Introduction to Mechanobiology
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Week – 08
Lecture – 38
Techniques in Mechanobiology: Traction Force Microscopy, Trypson Deadhesion and Laser Ablation

Hello and welcome to our today's lecture of introduction to mechanobiology. So, since the last two lectures I have started discussing of some of the tools and techniques that are useful for studying mechanobiology and in the first lecture I introduced about hydrogels.

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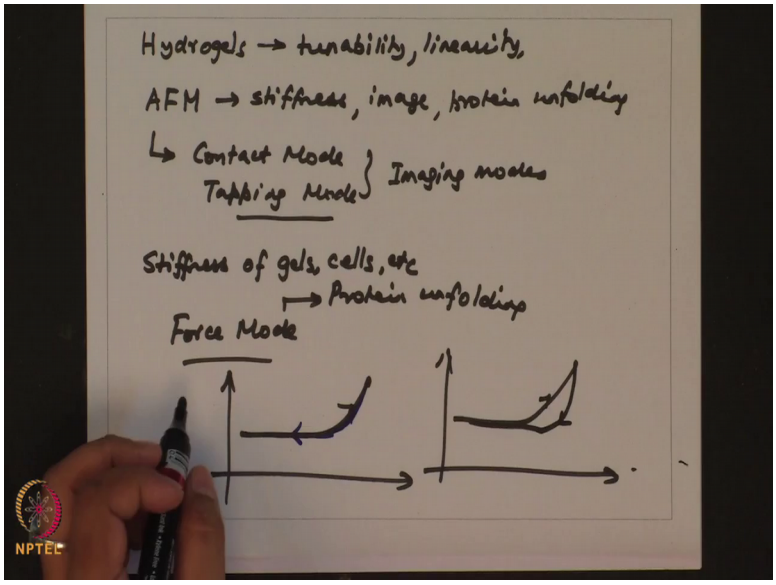

What are hydrogels? What are their properties? Particularly what are some of the properties that you need to follow in order to make them in terms of tunability, linearity of stiffness, etcetera. After hydrogels I introduced AFM once more, but this time I introduced it how to measure properties of in how to measure stiffness of samples with AFM and also to image. So, in AFM you have once again you could do protein unfolding as well. In AFM, you could operate it in contact mode or tapping mode these are two imaging modes.

So, these are two imaging modes; contact mode being more suitable for flat rigid samples where there is no chance of damage to the sample because you are in perpetual

contact with the sample and you have the tapping mode in which your intermittent contact and what you control is the amplitude set point. So, this is tapping mode is more suitable for imaging cells which are loosely adherent and can be dislodged upon lateral friction. And for measuring stiffness of samples of gels, cells, etcetera you use a force mode of operation. So, this is the same mode you also use for probing protein unfolding.

And I had mentioned that in these depending of your material is elastic you get a curve which follows the indentation. So, the curve is indistinguishable between the indent and the retract zones and if the material is viscoelastic like a cell you get a curve like this, but there is one path of indentation and one part of retraction; suggestive of viscous damping.

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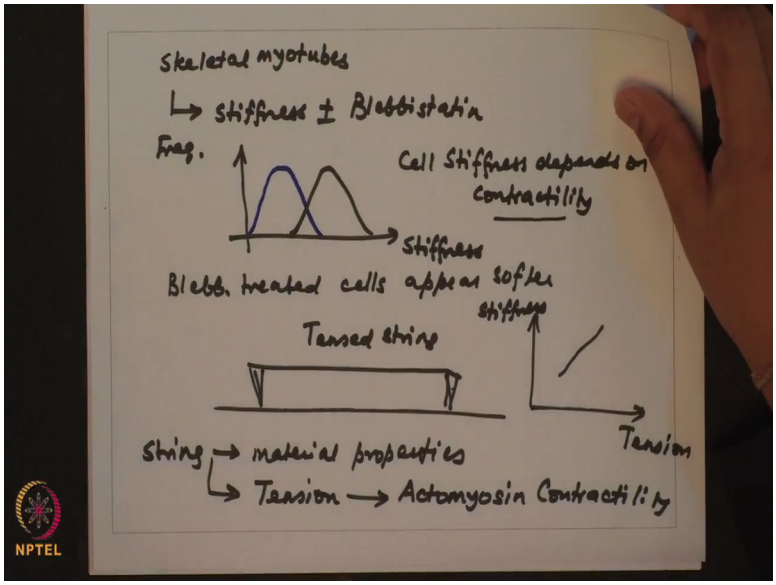

