Introduction to Mechanobiology
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Week– 08
Lecture – 40
Techniques in Mechanobiology: FRET

Hello and welcome to a last lecture of the course Introduction to Mechanobiology. In last lecture over the last 2 lectures we are discussing about various tools in Mechanobiology.

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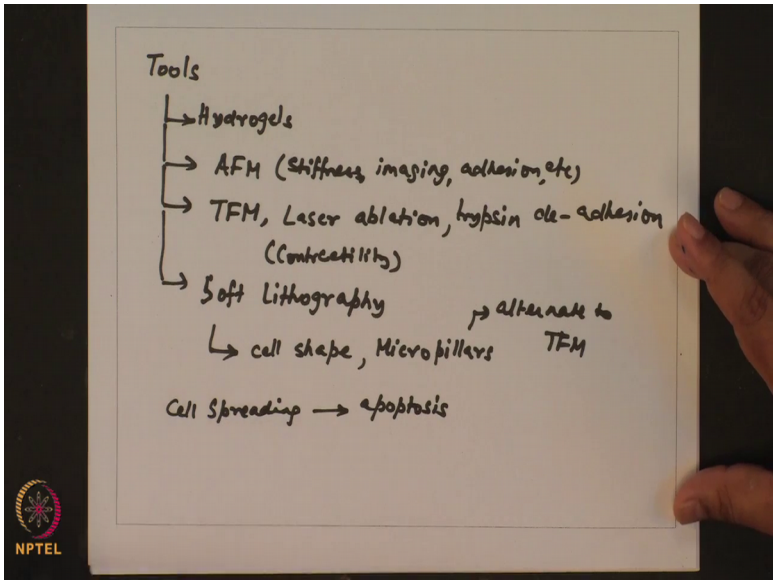

Among the tools introduced Hydrogels, AFM access like traction force microscopy, or laser ablation, or trypsin the adhesion for proving the contractility of cells AFM for measuring stiffness, of samples, for imaging, topography, for also doing adhesion measurements etcetera.

And the last class spoke about Soft lithography and using this you can regulate cell shape or you can use micro pillars, you can fabricate micro pillars for traction as an alternative to TFM. I had briefly talked about fun study which linked cell shaped or cell spreading area with apoptosis dictated by the extent of cell spreading. Similarly there are multiple other studies which are focused on using micro fabrication to glean insight into molecular processes.

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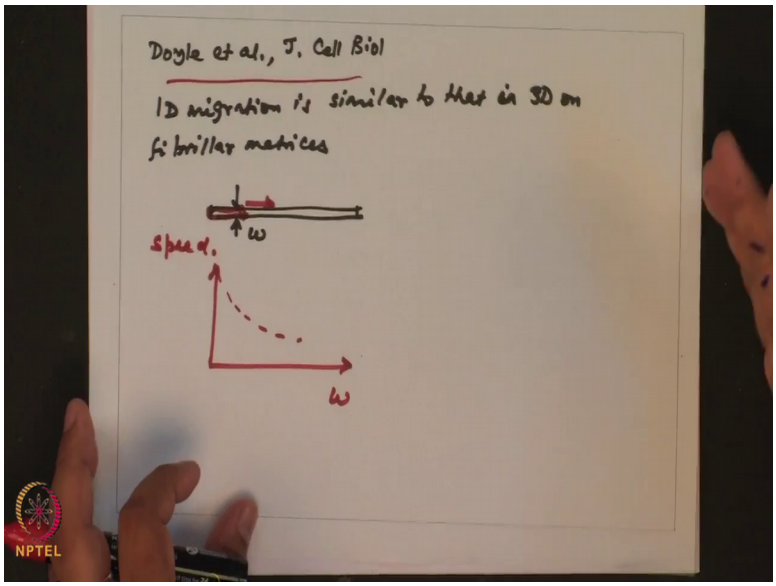

One other study by Doyle at all, published in J Cell Biol demonstrated that 1D migration is similar to that in 3D on fibrillar surfaces.

