Introduction to Mechanobiology
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Week - 05
Lecture - 22
Adhesion Independent & Collective Cell Migration

Hello and welcome to our today's lecture of introduction to mechanobiology. So, in last lecture we had started this alternate mode of migration called amoe boidal migration, amoe boidal motility.

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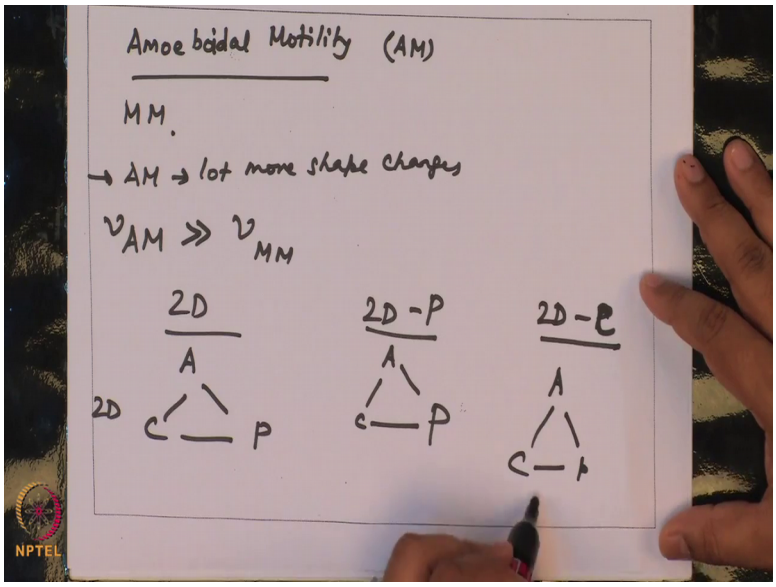

So, in compared to mesenchymal migration. So, I am just writing MM. So, this is AM. So, MM where, it is adhesion dependent; in AM you have lot more shape changes and adhesion amoe boidal migration is characterized with cell speed.

So, VAM is typically much greater than VMM. So, these can migrate in amoe boidal motility cells might migrate hundred times faster than mesenchymal cells. So, then I said that what are the different modes of amoe boidal motility and in that regard. So, in 2 D, I said that there are three different possibilities. You might have A C P where, just like mesenchymal migration, cells protrude, sense actimness protrusions, stabilize the protrusion and use contractory to the cell forward.

So, this is just 2 D normal mesenchymal migration. You can have 2 D protrusive where, your protrusion force is much significant and adhesion is there and contractility is insignificant and this is possible because these cells are small in size. If the cell was huge then this kind of localized protrusion would not lead to accumulation of membrane tension as a consequences cell would not move forward.

And the third kind of motility in 2 D, we said its two d contractile; where, your adhesion is required, C is required, but p can be very small. So, this kind of migration is called a bleb based migration. In 3D similarly, you can have regular 3D migration.

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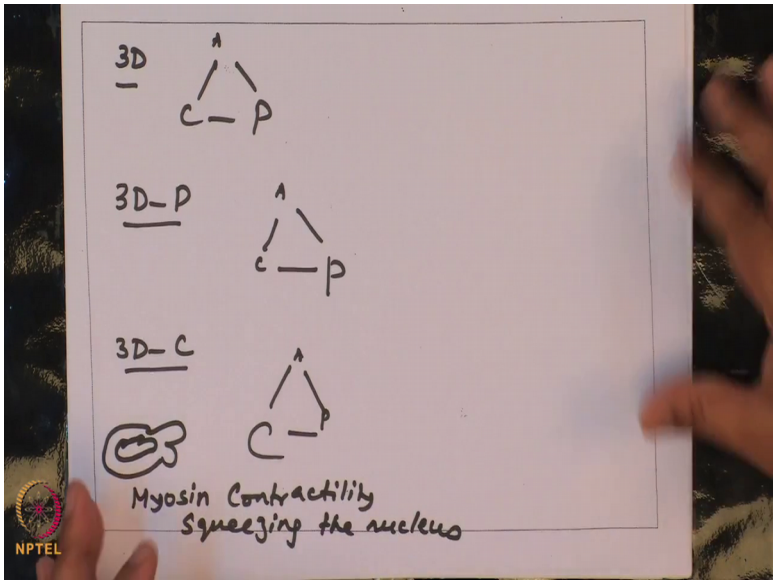

So, in 3D normally adhesion is not that important partly because the surrounding matrix can provide you the resistance for the cell to push against it.