So now, one thing when you measure the stiffness, what has been observed let us say if you take skeletal muscle tubes; if you take skeletal myotubes and you measure their stiffness plus minus blebbistatin. So, what you would observe is in the presence of blebbistatin. So, this is your stiffness axis and this is your frequency axis, this is your output data. If this is how your stiffness distribution looks in control or untreated cells in blebbistatin treated cells the curve will shift to the left or in other words the cells will appear softer. So, blebb treated cells appear softer, but how do we think about it. Why is blebbistatin with inhibits myosin to activity lead to a software cortex? And the reason is as follows.

So, if you think the cell as a tensed string, which is anchored at two points on a substrate. So, what you will see? So, the string has its own material properties on top of which you are tensing the string, you are adding some tension in this string. So, what is the source of this tension? If you anchor it to the substrate the source of this tension is actomyosin contractility . So, if you inhibit the tension. So, in a sense if I relate the tension to the overall stiffness that you measure they should be a straight line.

So, greater amount of tension you impose. So, if you pull the string then if you try to deform it you need extra force to deform it, in other words the string appears to be stiffer. So, this tells you that cell stiffness depends on contractibility. So, this suggests that in a sense you can get an idea of how contractile a cell is by measuring its stiffness. However, you will not see the effect of contractility on stiffness if your cortical contractility does not change, is unchanged.

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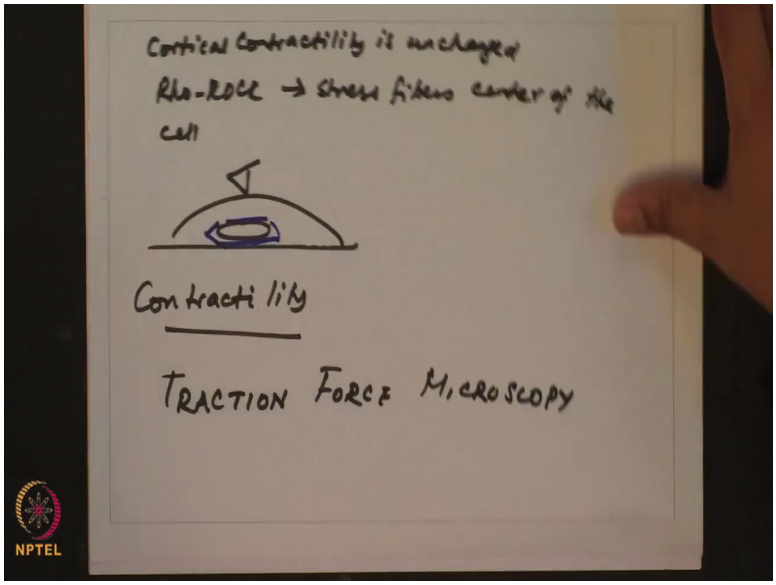

But you have up regulation of rho or rock such that there is stress fibers which are built up in the center of the cell. Why because, if this is your nucleus you are probing from the top. So, if you have stress fibers which are right next to the nucleus you cannot these will not contribute as much to the overall stiffness that you are proving.

So, they has to be a different way of probing contractility independent of measuring stiffness and one of the most well established ways of probing contractility is traction force microscopy. So, let me explain what exactly is TFM. So, if you have a cell sitting

on a substrate of given stiffness E and what I can do is let us take a section of the cell here.

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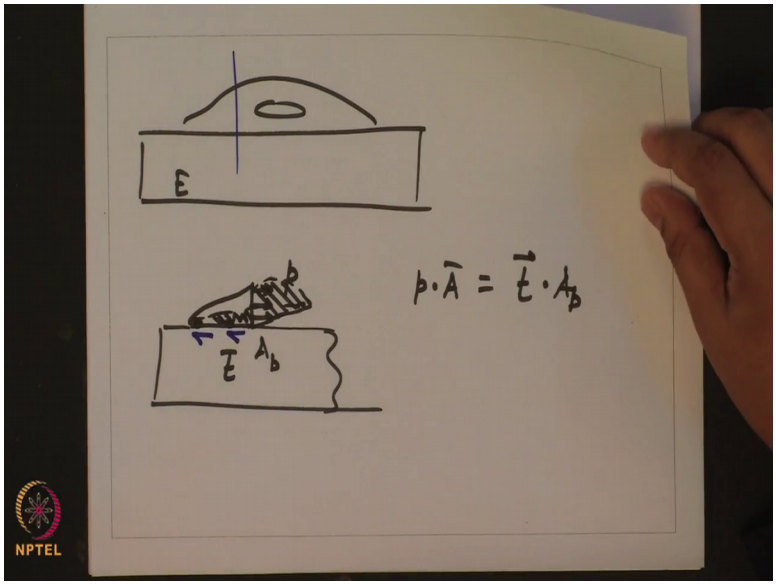

