Introduction to Mechanobiology
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Week - 05
Lecture - 25
Mechanobiology of Stem Cell Fate – II

Hello and welcome to todays lecture of Introduction to Mechanobiology. In the last lecture we are started discussing about how Physical factors can regulate Stem cell fate.

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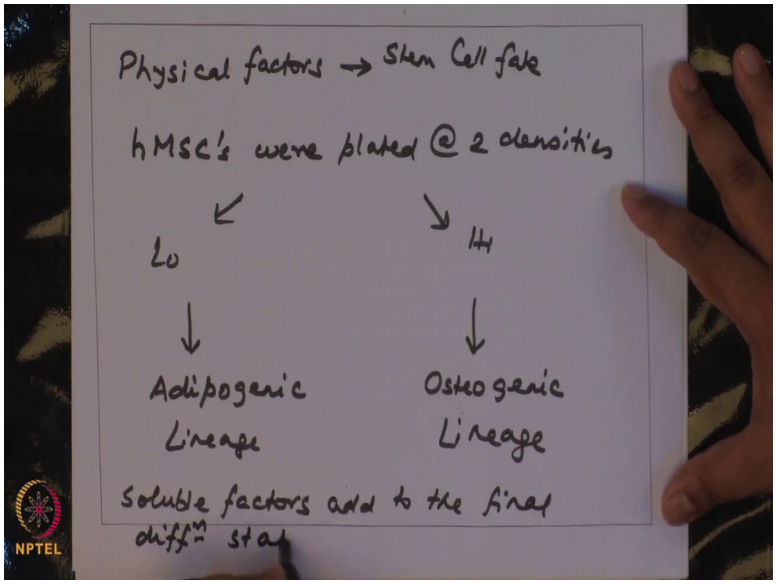

In this regard we discussed a paper where human Mesenchymal Stem Cells were plated at 2 densities; you have low density and high density. And what the authors discovered was at low density, the cells tended to differentiate into an Adipogenic Lineage, and on high seeding density they differentiate it into an Osteogenic Lineage. Of course, you have soluble factors add to the final differentiation state. So, to understand why this density dependent effect was observed the authors speculated that indirectly density or seeding cell density is regulating cell shape.

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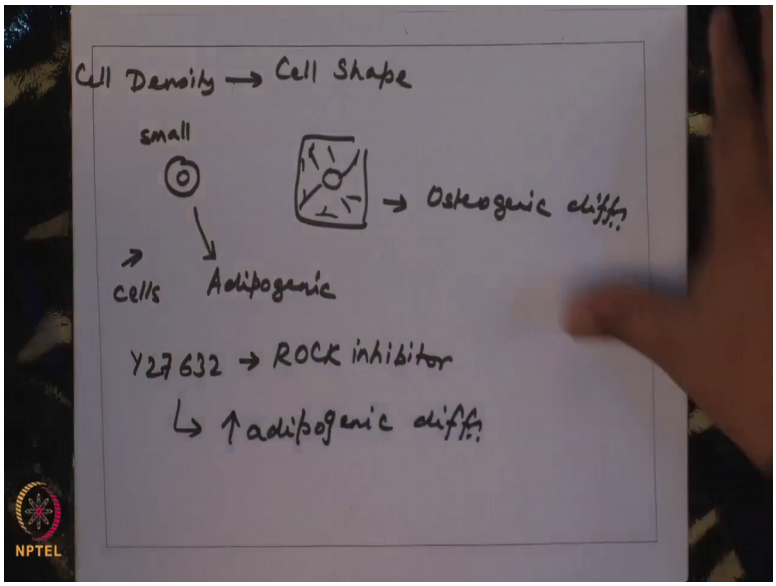

To test what the authors did was they took patterns either small or large this is that when you seed the cells, you would have a cell, with well developed focal adhesions and stress fibers and what they found was again consistent with the hypothesis at this small when the Island size was small you had a Adipogenic differentiation and on this Osteogenic differentiation. If the on the bigger Islands if the cells treated with Y2 7632 which is a ROCK kinase inhibitor. So, what they found was increase in Adipogenic differentiation. Together what this studies suggest is you have cells if I take an each MSC.

