called as either lobular carcinoma. In incase of which if you have these ducts, these lobules the structure falls apart and the cells start to invade. So, these are you epithelial cells and you have

increased proliferation of these cells and then these cells try to start to migrate and eventually very far off you would have the vasculature, and in and around you have the ECM. So, these cells invade and move towards the vasculature. Same thing happens in case of ductal carcinoma. So, what you have is these cells, the structure is lost, the basal lamina is disrupted and these cells start to migrate. First of all proliferate, so first step is proliferation and then they breach the basement membrane and start invading. So, this is the picture that you have two different kinds of cancer.

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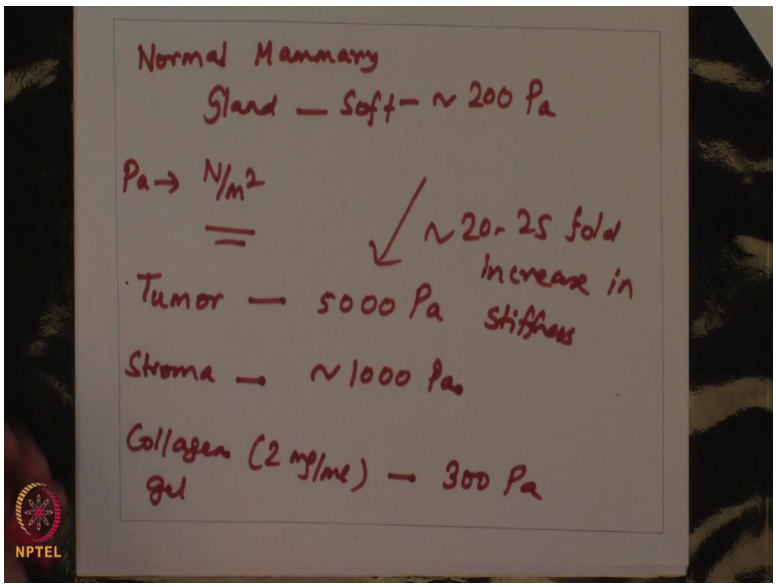

So, now if you look at the how properties of the tissue in and around the tumor get perturbed. So, normal mammary gland is very soft this orders 200 pascals in stiffness. So, again just to remind you pascals is newton per meter square this unit is called pascals. So, these are very soft this 200 pascal is comparable to soft you know neuronal tissue brain tissue. In case of the tumors, this gets increased almost many folds and you can have order 5,000 pascals. So, this is order 20 to 25 fold increase in stiffness the stroma which is attached to the tumor is order 1000 pascals. While, if you look at collagen let say a 2 mg per ml collagen gel collagen gel if you may this has ordered 300 pascals stiffness. So, what you see is there is a drastic increase in the stiffness in case of cancer. The question then arises is, is this increase in stiffness driving cancer progression, driving cancer invasion or is it a consequence of cancer?

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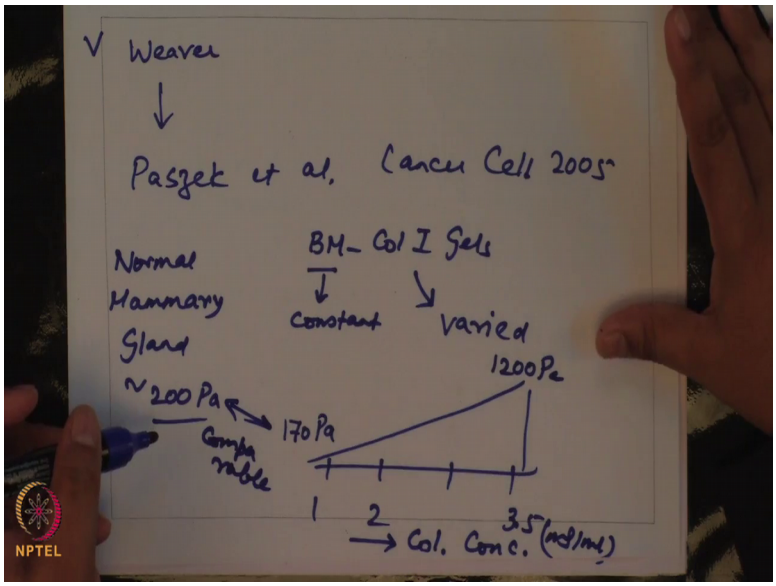

So, to answer this question Valerie Weaver, so she published this landmark paper in 2005. So, what they began by doing was. So, they first probe the properties of the mammary tissue mammary gland tissue which was order around. So, mammary normal mammary gland is ordered 200 pascals. So, what she did was she made these collagen gels. So, she made a range of basement membrane, collagen gels collagen 1 gels, where this was kept constant and the concentration of collagen was varied.

