Introduction to Mechanobiology
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Week - 06
Lecture - 26
Mechanobiology of Stem Cell Fate – III

Hello and welcome to our today's lecture of Introduction to Mechanobiology. So, in the last lecture, I started discussing the effect of physical factors and among this specifically stiffness in regulating stem cell fat.

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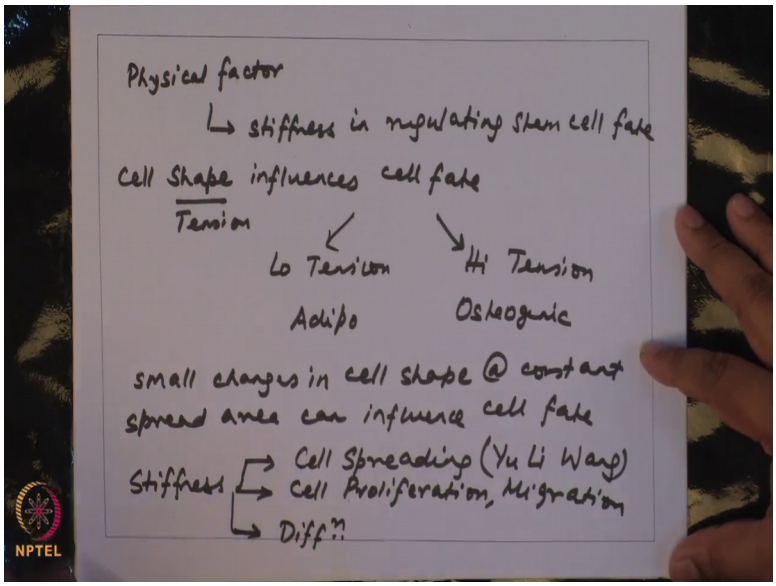

So, previously I have shown that cell shape influences cell shape and cell tension influences cell fate. So, with higher tension when you have low tension, you have adipogenic differentiation and you have high tension you have osteogenic differentiation. Also I discussed a paper where we showed that small changes in cell shape at constant cell area spread area can influence cell fate. And towards the end I started discussing about stiffness. So, ECM stiffness has been shown to direct cell spreading, this is the Yu Li Wang paper who first demonstrated it. And this is the increased cell spreading is driven by greater focal addition signaling. Lot of other studies have demonstrated the effect on cell proliferation, migration. And it has also been described the stiffness regulates differentiation.

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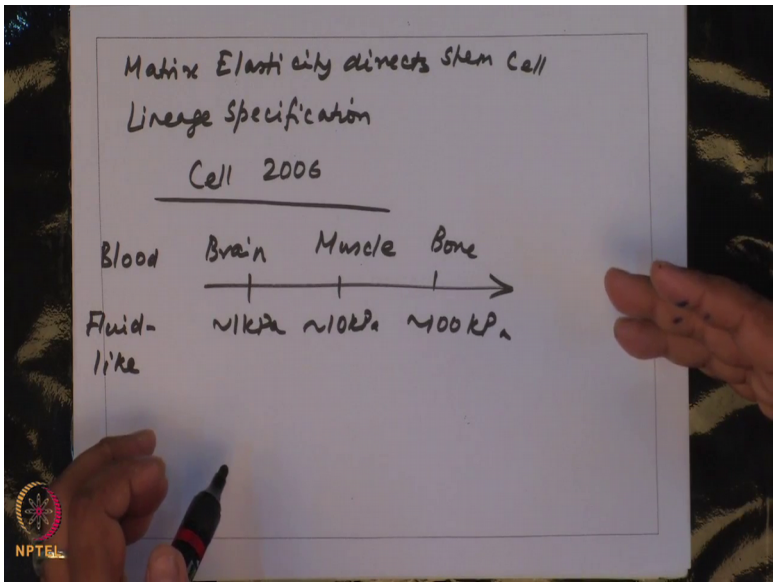

So, today I will start discussing this paper Matrix Elasticity directs stem cell lineage specification, Cell 2006. So, the question the authors asked was given that within in vivo you have orders of magnitude. So, brain being soft, muscle being intermediate stiffness bone being stiff, so this is order 1 kpa, order 10 kpa, order 100 kpa; and in the extreme you have blood which is fluid like. So, given these environments which exist in vivo, how do stem cells respond to the stiffness if at all and if yes how.