So, what you have is a section of the cell, the cell is exerting internal forces. This is the source of your p stress p and these forces are stabilized by traction at the substrate at the interface of cells and gels transmitted to the focal adhesion. So, this is your t bar. So, you must have a force balance required by p. So, if I were to draw the whole cell in this way, this is a three dimensional surface then p into this cross sectional area; let us say A bar must be equal to t dot this base area A b; A base. This is the force balance and this is the basic ideology of doing fraction force microscopy, but how do you measure these fractions or these forces on the substrate?

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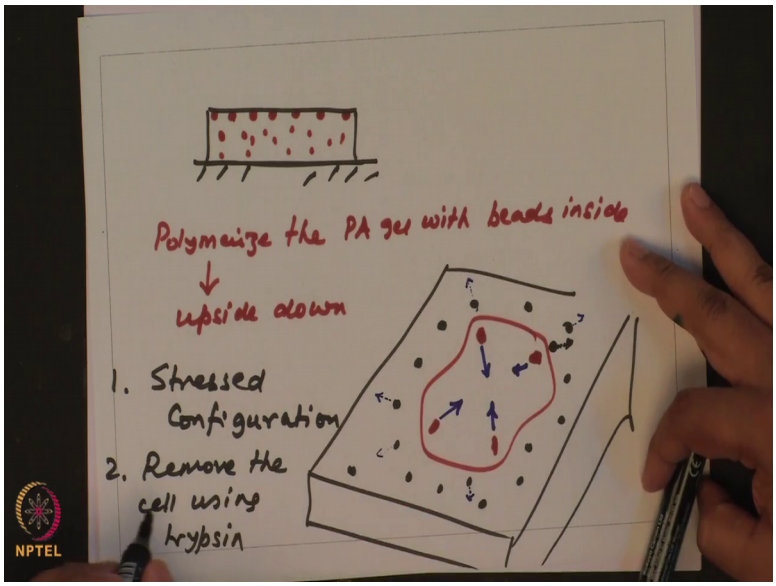

What is typically done is in the PA gels or PDMS cells that you fabricate, you put some fluorescent beads on the surface.

So, you might and how do you get these beads on the surface? You polymerize it, polymerize the PA gel, polyamide gels with beads inside. So, there are of course, a lot of beads in other planes as well, but you are more focused and you polymerize this gel upside down. So if you polymerize the gel upside down then because of gravity all these beads will remain at the top surface of the gel. And then, so if I were to draw the cell in 2 D and this is your substrate. So, you have these beads sitting in an around the cell, on the top surface. So, if there were individual focal adhesions through which the cell is exerting forces inside if let us say these are four focal adhesion through which cells are exerting forces in the given direction.

So, what you do? After you played the cells on these substrates you take one image in the stressed configuration. So, imagine these black dots are the positions of the beads in the stress configuration. Then what I do? I remove the cells using trypsin. So, if these were the original directions in which the cell was exerting forces, once I remove the cells these beads would move in the output directions, but the beads which are far from the cell. So, this bead may not have effective any displacement, but these beads which are positioned close to the cell will move in output directions.