So, she varied the concentration of collagen and this allowed her to generate a range of different stiffness's. So, this is my collagen access this is 3.5 this is my collagen concentration in mg per ml. So, she generated a range of different stiffness of the bulk stiffness of this collagen gel ranging from 170 pascals for this 1 mg per ml to nearly 1200 pascals for the stiff gels. So, this 170 pascals is very comparable to the normal mammary gland properties.

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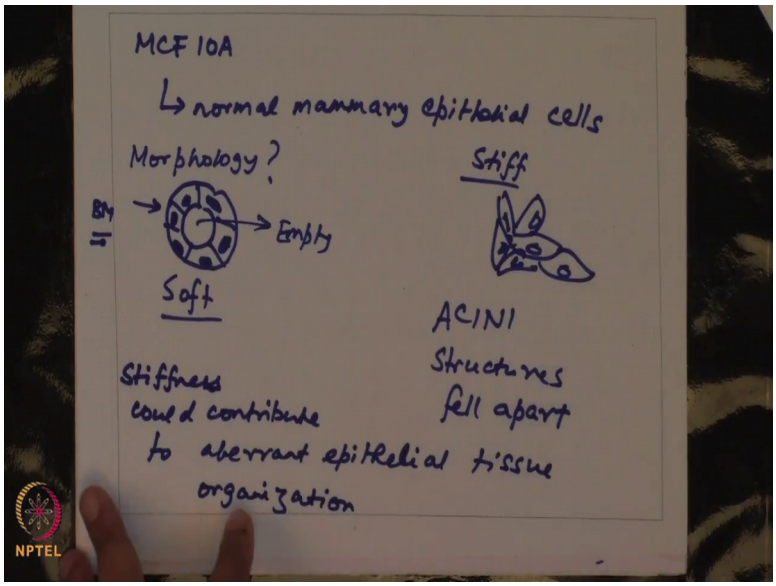

So, she asked, so what she did was she plated cells MCF 10A, these are normal mammary epithelial cells. And she asked what is the morphology of these cells. So, on the 1 mg per ml gels on the on the soft gels, what she found was the cells formed this acini like structure. So, in this acini, the center portion this is empty and these are individual cells which are connected together. So, you have basement membrane at the periphery of these cells and these cells each of these represents the nuclei, but when she looked at the morphology on the stiff surfaces, what she found was a group of cells a very invasive phenotype, the acini structure was gone. So, on the stiff surfaces, acini structures fell apart. So, there was no acini structures.

So, these suggest that your tissue stiffness could contribute to aberrant epithelial tissue organization. So, there was one caveat about this experiment that when she plated because she was changing the collagen concentration. So, collagen is also changing the ligand density. So, while the bulk stiffness is changing, the ligand density is also changing; so to rule out the fact that these differences are the consequence of lie alterations in ligand density.

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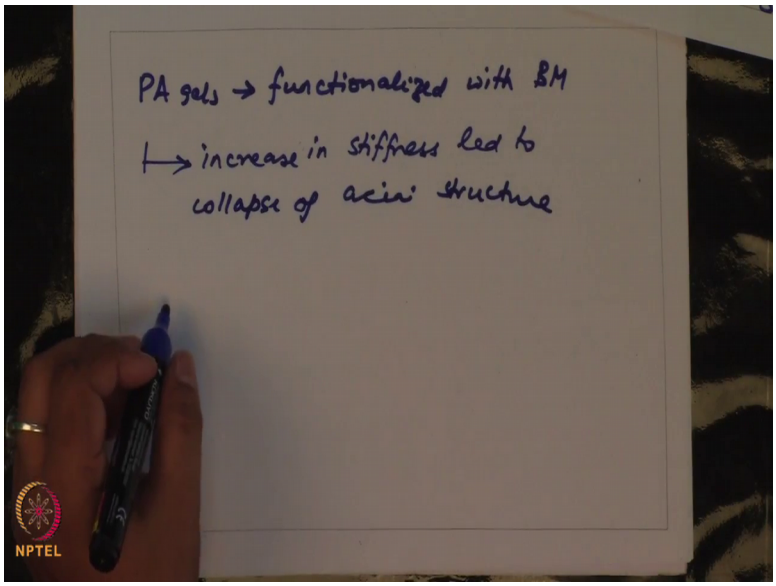

She repeated the same set of experiments in PA gels - polyacrylamide gels which were coated, these were functionalized with basement membrane. And once again she observed the same thing, so increase in stiffness led to collapse of acini structure. So, we will stop here for today. In the next class, we will continue from this part.

Thank you for your attention.