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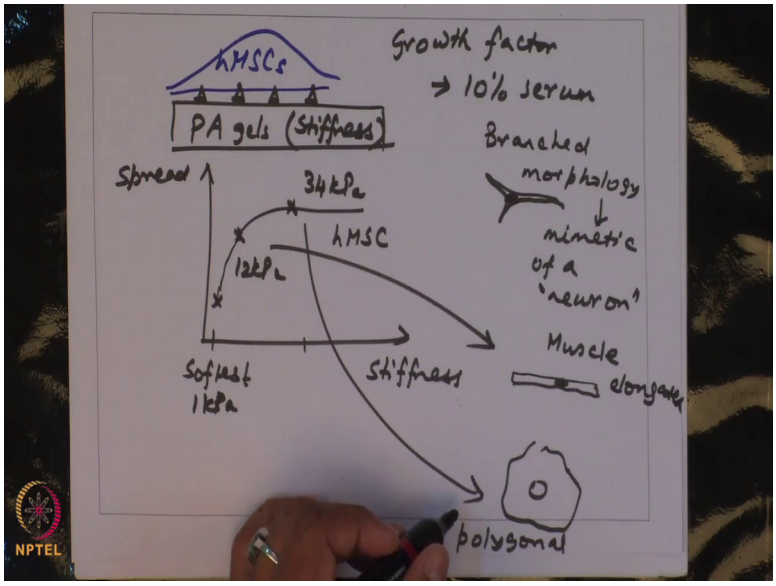

So, to ask this to answer this question, what authors did was they took PA gels they functionalized it with collagen and then they plated cells on top hMSCs were plated. So, growth factor was a constant was 10 percent serum as it is present in normal cell culture, but there was no other extra added factors. And what was tuned was the stiffness of the PA gels. So, first of all like other cells as a function of stiffness so cell spreading if you track after 24 hours, you will always see a saturated profile cells increase to spreads on the softest you have the least spreading area of these cells. And as you increase the stiffness spreading increases and beyond some stiffness spreading is constant. Interestingly so this has been observed in other cells also. So, this is your hMSC response, but you see in most other adherent cells including fire blast.

However, if you look at the shape, so this is soft 1 kPa stiffness, this is 12 kPa stiffness and this is 34 kPa stiffness. So, when you ask what is the shape of the cells what the authors found was on the softest 1 kPa gel, the cells had a more elongated or a branched morphology on the intermediate. So, branched morphology pneumatic of a neuron, this is a nucleus. On the intermediate surface, the cells looked more like that of muscle from 12 kPa, the cells looked more like this. Around 34 kPa, cells looked more polygonal this was elongated morphology or muscle like environments. And polygonal on the stiff environments and similarly we observed the similar shape on glass. So, they then did some detailed morphological correlation. So, they defined something as spindle factor.

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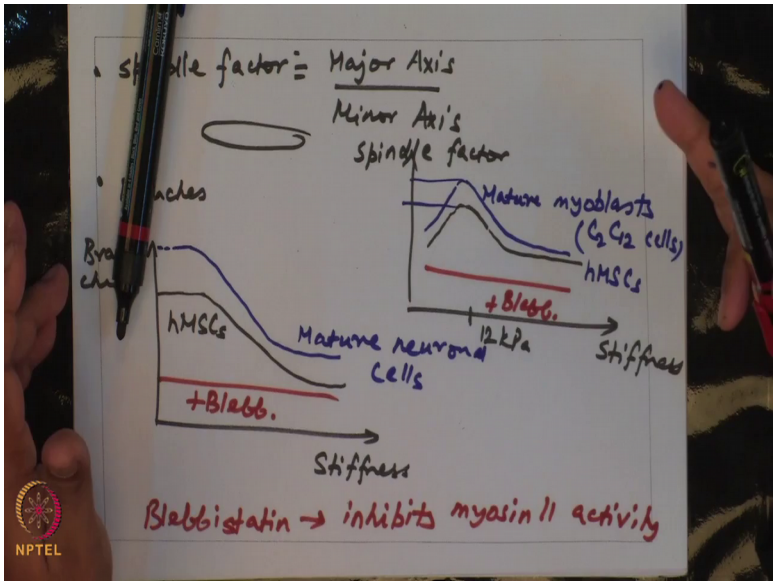