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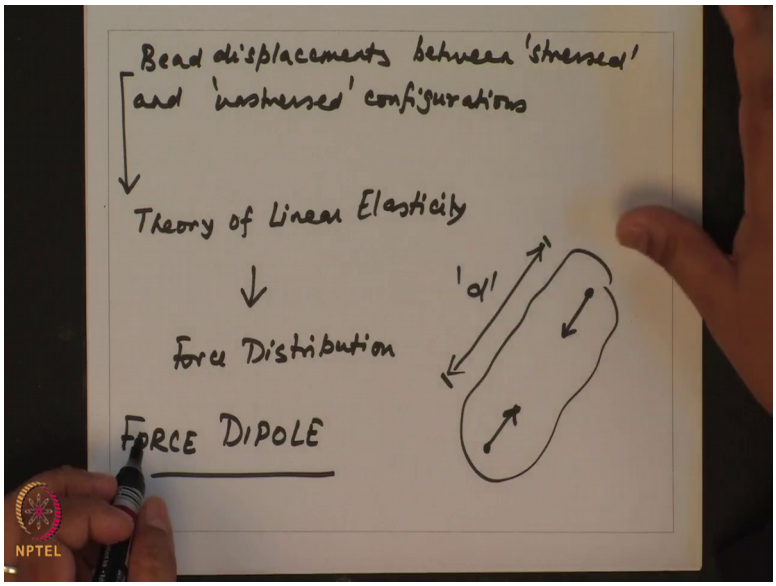

So, from this what I can do is I can track, what is the bead displacements between stressed and unstressed configurations? So, unstressed corresponds to the configuration when the cell has been removed and stressed corresponds to the configuration if this is the cell is actively exerting forces on the cell. So, using this once you get the bead displacement then, you can use theory of linear elasticity to find out what is the force distribution? And these experiments have been done for multiple different cell types as a consequence of which say for example, if this is a muscle cell, you would find broadly two bead forces which are equal and opposite in direction, but the points of exertion of these forces are separated by a distance d.

So, this is for a muscle cell. So, this corresponds to what is called as the force dipole. So, this is an example in which you can exert contractile forces along one direction. This is applicable for cells which are elongated like fibroblasts or muscle cells.

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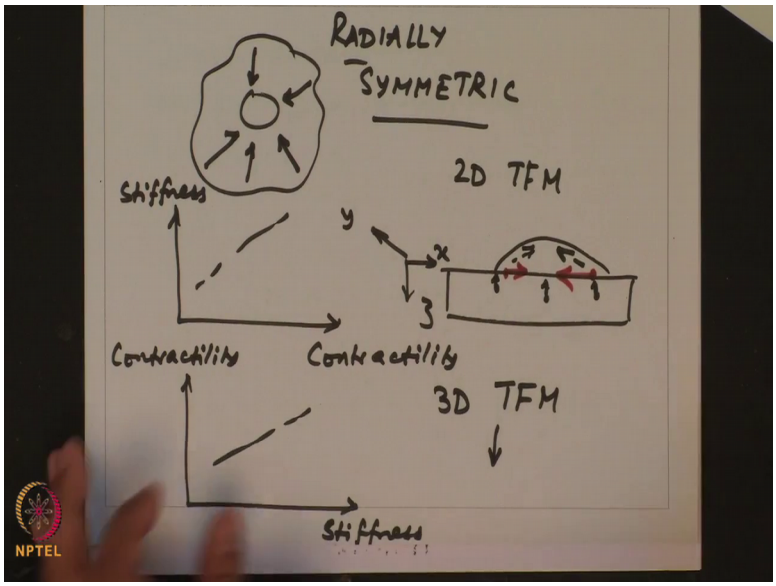

If you were doing the same experiment for your osteoblasts, so which is a more the morphology of an osteoblasts is more polygonal in nature and if you do the force distribution, you would see the force distribution as radially symmetric and using these experiments a lot of people have shown that again that there is a linear relationship between contractility and stiffness; you should have a straight linear relationship.

So, this means that if you plate. So, one more observation has been for gamut of cell lines, if you do with stiffness substrate stiffness you always see contractility increasing contractility exhibits increase with stiffness. So, this approach is very powerful, but in this particular type of traction force microscopy that we discussed this is called the 2 D traction force microscopy. So, what it assumes is when you have a cell on a substrate that cells are exerting inward direction forces in the x y plane. So, this is your x y plane and this your, so you have x, y and z. So, all the forces are being exerted by the cell in the x, y plane which is not necessarily true. So, here you do not account for bead displacements which are in the z direction. So, instead of pulling like this, you could very well imagine the cell exerting a force like this in that case there will be z displacement of the beads as well.