So, adhesion can still be small and cell can move, you can have 3D protrusive where, protrusion is major; A is insignificant, C is insignificant. And this is through matrices where the pore size is comparable or bigger than the nucleus because the nucleus is the stiffest component. And the third one is pretty contractile where you have the formation of blebs and the nucleus is actively deformed.

So, in 3D contractile you have myosin contractility, squeezing the nucleus. We will discuss this in greater detail later in the course. With that I again get started with the paper we started discussing on adaptive force transmission.

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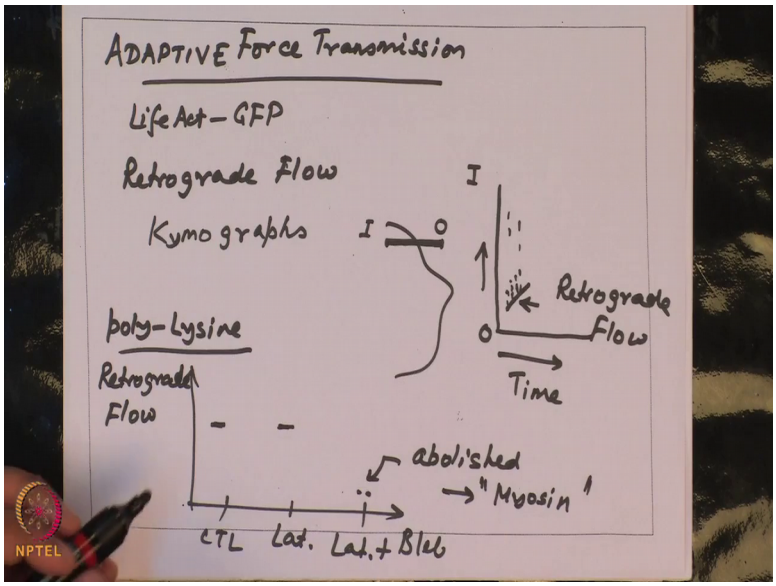

So, what the Arthurs had done was transfected cells with dendritic cells with GFP Life Act and monitored the retrograde flow and using kymographs.

So, once again what is the kymograph? You have a membrane, you draw a very small strip somewhere and along the length, so you have outermost point and this is innermost point; you track it over time. So, this is your time axis and this is your length. So, this is your outer point, this is your inner point. So, if you had a fluorescent signal then, what you would have as a consequence, you have a retrograde flow is you will have a slope which you can draw. You can see the intensity which is at certain point here it goes on getting inside and inside. So, this is the signature of retrograde flow; this slope and the slope of this line will give you the exact magnitude of the retrograde flow.

So, when the Arthurs did these experiments on poly lysine or adherent condition, what they observed was this. In control cells you had certain magnitude of retrograde flow, when you inhibited polymerization with latrunculin you had comparable value, but when you added latrunculin plus bleb retrograde. So, this is my retrograde flow axis.

So, this was completely abolished, suggesting that you have myosin motors once again similar to mesenchymal migration. You have myosin which contributes to retrograde flow. Now to check, so this is under adherent conditions to check how the cells would migrate under non adherent the Arthurs use to set up. So, in side view, what you have?