So, what is spindle factor it is nothing that if you approximate a cell shape like an ellipse; spindle factor is major axis by minor axis. So, the higher the value means it is more elongated in one direction. And what they also tracked was spindle factor also tracked the number of branches, average number of branches per cell. So, what they found was in terms of branching in terms of branching you had the maximum branching on the soft surfaces and then there was a drop. They observed the identical behavior with neurons mature neurons. So, this is matured neuronal cells, these are your hMSCs. So, you had maximum branching on the softest surface; not only that, if you plotted the spindle factor, this is your stiffness axis and this is your spindle factor. So, what we found so this is your 12 kPa which roughly correlates with stiffness of muscle tissue.

What they found was, so similar to that exhibited on similar to maximum branching on the softest surfaces. So, on this intermediate stiffness surfaces hMSCs exhibited the maximum spindle factor and this was similar to that of mature muscle cells, mature myoblasts which was c 2, c 12 cells. So, you had maximum elongation on the intermediate stiffness surfaces. Interestingly, when they treated the draw cells with the blebbistatin, so this is plus blebbistatin, what does blebbistatin do, inhibits myosin two activity.

So, suggesting that these morphological indices, they have not only correlate with in vivo stiffness, but require myosin two generated contractile forces for sensing. So, when you inhibit myosin two any kind of shape changes is completely eliminated. So, these quantification at early time points. So, the authors went onto do the transcript profile.

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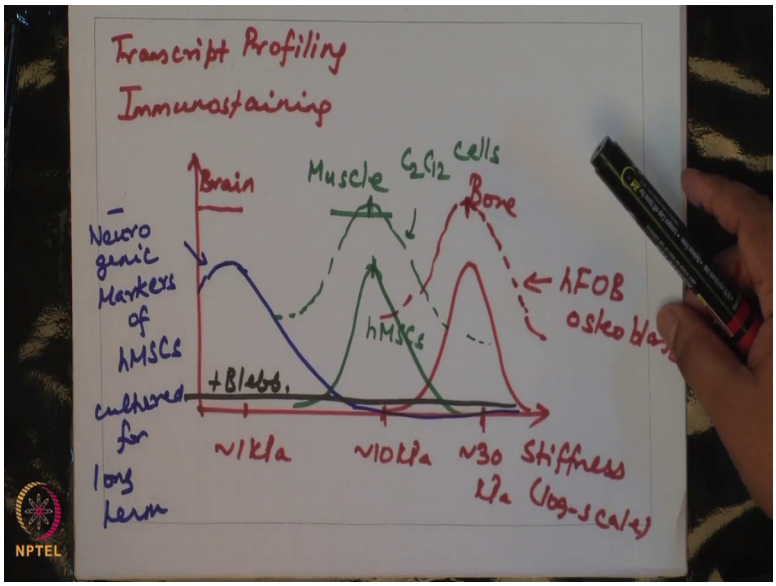

The authors did transcript profiling and immunostaining. And what they observed. So, if I were to plot as a function of stiffness. So, you have three zones, the softest zones in which you have brain pneumatic. So, it mimics the brain soft range. So, this is in log scale. You have a zone which mimics, so this is order 10 kPa order, 1 kPa an order 30 kPa. So, let me draw with different colors for each of the three things. So, the brain signal the muscle signal of neurogenic marker expression. So, these are the neurogenic markers of hMSCs which have been cultured for long term. So, what you find was on tissues or on substrates which mimic the brain tissue the hMSCs tend to over express neurogenic markers on substrates which mimic the muscle tissues stiffness you have a corresponding peak. So, this is your muscle, the third one is your bone.