So, by fibril matrices I mean aligned collagen matrices are similarly. So, how did they go about doing that? What they did was they pattern these lines which varied in their width w and they tracked, you have these lines you plate cells on top of these lines and you track their migration and what they observed what they observed as a function of width. So, what the observed is a function of width was as the width increased, as the width increased there was a drop in the extent of migration. In 1D lines as your increase in width your speed dropped I do not want to discuss this study anymore, but I refer you to this paper it would give you insight as to how cells migrate on fibrural nutrises.

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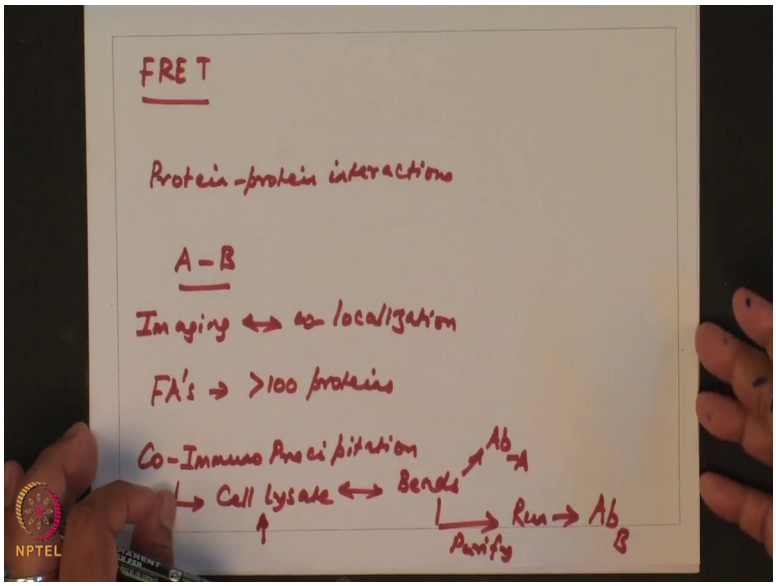

Today I would introduce another tool in mechanobiology, this is called FRET. What is fret used for. FRET is used for probing protein interactions. Essentially the question is how do you show that a protein A binds to protein B or like a lis10 to ligand interaction.

If you use imaging all you would get information about co-localization, but this is not enough to say and we interact because we know for example, at focal adhesions you have greater than 100 proteins which localized. It is not that each of these proteins is interacting with each other. So, one way to go about it to find out the interaction of 2 proteins is using Co-Immuno Precipitation. What is done in this approach is you collect the Cell Lysate and incubate with beads which are coated with antibody against a lysate.

We are proving the interaction of A and B. So, these beads will collect get rid of all those protein aggregates where A and B are attached to each other then you purify this component. So, you purify you run on a gel and then you can probe it with the antibody to B to C if you get a bend. So, this is of course, useful technique, but what this information what this technique does not give us information is about dynamic interaction. Because it depends at what time point you are collecting your lysate, but if this interaction is dynamic let us say different stages of time or specially regulated then you will not get this information because by collecting this you are getting an average response.

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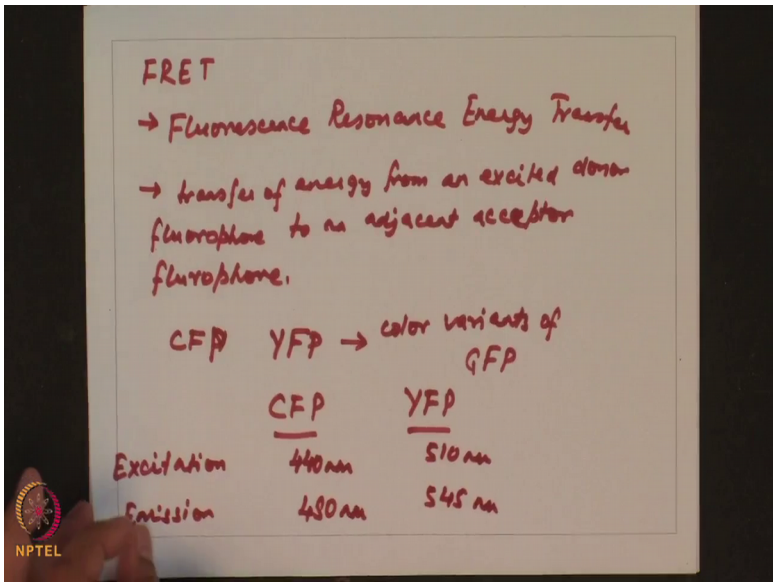