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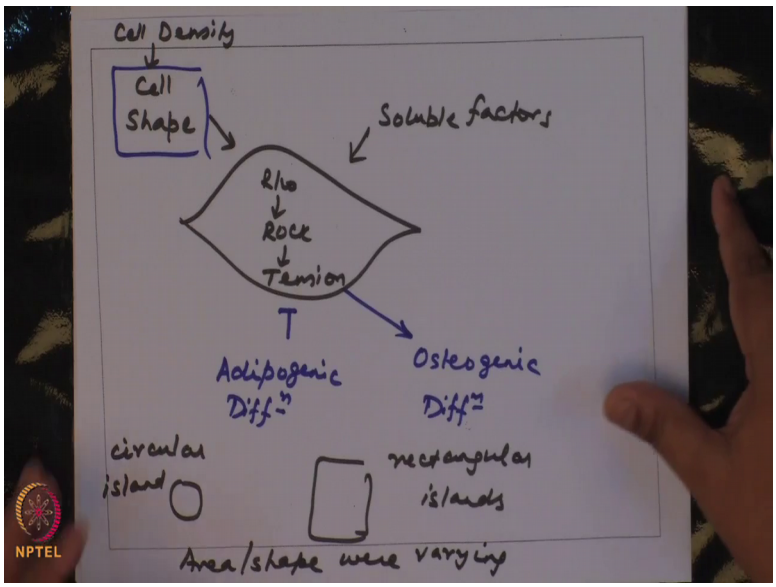

You have 2 regulators of this differentiations with one being cell shape and indirectly your cell density is regulating cell shape you also have a soluble factor and internally what they do is they stimulate actamyosin contractility

If you inhibit this tension; if you inhibit this tension then what you will get is if you will get an Adipogenic differentiation, while if tension is promoted then it leads to osteogenic differentiation. This study shows how you can have a physical factor like cell shape regulate the differentiation fate of stem cells. Now in this study the authors are actually used Islands of different shapes and sizes right, you had a circular Islands or you have rectangular Islands. In there is a possibility that this difference that the authors observed is because of this shape also how would you here both area and shape were varying.

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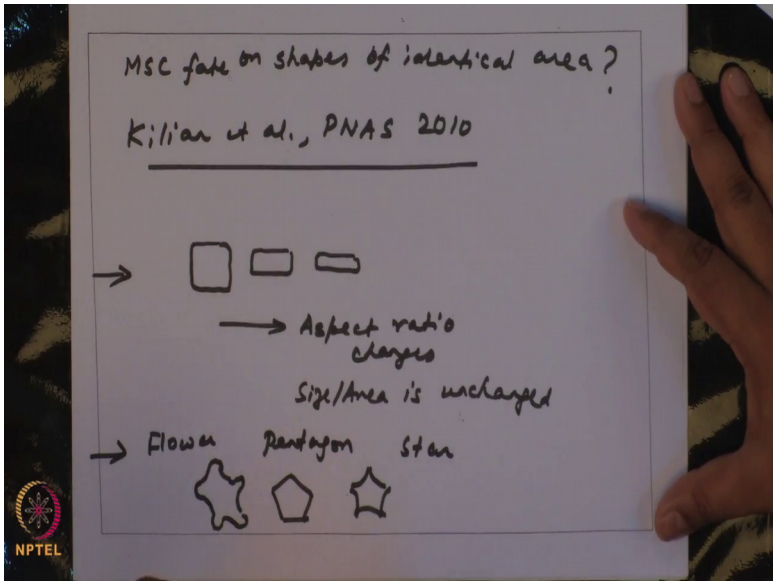

How would you speculate would happen to MSC fate on shapes of identical cell area? So, to answer this question what are different group do, the paper is again a PNAS paper.

What the authors did was they took different patterns rectangular patterns you have rectangular pattern and what you do is you keep changing the aspect ratio. Your aspect ratio is changing, but size or area is unchanged. Similarly this was one set of geometry stutters that the authors chose, in another set of geometries what they did was they took a pentagon and then they generated 2 different geometries from it one in which they rounded the interior to form a star shaped or there. So, they made a flower this is a pentagon and this is a star.