So, on soft substrates the hMSCs exhibit neurogenic lineages; on intermediate surfaces the cells exhibit muscle specific lineages; and on stiff surfaces they exhibit bone specific lineages. Again if you treat with blebbistatin, so it is plus blebb, it inhibits differentiation across the board. Now, does this mean that this earlier differentiation is enough to become functional muscle. So, what would be the profile if you played the same for a muscle cell line. So, what you find is for a muscle cell line, so it exhibits the same peak at the same place which is ordered 10 kilo pascal, but muscle expression. So, this is of c 2 c 12 cells, which is skeletal muscle cells.

The average expression of c 2 c 12 cells is higher than hMSCs, but both c 2 c 12 cells and hMSCs exhibit the peak at the same point which is 10 kpa. So, on muscle elasticity hMSCs over expressed muscle like exhibit a muscle like phenotype, but they are not yet functional muscle same on the stiff surfaces. If you plot the bone signals for a committed cell line. So, these are hFOB osteo blasts again the expression of hFOB osteo blats for bone specific lineages are much higher than that of hMSCs. However, the trend is the same we observe peak when cells are plated on bone like surfaces. So, this raises the question, so if physical factor is so important it has chemical factors not come into the play. This is counter to what most people think that that chemical factors are all that that regulates stem cell fate.

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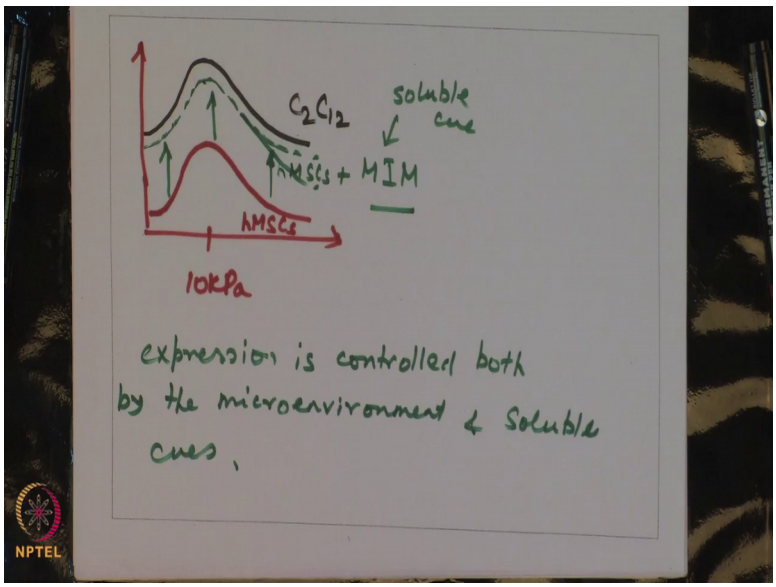

So, what the authors did was they did experiments in which so this is your MHC signal let us say this is your MHC signal with a peak at 10 kPa. When you do it for committed muscle cells c 2 c 12 cells, what you see is. So, this is for c 2 c 12 cells which are muscle cell lines and this is your hMSCs. If you add a muscle induction factor the expression profile, so this is hMSCs plus muscle induction media. So, this is your soluble cue this suggests that when you add a soluble cue your state increases in a stiffness independent manner. So, you just have shifted the baseline across all the surfaces, but the peak remains the same, the peak is the physical signal. And when you add muscle induction media together with the appropriate stiffness, what you see is on these surfaces, your signal is very close to that of a c 2 c 12 cell line. So, this shows that your final expression

is controlled both by the micro environment and soluble ligands, soluble cues. So, this is an example where two signals had a synergistic effect.