So, this is what is taken into account in 3 D traction force microscopy where, cells are embedded in gels and you have beads in around the cell in all different directions and you track the x, y, z movement of the beads. So, this is of course, way more complicated

and it requires important particle tracking algorithms to find out, to detect the position of individual beads and to track their displacement. So, one of the caveats the challenges with traction force microscopy is imagine how can you do use traction force microscopy if cells are cultured on glass?

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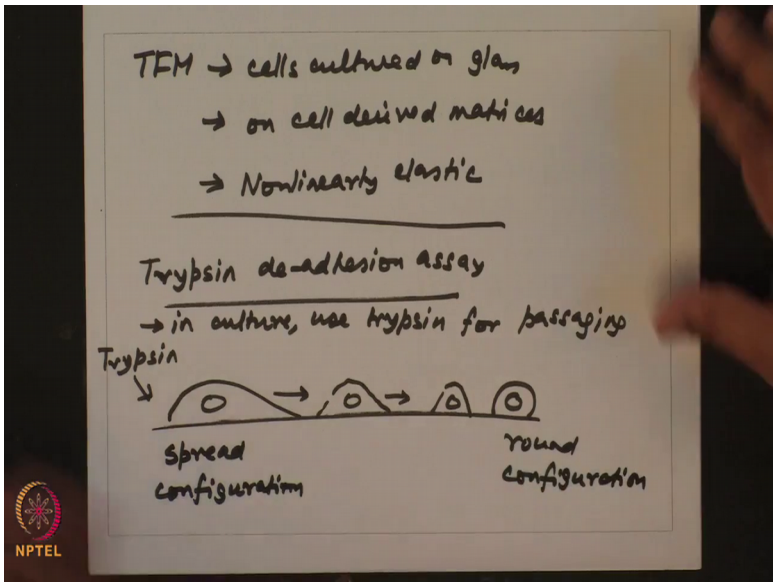

Or let us say on cell derived matrices, so where there is no way of adding beads into the material or one more example is if your material is non linearly elastic because the formulation for traction force microscopy assumes linear elastic substrates. For all these cases, so there is a very simple alternative approach which you might follow is called a trypsin deadhesion assay. So, what is the trypsin in deadhesion assay?

So, this is what you do leadingly get. So, in culture you use trypsin in for passaging. So, if you have a cell which is an adherent and you add trypsin give then at some point of time it should round up and in between you would have a range of different configurations where, the extent of rounding up is increasing. So, cell goes from a completely spreads configuration to a round configuration.

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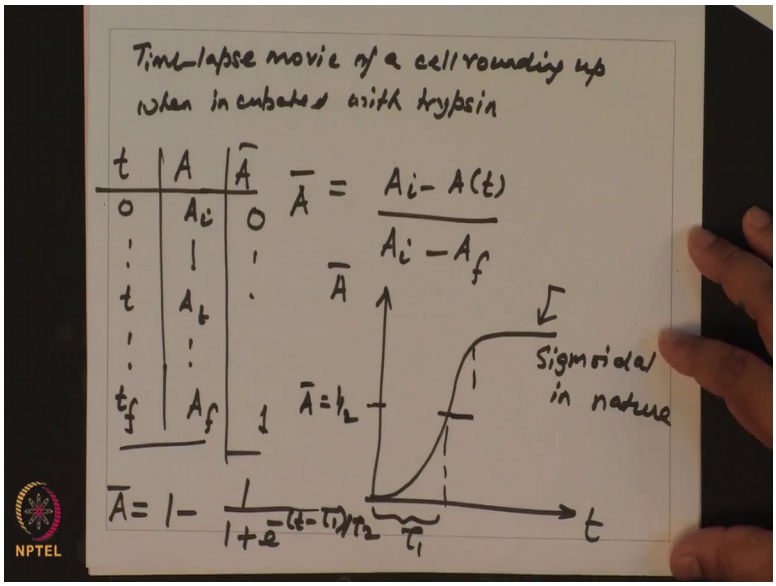

So, you can actually track the dynamic. So, what if you take a time lapse image, time lapse movie of a cell rounding up when incubated with trypsin. So, what you will have is a plot of time and area A. At t equal to 0, the initial area of the cell is A, at some time t it is some A t and finally, at some time t f, you have a final of A f.