This is where the technique of fluorescence resonance energy transfer is useful. FRET is short form for Fluorescence Resonance Energy Transfer. And this involves the transfer of energy from an excited donor fluorophore to an adjacent acceptor fluorophore. Generally what is commonly used is CFP and YFP these are all variants these are color variants of green fluorescent protein.

What you have is for these 2 proteins these 2 fluorescent molecules CFP and YFP you are x Excitation is that 440 nanometers, Emission is that 480 nanometers for CFP, but the expectation peak for YFP is 510 nanometers, and the emission is that 545 nanometers . Now, what you can do. So, what I what he has been showed?

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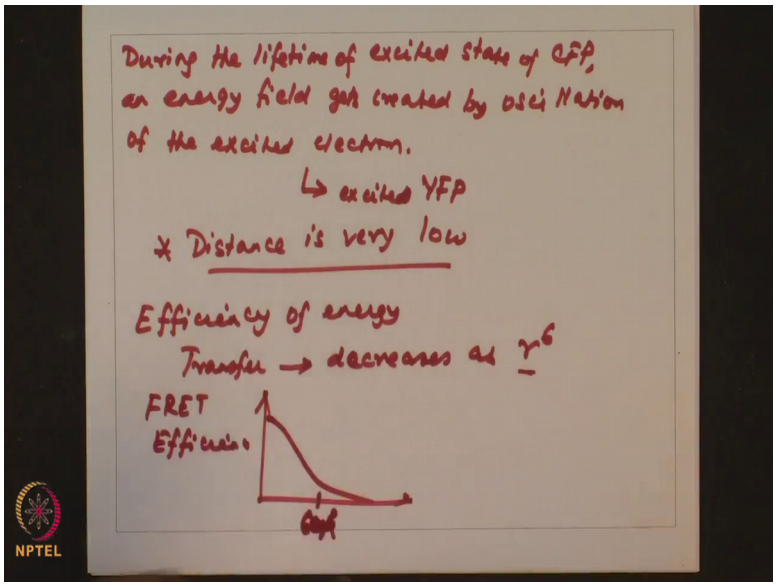

So, for if you have during the lifetime of excited state of CFP, an energy field is created gets created by oscillation of the excited electron. Now why if piece of this energy can then be used to excite YFP provided. There is one clause the distance is very low now how low is low. So, what has been shown that the efficiency of this transfer of energy you can have this decreases as r to the power 6 power.

Which means if r is the separation between these 2 fluorophores your energy will decrease reduce as r to the power 6. So, you have an efficiency FRET efficiency which falls off really fast and this fall off. Let us say beyond 60 nanometers 60 angstrom sorry 60 angstrom your fret efficiency is close to 0 60 or 70 angstroms. Essentially you are floor to floor for distance has to be less than 70 angstroms to be detected by this method. So, now, what do you do in this fret take?

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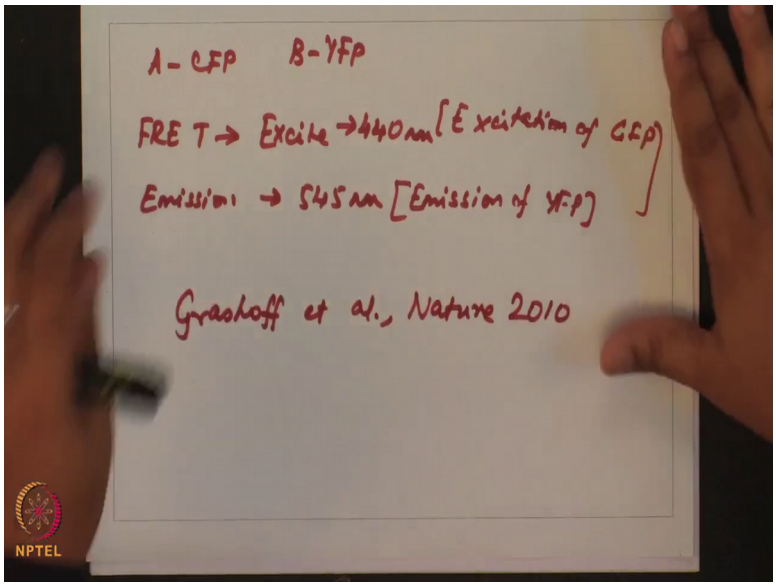