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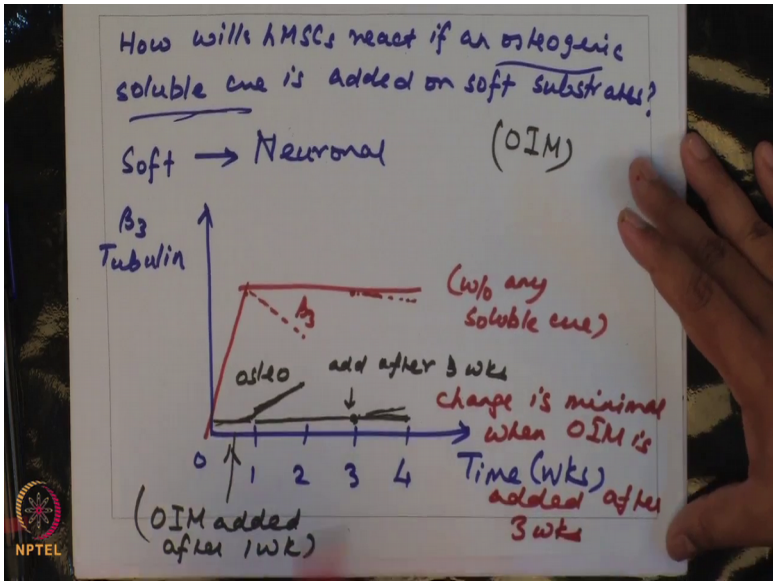

What would happen if you have two signals which oppose each other. What would happen, so the question is how will hMSCs react if an osteogenic signal soluble cue is added on soft substrates. So, on soft you know the cells has a. So, from the physical cue the cells tend to differentiate into neuronal lineage. And you are confusing the cell by putting a osteogenic soluble factor. So, what the study demonstrated. So, this is time in weeks; and you have for a 4 week span. If you were to track beta 3 tubulin, which is a neuronal marker.

So, what does authors observed was neuronal marker increased in a span of 1 week and stayed flat. So, this is without any soluble cue. If the authors added osteogenics CBFA alpha 1, if the authors added osteogenic media, so OIM is osteo induction media at 1 week what the authors observed was after 1 week, you have osteogenic factor increasing and adipogenic neuronal factor decreasing. So, this case corresponds to OIM added after 1 week. So, yeah this was baseline and you have an increase in OIM. So, this is osteo. So, this is an osteo signal and your beta three tubulin keeps going down there is a drop.

So, drop in neuronal marker increase in bone marker. However, if you add after 4 weeks, if you add after a long time, so what you see is later time points we are add after 4 weeks or you add after 3 weeks. So, from this point, there is hardly any drop and there is hardly

any increase in osteo marker. So, this change is minimal. So, change is minimal when OIM is added after 3 weeks. So, this shows that this is a dynamic process. So, the strength if you mechanically condition the cells for 3 weeks, they are committed enough then the osteo signal is not going to have much of an effect. So, once again as I said that if you inhibit with blebbistatin, there is no differentiation. So, suggesting the differentiation is myosin 2 dependent.

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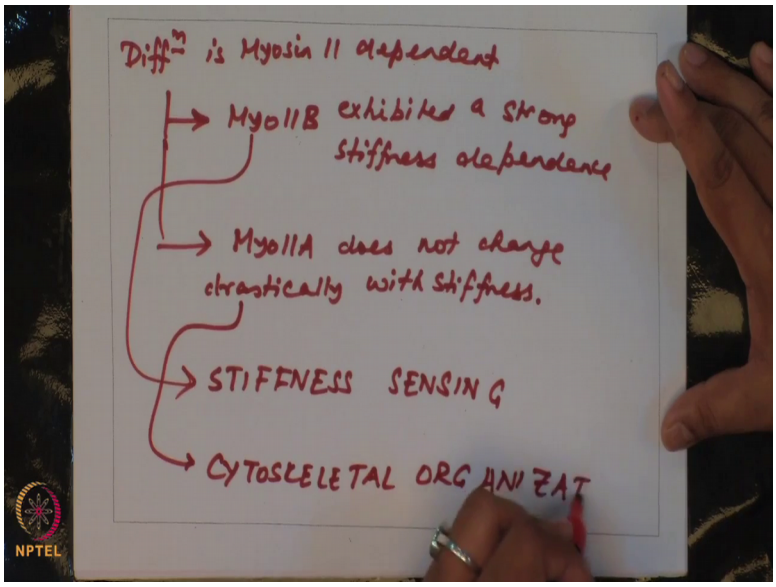