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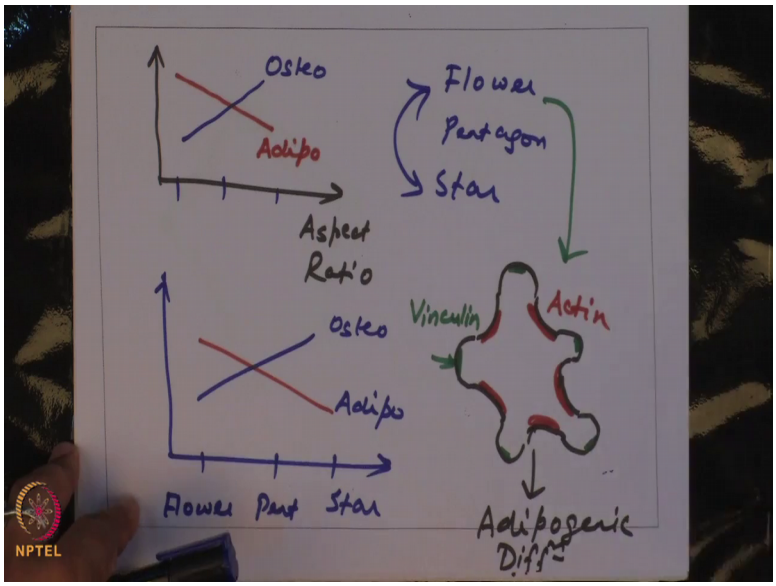

So, you in both these cases you have different shapes, but identical areas. What the authors observed was again; if I plot it as a function of aspect ratio on the rectangles on lower aspect ratio in both the cases, you had a mixed fate. So, this is Adipogenic differentiation and this is osteogenic differentiation.

What the authors observed was more aspect with increasing aspect ratio the cells tended to differentiate wound into an osteogenic lineage and same was true here you have the Flower, Pentagon and Star. You observe the authors observed a similar trend osteogenic differentiation Adipogenic differentiation. This suggested that something must have been changing because of which you have a drastic change in the eventual fate, now please note that this is a mixed fate it is not that you have 100 percent Adipogenic 100 percent or Osteogenic differentiation. So, to probe into the basis what the authors chose was these 3 shapes the Flower, the Pentagon and the Star. And the compared between the Flower and the Star what has changed in terms of the cytoskeletal organization and the focal adhesion organization.

What they found was on the flower patterns; on the flower patterns, if I were to draw a heat map, what they found, was the red denotes the actin signal on the flowers you had increased actin signal on these edges, but for the star shaped. This is the actin signal and the focal adhesions were towards the periphery. So, this was what the signal that they

observed. So, on the flowers on the flowered geometry this is what they observed. So, the green are vinculin or focal adhesion and the radious actin.

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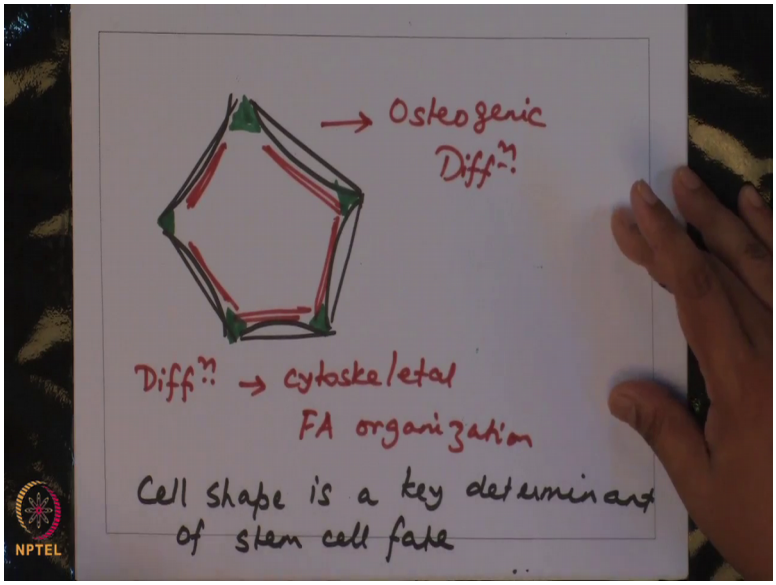

On the star shaped Island, what they found was the focal adhesion signal, they generated the star shape like this you have a slight curvature. What they found was focal adhesion signal was much more prominent for the stars and accordingly the stress fiber signal was also much more put it.