If you have 2 typed proteins let us say protein A which is stack to CFP, protein B which is stack to YFP. In FRET what you do you excite at 440 nanometers and you collect the emission spectra of 545 nanometers. So, this is emission of YFP and this is excitation of GFP. So, this fret efficiency will work this fret efficiency is good when these 2 molecules are very close enough of the order of 60 70 nanometer angstroms (Refer Time: 11:23). If they are so close then you can safely make an assumption that these 2 proteins are really interacting.

I will said that I would next come to how can we make use of FRET for mechanobiology studies in that regard I would like to discuss this particular paper grashoff at all this is a nature paper 2010 paper .

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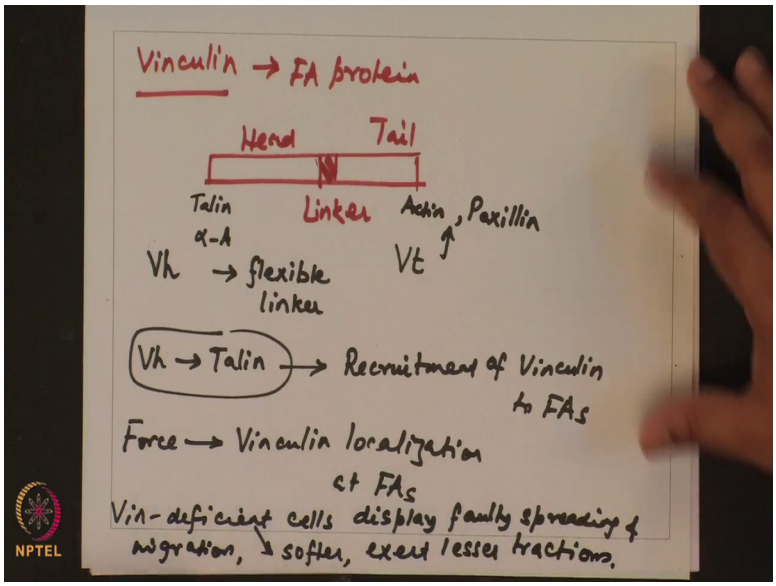

So, by now through this class you have got to know about protein called vinculin. So, vinculin it is a FA protein and if you want to look at it is structure, you have a head domain, and a tail domain, which are connected by a flexible linker. In the head domain you have binding partners like talin, alpha actinin and in the tail domain you have actin as one of the binding partners.

These head and tail let us a Vh corrsponds to the head domain and Vt corresponds to the tail domain if they are separated by a flexible linker now binding of Vh. When Vh binds to talin this event leads to recruitment of vinculin to focal adhesions and Vt as I said binds to actin and paxillin. Now what people have found that force is an important determiner of vinculin localization at focalizations, and vinculin deficient cells the display faulty spreading and migration also vinculin deficient cells are softer compared to controls and they also exert lesser forces.