So, this differentiation is myosin 2 dependent. So, what the authors found was myosin two b exhibited a strong stiffness dependence in comparison to 2A does not change drastically with stiffness. So, suggesting that probably 2B is associated, so 2 B is associated with stiffness sensing and 2A probably contributes to the cytoskeletal organization.

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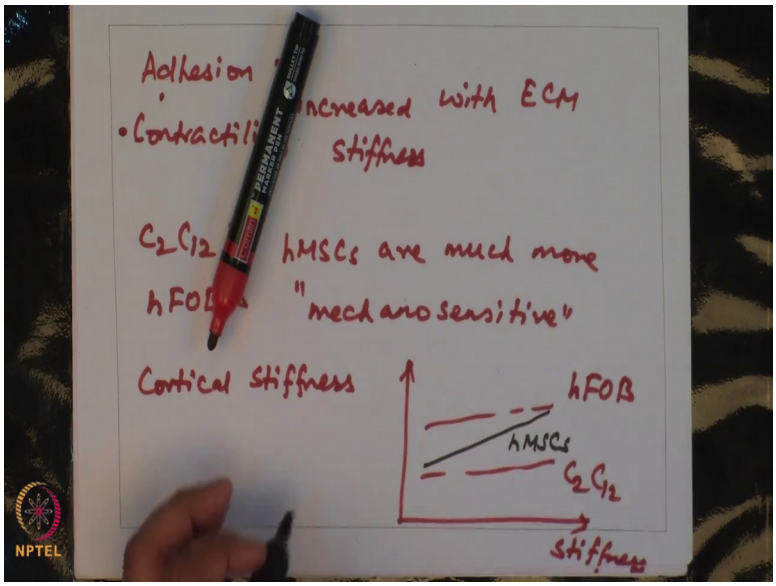

So, what the authors also demonstrated was for hMSCs both adhesion and contractility. So, both these terms increased with ECM stiffness. So, you see stronger additions and stronger additions are required to stabilize stronger contractile forces exerted by the cells. What the authors also observed was in comparison to mature cells like c 2 c 12 or hFOB, so these are muscle cells these are osteoblast, stem cells hMSCs are much more mechano sensitive. So, they reach these observations by probing the cortical stiffness of these cell lines. And what they observed was so if this is my stiffness axis, so these are c 2 c 12 cells and this is the response of hFOB. However, your stem cells exhibit a intermediate phenomena. So, hMSCs they possess properties of muscle cells on softer surfaces and possessed properties of hFOB comparable to that of hFOB stiffer surfaces. So, this suggests that the stem cells are more mechanosensitive.