What you see is the differentiation is associated with cytoskeletal and focal adhesion organization. On the star where there was more amount of stress fibers this induced an Osteogenic differentiation, while on the flower you had an increased precedence of Adipogenic differentiation and consistent with this and consistent with the idea that increase in tension stimulates Osteogenic differentiation you had lesser tension or lesser accumulation of stress fibers, in case of flower shape patterns and greater stress fibers and bigger focal adhesions in case of the star shaped pattern. This once again demonstrates the fact that cell shape is a key determinant of stem cell fate.

In adhesion to this if you think of the other physical cues, this kind of cell shapes for example star or flower you can engineer these shapes, but these shapes do not naturally occur inside out in vivo context. What is one of the factors are in view relevant factors which influences stem cell fate. And one of such factor is the Stiffness of the Tissue.

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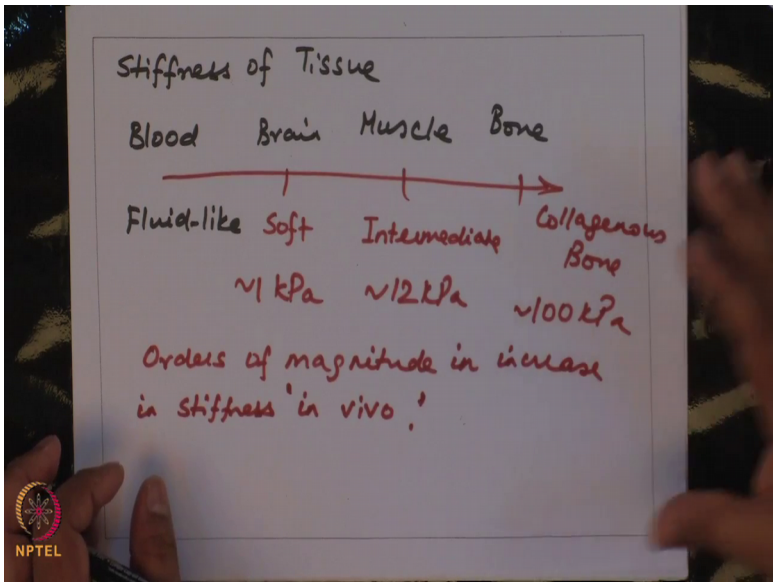

So, you know by default that Blood is Fluid-like, Brain is soft, but it is stiffer than blood because blood is like a fluid. Muscle tissue is stiffer than brain and of course the stiffest is bone. If I were to draw a stiffness scale forward to or stiffness scale you have brain muscle and bone which have distinct mechanical properties. Brain is soft by soft I mean order one Kilo Pascal. Muscle is intermediate stiffness order 12 Kilo Pascal, and bone rather Collagenous bone is stiff order 100 Kilo Pascal. So, you see there is a natural increase orders of magnitude in increasing stiffness in vivo.

So, adult stem cells they exist in all these tissues is it possible that these stem cells are influenced by this property of stiffness. One of the very first studies which was tried attempted to understand this question was done by Yu Li Wang.

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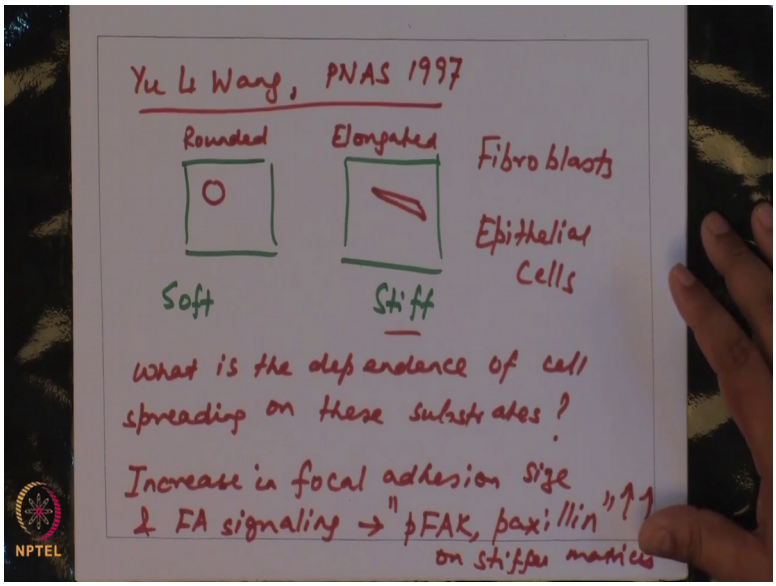