So, I can define this normalized change of area defined as, I can define this normalized quantity A bar as A i minus A f time t by A i minus A f. So, if I do this then an if I plot A bar at t equal to 0 A f t is same as A i. So, you have a value which goes from 0 all the way to 1. So, if you plot A bar as a function of time, you would have a curve you are A bar curves look something like this and what has been observed is this curve is sigmoidal in nature. So, for a sigmoidal curve what you can do, you can fit this data by the following expression. I can have this particular function A bar fitted to this expression where, I can find out two time constants; tau 1 and tau 2 is.

So, what is tau 1? Let us see at t equal to tau 1, this entire variable becomes e to the power 0. So, that is 2. So, 1 minus half becomes half. So, tau 1 corresponds to the time at which you have reached a value of A bar equal to half and tau 2 is the time constant for the second portion of the curve. So, what has been observed? So, you can backtrack two time constants; tau 1 and tau 2.

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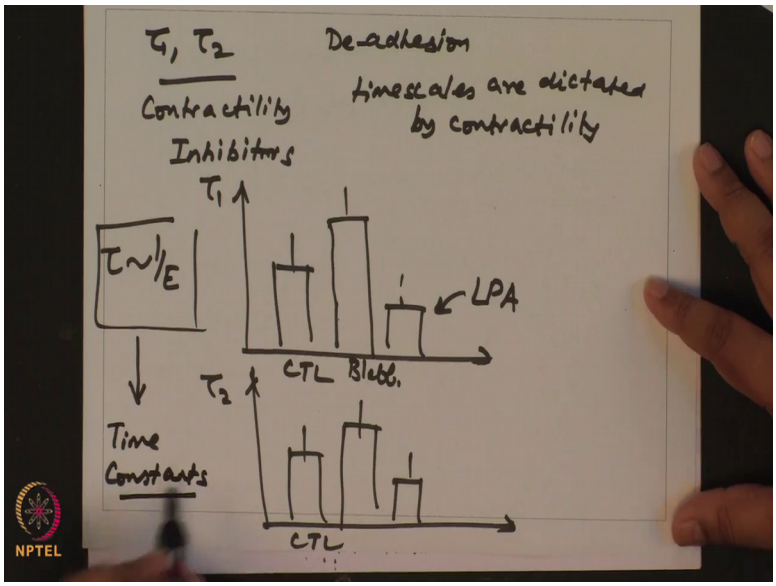

What has been observed is when you perturbed cells with contractility inhibitors. So, what you see? Let us say if this is the value of tau 1, let say this is my tau 1 and this is my tau 2; for control cells let say this is my value of tau 1 and tau 2 when, I treat them with drugs like blebbistatin like blebb which inhibits contractility both these time constants tend to increase. Again when I treat with drugs which promote contractility there let us say lights of lysophosphatidic acid which promotes the role of pathways then what you see is your tau 2 tau value is decreased. In other words deadhesion timescales are set, are dictated by contractility.

So, if a cell is more contractile in nature then. when you treat the tryspin little round of that much faster. So, what has been also observed is by correlating these time constants with the value of stiffness using AFM, what is observed is tau is generally inversely correlated with that of stiffness. So, for a cell which is stiffer and more contractile its deadhesion timescale will be lower. So, this is a very simple assay based on which you can estimate how much is the contractility. The caveat is, so one of the caveats is you are estimating contractility indirectly in terms of time constants and not in terms of actual amount of forces exerted by the cells. The other caveat is that tau is not only dictated by contractility, it is also dictated by adhesivity.

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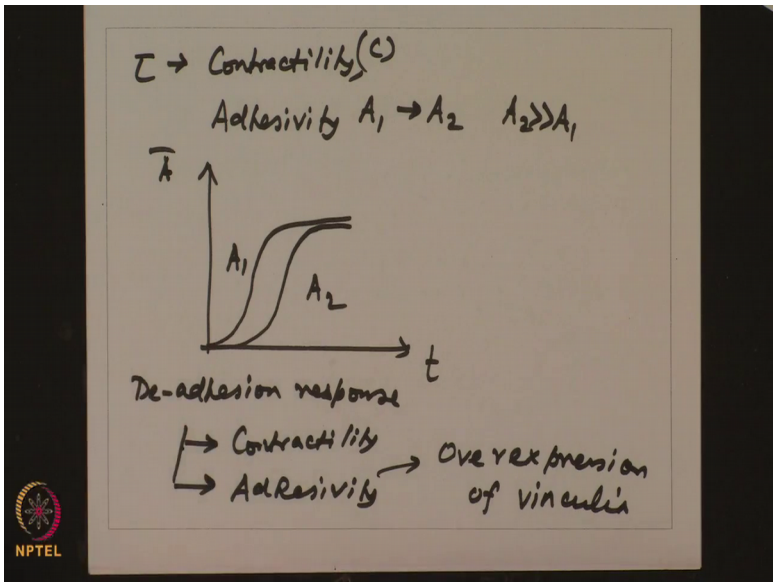