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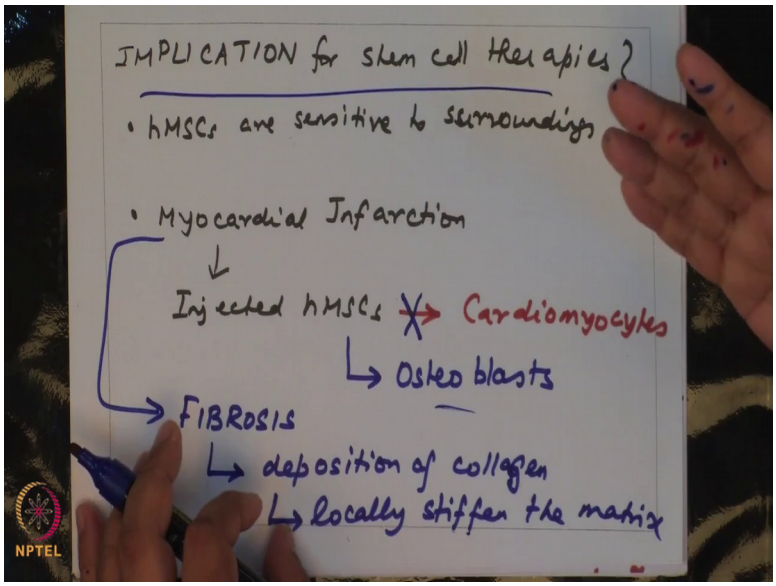

So, what is the implication. What implication does the study have for stem cell therapies. So, first of all if hMSCs are sensitive to surroundings, so then where you are injecting will have a dramatic effect on what the hMSCs differentiate into. So, there was one study in which they did they simulated a myocardial infarction injected hMSCs. So, ideally, so you ideally want the hMSCs to differentiate into cardiomyocytes. However, to their surprise the author showed observed that the hMSCs were not differentiating into cardiomyocytes were differentiating into osteoblasts.

So, this would be completely dangerous and the reason why the cells were found to differentiate into osteoblasts was because whenever you have myocardial infarction you have fibrosis. So, fibrosis is lot of deposition of collagen. So, this would locally stiffened the matrix. So, consistent with this particular study, so hMSCs then would think that they are in a more stiff environment and they will differentiate into osteoblasts. So, this is of key relevance. So, the implication for stem cell therapy is humongous.

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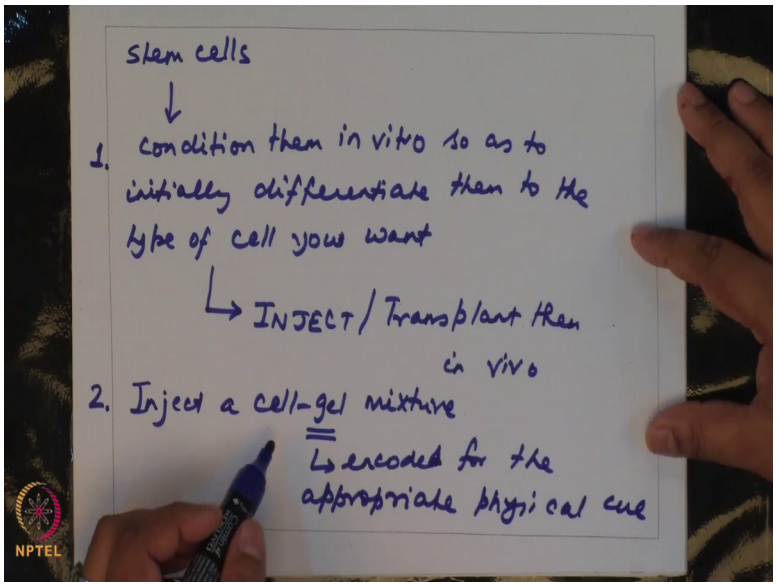

So, you cannot just inject stem cells anywhere and you expect them to form the a probe or the differentiate into the appropriate cell type that you want. So, one way to get around this problem is first of all what you can do is you take stem cells you condition them in vitro. So, as to initially differentiate them to the type of cell you want and then once you have observed this kind of differentiation in vitro then you can inject them inject or transplant them. So, this might be one strategy. The other strategy is what you do is you inject a cell gel mixture. So, such that the gel encodes for the appropriate physical cue that you want and with the hope that once the cells are differentiated enough they can remodel the gel in the way that is possible.

So, with that I stop here. In the next class, we will get started with mechanobiological regulation in case of diseases will start with cancer and how mechanics is relevant to cancer.

Thank you for your attention.