What he did was he created 2 substrates? He used 2 substrates one was Soft and one was Stiff, and on these he plated Fibroblasts as well as Epithelial cells and he asked that what is the dependence of cells speeding on these substrates and what he found was the same cells on a soft environment, exhibit much smaller or more rounded, and exhibit smaller cell areas why the same cells on a stiff matrix tend to exhibit much more elongated. So, this is more rounded phenotype, more elongated phenotype on stiff surfaces. There is increase in stiffness drives increase in cells spreading and this increase in string cell spreading on stiffer surfaces is associated with increased in focal adhesion size and FA signaling adhesion signaling.

So, what the observed was on stiffer environments you had phosphorylation of "FAK or paxilin" this integrin signaling molecules were much more phosphorylated on stiffer matrices. So, they expression was over up regulated on stiffer matrices. This was one of the first studies; this was in a previous paper in 1997. So, this was one of the first studies to demonstrate the effect of stiffness in driving cell spreading. So, subsequently there have been lot of studies, but the question automatically arose that what would happen to other cell types if they were placed in such environments, and spreading is of the order of a you know 5 6 hours cell spread what would happen to longer time events a subsequent study working on muscle cell differentiation.

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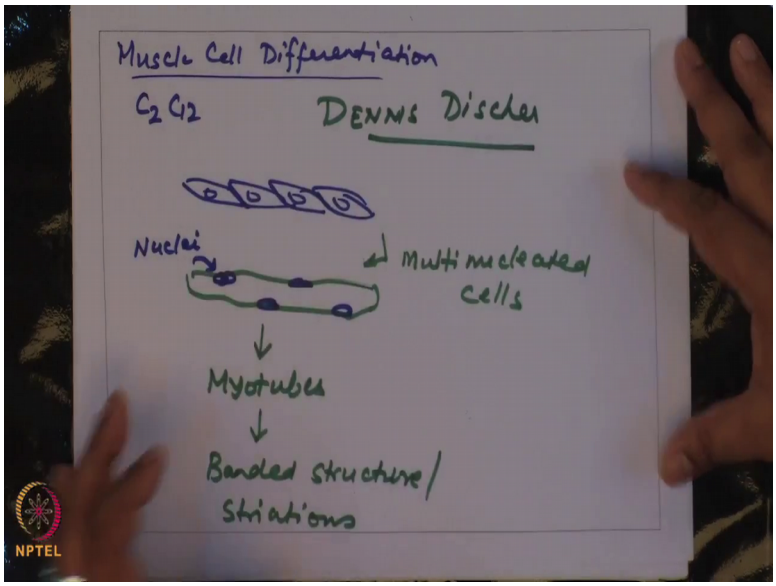

What they asked for muscle cells to differentiate particularly cell skeletal muscle cells like C2 C12 cells, you have individual cells which kind of come together and then they eventually these guys fuse to form a Multinucleated Cells. Here by blue these are the Nuclear and then after found these multinucleated structures, these guys differentiate to form myotubes, where you can see the banded structure also referred to as striations. So, this was done in the group of doctor Dennis Discher.