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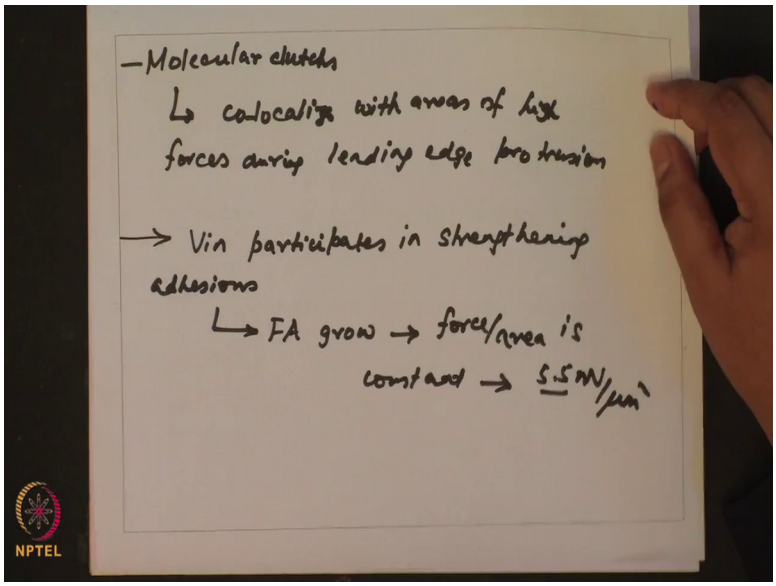

Now, vinculin is also assumed to be an important part of the molecular clutch and vinculin is known to colocalize with areas of high forces during leading edge protrusion. So, based on all of this people have thought that vinculin participates in strengthening adhesions such that if there is increased force then you have increased recruitment of vinculin. So, focal adhesion will grow in a way that force per area is constant this we discussed that paper we showed that you have on an average of 5.5 nano newton per micron square of focal adhesion. So, they wanted to understand what happens to the structure of vinculin when you expose it to force. So, in this regard they made use of a construct which they named as the tension sensor.

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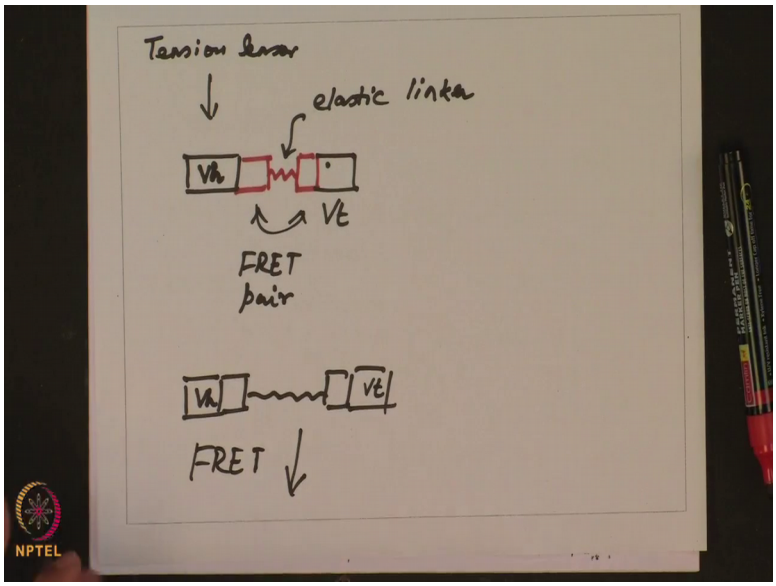

What is the tension sensor design? So, what they did. So, you have the tail domain of vinculin the head domain of vinculin let us say vt you have. So, this is a FRET pair and we still linked using an elastic linker. So, if x this is the tail domain head and tail domain is connected by this constructor tension sensor which has an elastic linker in between. If the elastic do the idea being that the if the elastic domain is stretched. So, when you have stretching of this elastic domain in this compression you are FRET is going to FRET signal is going to kolo. So, this is the idea of the tension sensor design.

What they first did was they wanted to evaluate the responses of this vinculin to cellular forces.