In other words, imagine I have two conditions in which contractility is constant; C is constant, but adhesivity changes from A 1 to A 2 where, A 2 is significantly greater than A 1.

So, what you will observe is if this is the profile, deadhesion profile for a given cell with A 1 as you make it A 2, so what you will see is this deadhesion will be delayed. So, this is for value of A 1, this is for value of A 2. So, in other words the deadhesion response is not only dictated by contractility, but also dictated by adhesivity. Now you can put up the adhesivity might change because of over expression of vinculin let us say, of a focal adhesion protein like vinculin which is a structural protein, but you can also change this by adding or adding trypsin which is ten times more active. In that ways the chopping rate of focal adhesion is going to be higher.

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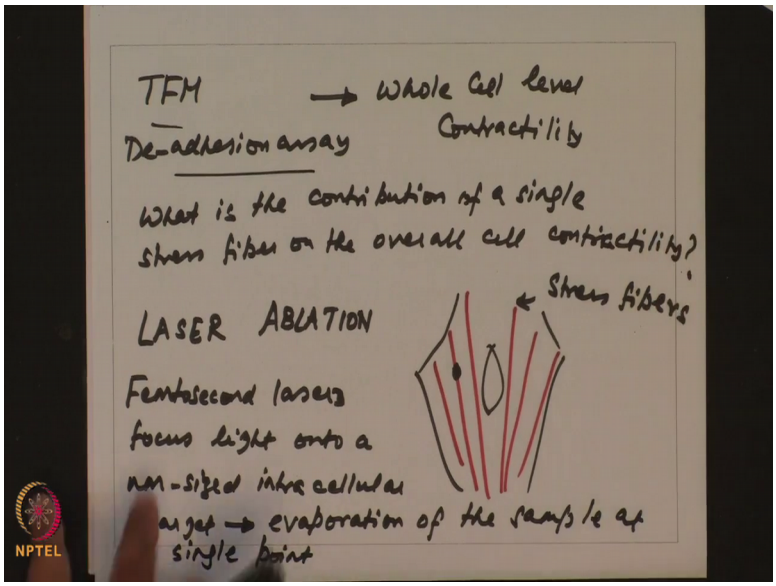

So, compared to traction force to both TFM and these deadhesion assay, they kind of estimate the whole cell level contractility. Is it possible to estimate the contractility in a single stress fiber? So, in other words, what is the contribution of a single stress fiber on the overall cell contractility? So, in these cases it is difficult to use TFM or deadhesion assay to estimate. So, there is a new technique which has been introduced called laser ablation. So, imagine you have a cell which is transfected with where you have transfected cells with Life Act, you can have very prominent stress fibers. So, these are very prominent stress fibers.

So, what laser ablation does is it focuses laser light at a single point on a stress fiber. So, the when you focus laser light on a single point on a stress fiber the energy is so high that locally the material just evaporates. So, using femtosecond lasers you focus light onto a nanometer sized intracellular target. So, this light is so focused this will lead. So, this will lead to evaporation of the sample at a single point, at the point where you have focused. So, what you will have?