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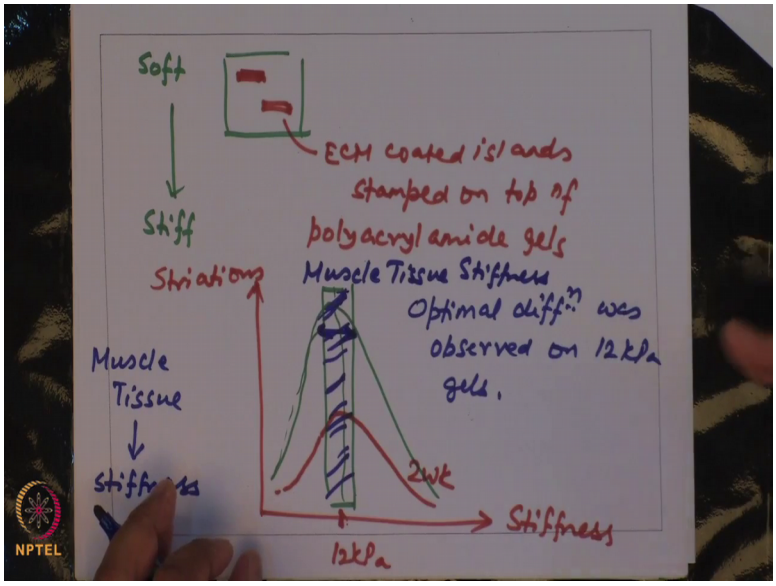

What the study demonstrated was if you took different substrates from soft to stiff all the way to stiff and you on these gels what the authors did was they pattern these Islands. So, these are ECM coated island stamped on top of polyacrylamide gels.

What the authors observed was as a function of stiffness and as a function. So, the Y axis were striations, what they observed was as a function of stiffness you see at early time points these cells exhibited a peak at a 2 week time point on gels which were roughly 12 kilo paxalin stiffness and this as time went on the differentiation profile grew even more prominent, but the position of the peak remained unchanged. This zone on which the cells optimally differentiated, optimal differentiation was observed on 12 kpa gels. So, the obvious question was why is it that the cells are exhibiting, maximum fashion or maximum reorganization on these matrices.

What the authors did was they excise muscle tissue and measured it is stiffness, and what the authors found was the stiffness of muscle tissue was exactly in the zone in which maximum amount of striations was observed on these gels suggesting that muscle cells differentiate optimally on muscle like environments. So, this study suggested that muscle cells differentiate optimally on muscle like environments.

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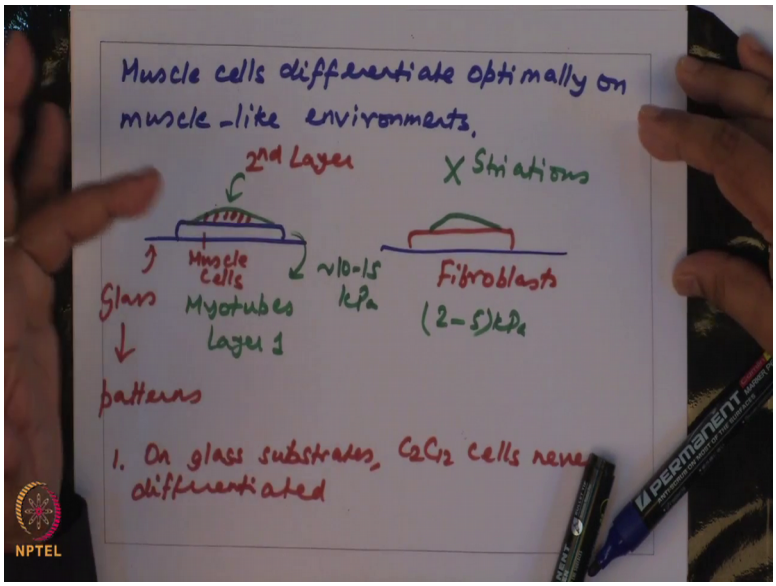

They also did one set of experiments in which on these patterns which we have a bottom layer of cells you have a bottom layer of myotubes and on top layer you put one more cell layer you have a top layer. So, the bottom layer is myotubes, on the top layers this is

the first layer myotube layer one, and you on what you do is after you get a bottom layer these experiments were done on glass this is a glass substrate where you again have patterns so, that you can get well defined myotubes.