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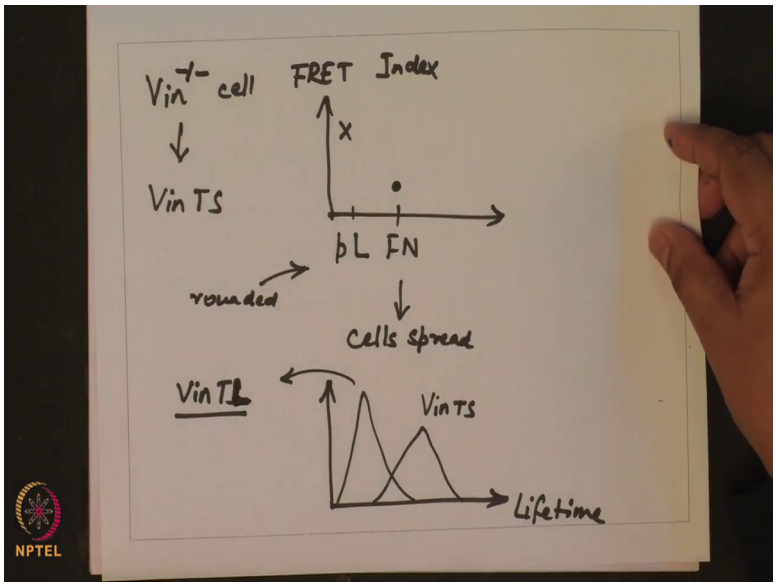

So, for that they first generated a vinculin minus cell which has does not have any vinculin of it is own and then they transfected it with this vinculin tension sensor is the construct. And what they showed what they showed was when they plated these cells and what they were looking at is the FRET index or the FRET efficiency.

When cells were plated on polylysine whatever with the fret index when they plated it on fiber nectarine the fret index dropped. So, this was one of the big things. So, on fiber nectarine the cells were spread. These results suggest that florescence you know under force. So, you know that once the cells spread out they will exert traction forces and so on and so forth, that should lead to a decrease in the fret index in polylysine surfaces cells were much more rounded and consistent with this the fret index was much higher you could also track the lifetime.

So, they track to the fluorescence lifetime of this and found the lifetime of. So, of the tension sensor this is my lifetime how long they exist at focal adhesions this was much longer compared to a control construct, which they call as Vin Ts sorry Vin TL contracts for Vin TL where only at the tail domain connected with the fluorescent construct.

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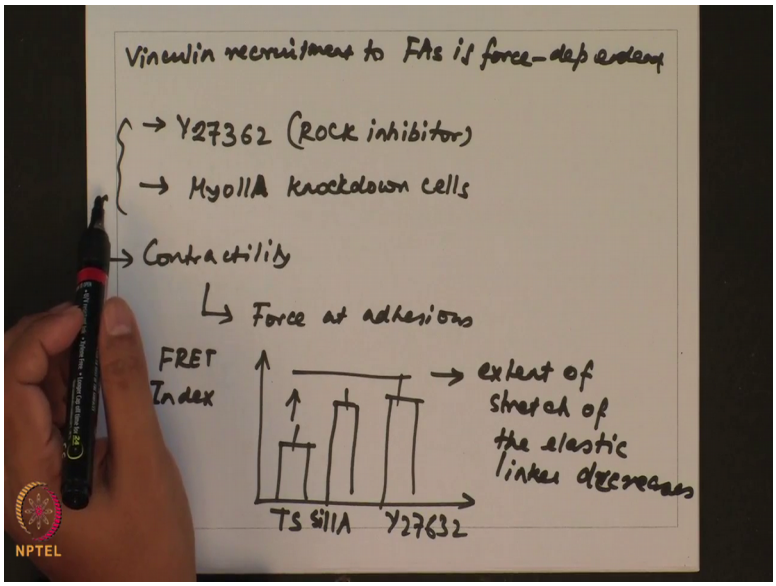

This was the first demonstration that when you play it on ecm coated substrates this vinculin is under tension. What did it next was that to demonstrate whether vinculin recruitment to focal adhesions is force dependent or not.

Whether recruitment correlates with force transmission what they did was they took cells and treated with 2 contractility fortur in drugs one is Y 2 7 3 6 2 which is the rock inhibitor and they used myosin to a knockdown cells. So, in both these treatments your contractility should go down. If the contractility goes down then the force at adhesions is supposed to go down. So, compact. So, consistent with this idea what they found was the when they tracked the fret index. If this was the average fret index when you have the tension sensor construct on the control conditions when they knocked down to a or Y 2 7 6 3 2. In both these cases the fret index went up.