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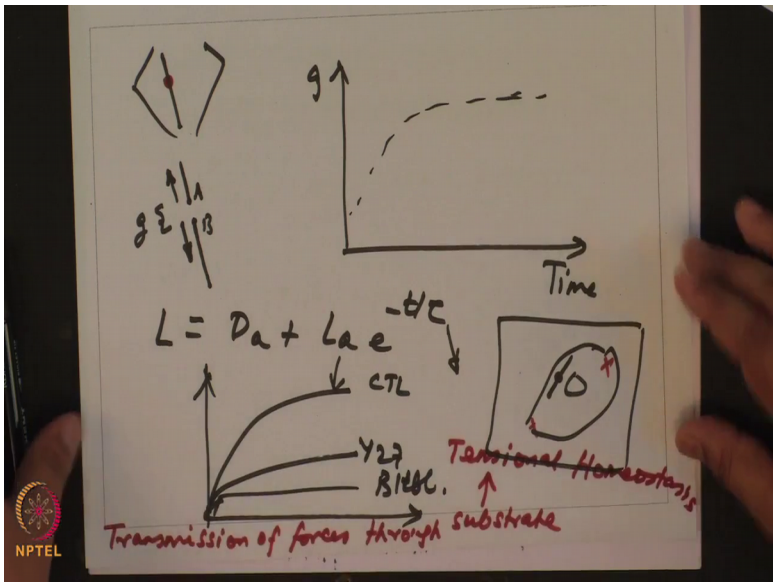

So, imagine I have the cell and this is one such stress fiber in which I had focused light at the single point. So, and you acquire the fluorescence images as a function of time.

So, what you will see is this stress fiber it will relax so both these ends A and B. So, you have you have locally disrupted this stress fiber, this point will retract along the line backward and this point will retract along this line in this direction. So, as a consequence this length if I actually track the gap g as a function of time, what has been observed is this g as a function of time exhibits a saturation profile like this. So, there is an instantaneous increase in gap and then it slows down to some eventual value.

So, just like previous case you can fit this data with let us say D a plus L a e to the power minus t by tau, you can get an estimate of the characteristic time constant of relaxation and also you can estimate this L a value. So, what has been observed is when you treat cells with contractile agonist, if this was the response for a control cell when you treat with (Refer Time: 28:06) 762, it drops with blebbistatin also it drops significantly.

So, this suggests that this is also an example of how contractility at the single stress fiber is being modulated. Using this experiments, Sanjay Kumar demonstrated that when a cell is sitting on a compliant substrate when you have a single stress fiber being ablated as a consequence of the single stress fiber because the substrate acts as an elastic link for two different points within the cell. So, when you even if you ablate a single stress fiber, the in there is global rearrangement in the cell as a consequence of the single ablation, but

this is markedly absent, if the cell was connected in a or cultured on a glass substrate. Suggesting that transmission of forces, so this experiment suggests that transmission of forces through substrate regulates the force balance in a single cell.

So, this regulates your tensional homeostasis or the force balance. So, that just to summarize today I have discussed three different techniques; traction force microscopy, deadhesion and laser ablation.

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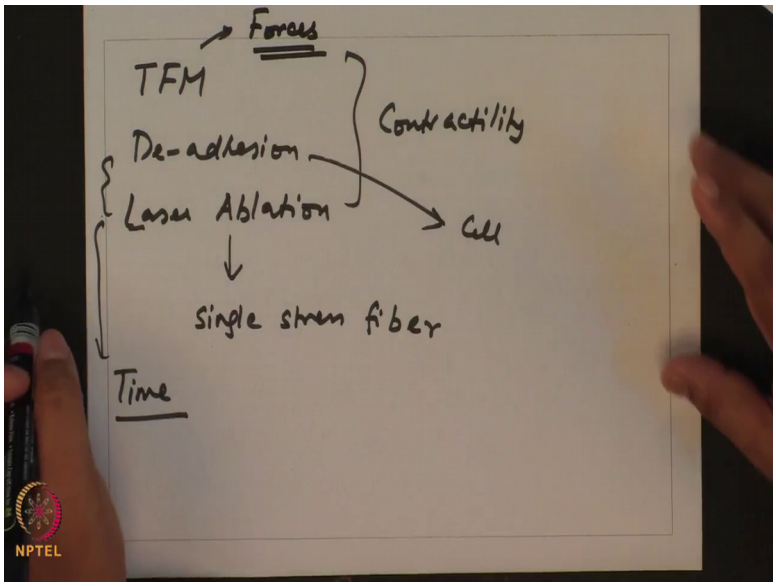

All of which provide some estimate of contractility. So, deadhesion provides the cell level contractility, TFM provides contractility information within the cell at different points and laser ablation also provides contractility, but form a single stress fiber. So, both these two techniques will provide this in information in the form of time. Leaser time is correlated with higher contractility while, in TFM you get actual estimate of forces exerted at different points within the cell.

With that I thank you for your attention and we will continue in next class with two other techniques; one is micro fabrication and the other is fret.

Thank you