So, what the authors observed first was on glass substrates C2 C12 cells never differentiated, but when you played the second layer of cells what they found was you had the formation of these striations it was very prominent when plated on this in bottom layer of muscle cells. So, these are my muscle cells only right. In a sense this suggested again the second layer of muscle cells are seeing a muscle like environment on which the differentiate; however, when the experiment was repeated with a layer of fiberglass at the bottom. So, the second layer did not exit did not have any striations. Fiberglass is known to be 2-5 kilo pascal in stiffness while muscle cells this is order 10-15 kpa in stiffness. This once again reinforces the idea that muscle cells differentiate optimally when placed on a muscle like environment.

Both these studies rear from these phenomena that these muscle cells behave like muscle cells the function like becomes functional muscle cells when they are placed in a muscle like environment.

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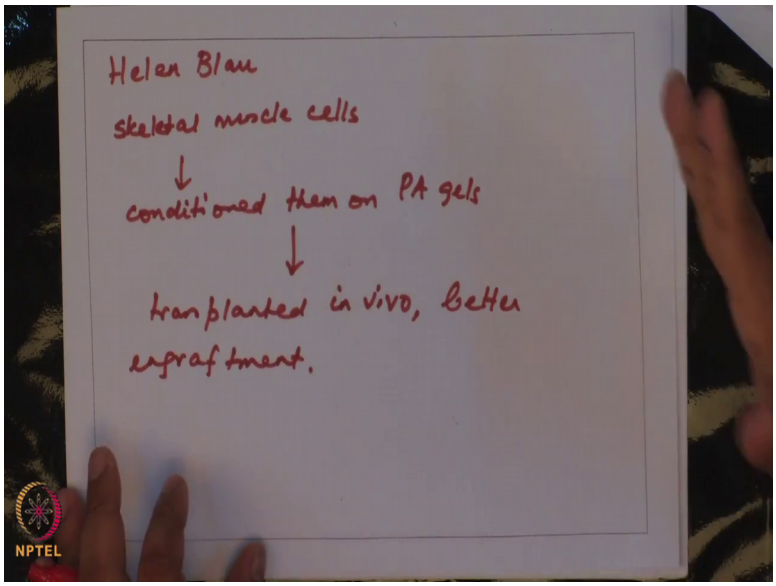

So, subsequently based on these words there was experiments done by Helan Blau. What she did was she took skeletal muscle cells once again, she conditioned them on PA gels which we make the tissue stiffness of muscle and she showed that these cells when

transplanted in vivo, exhibit better enpraftment. All these studies suggest that cells tend to have a preference for a certain kinds of environments.

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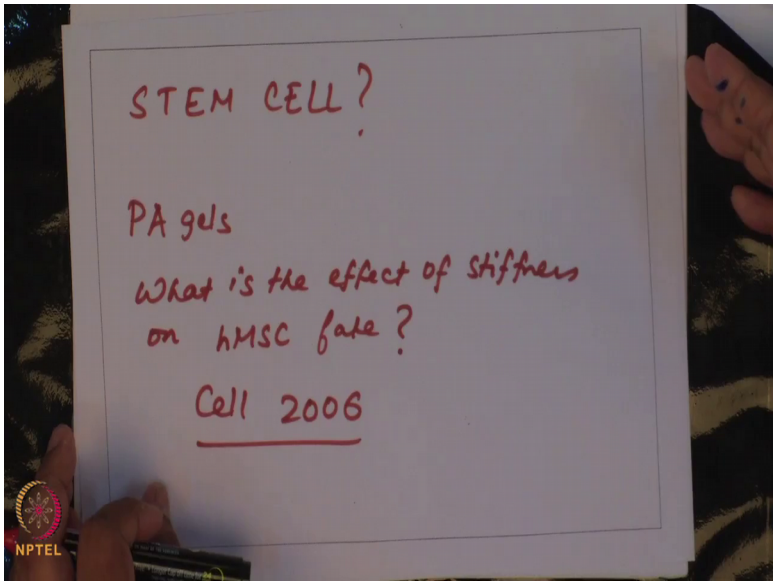

This raises the question what about stem cells? What about stem cells how would these stem cells believe, when placed on matrices of different elasticities. So, the group of dennis discher did this study on using PA gels using PA gels he asked that what is the effect of stiffness on human mesenchymal stem cell fate this is the question that they asked. This is the landmark paper this has been published in cell 2006 this would be part of your reading assignment I will stop here and I would start with this paper in the next class.

Thank you for your attention.