So, the fret index went up means that the extent of stretch of the elastic, linker, decreases. This shows that there is this association between forces and it is localization during adh adhesion. The extend of the study to ask that how does vinculin localization or tension in vinculin vary during cell migration.

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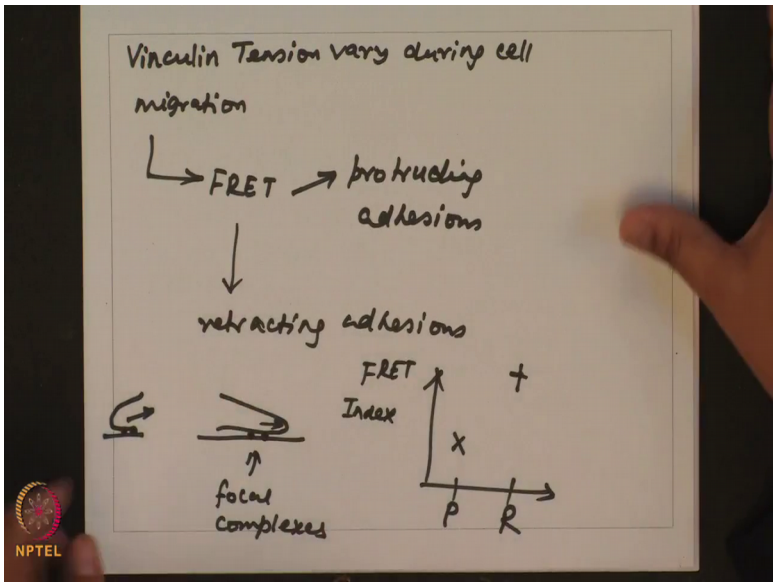

And for this purpose what there was they tracked the fret signal from the protruding side of the cell from protruding adhesions and retracting adhesions. As you recall that in the molecular clutch after the cell exerts the actimize actin dependent protrusion forces you begin to have the formation of these focal complexes at the leading edge, similarly at the trailing edge you have these adhesions which will break because of contractile forces at the rear. What they found was compared to the protruding edge and the retraction edge when they com compared the fret signal they found that the protruding edge the fret index was lot less compared to the retraction edge.

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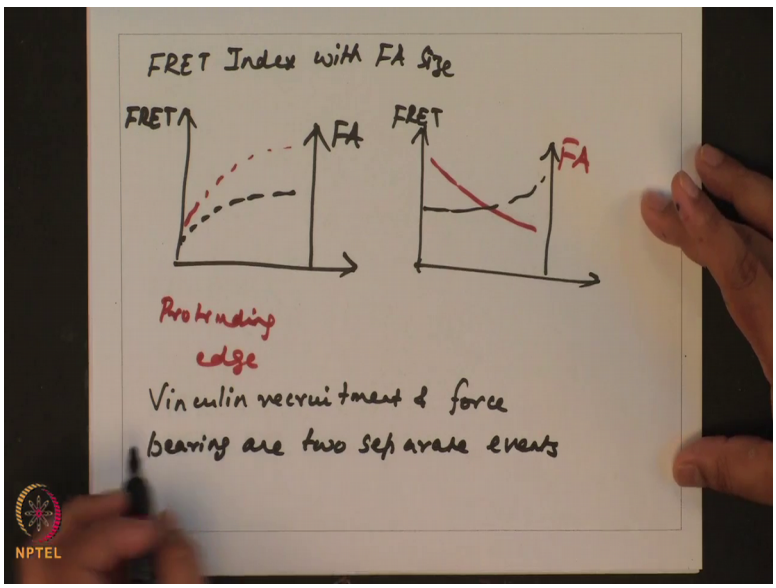

This shows that higher fret index means lower force. So, to look at this in greater detail what they did was they tracked they correlated fret index with focal adhesion size. And they found at the protruding edge they have 2 axes one is your fret efficiency and one is your focal adhesion size. So, found as a function of time if the fret index increased the focal adhesion size also grow. So, this was at the protruding edge at the other edge what they found this is positive correlation in this case. In the other case what they found was the fret index increased, but the focal adhesion size is decreased

These suggest that vinculin recruitment and force bearing at 2 separate events. So, this is a make major difference in the 2 separate portions of the cell. So, this concludes our discussion of fret.

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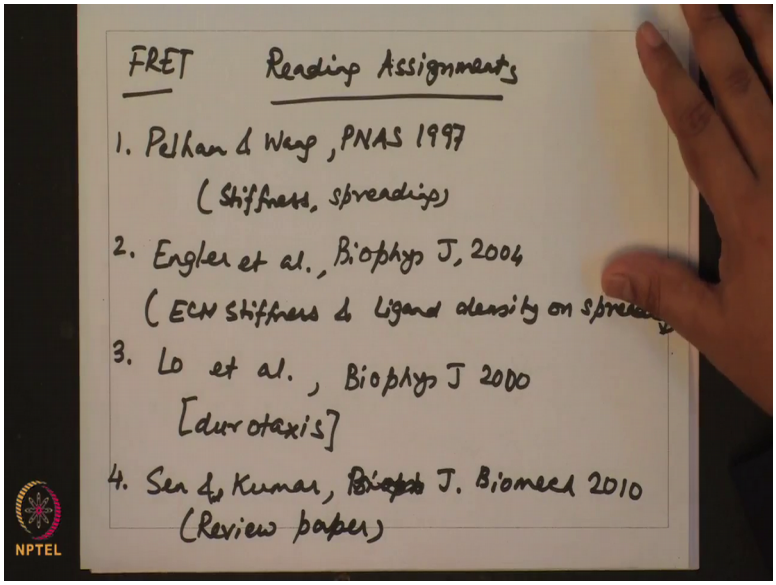

In the past few weeks; in the past few weeks we have discussed various papers I would like to list what are some reading assignments that you would have to do for the last one week first of all read the pelham and wang paper.

Which linked stiffness and spreading your biophysical paper, it showed the collective influence of ECM stiffness and ligand density on spreading; our Lo et al paper this was again grouped by wang. This is demonstrated sorry it is 2000 demonstrated durotaxis, this is a 2000 again biophys j sorry this is journal of biomechanics. So, this is a review paper which discusses how you can combine optics and mechanobiological tools for probing mechanobiology.

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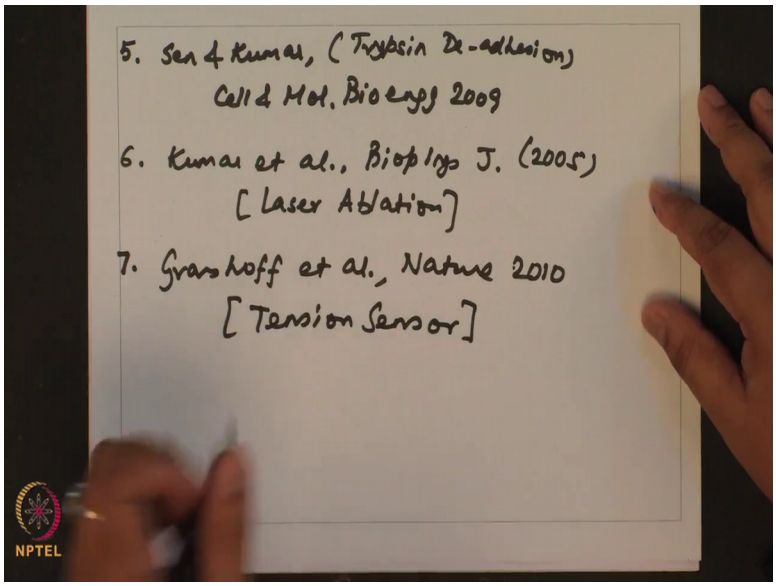

This is a paper introducing trypsin de adhesion as an assay for probing contractility you have this is a 2005 paper which introduced laser ablation for probing contractile mechanics, and the paper I just discussed on vinculin tension sensor. This concludes our course I hope you have enjoyed this course and I welcome you to read many of the papers which are listed in one or more of the paper that have suggested a reading assignment where you have reference to references to many other papers which discussed some more other mechanobialogical contexts relevant to cell behavior.

Thank you for your attention.