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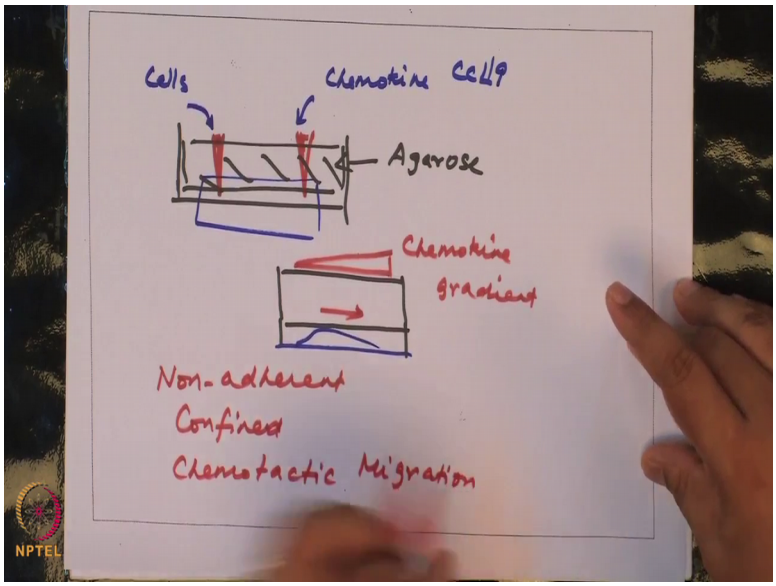

So, this is a non adherent plate, they polymerized a layer of agarose.

So, this is agarose which is inert agarose is inert. So, the cells cannot adhere to agarose and what they did then was there punched two holes on two ends of the well in one end they introduced cells and the other end they introduced chemokine CCL 19, which attracts the cells. So, in this setup, so if I zoom in this zone then what you have is a cell which is migrating under a layer of agarose and under the influence of a chemokine. So, gradient will be font.

So, this is my chemokine gradient, the cells will migrate from left to right under confinement and non adherent conditions. So, it is non adherent and confined and it is chemotactic migration. So, under these conditions when they tracked the retrograde flow, so if I plot my retrograde flow.

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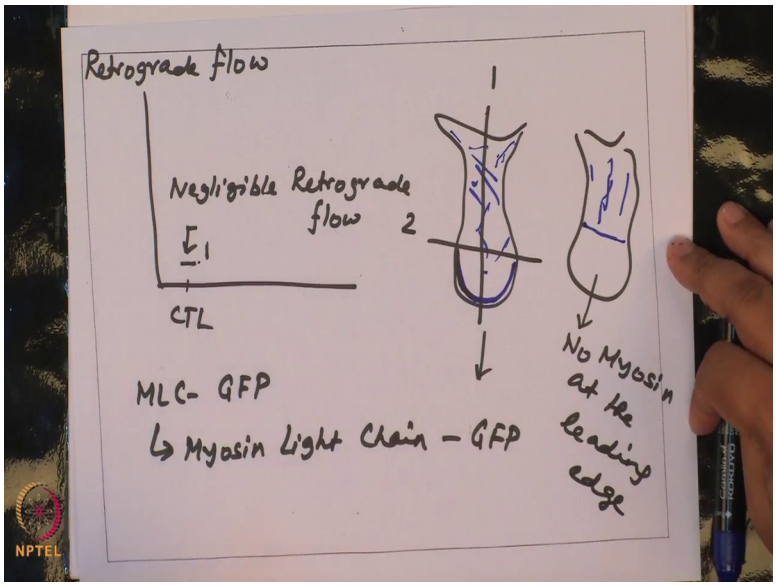

Was negligible? Retrograde flow in control cells was niggling.

Now, what they did was if you have a cell like this which is migrating in this direction, you can draw two lines; one is along (Refer Time: 08:32). This is the retrograde flow that they measured along line 1, what they also did was they did the perpendicular section which is along line 2 and along line 2, they found less amount of retrograde flow. So, there is negligible retrograde flow.

So, they did not transfected cells, so this was Life Act GFP, when they transfected with MLC GFP; MLC is myosin light chain tagged with GFP, what they found was the same cells. So, similar cells I can draw the cells. So, these cells did not have. So, if I want to draw the actin distribution in this previous cells you would have a flow a very a prominent actin zone here and otherwise you have diffused zones everywhere.

In the case of myosin; myosin was actually started this distribution started from far off and then all over the place. So, at the leading edge, no myosin at the leading edge , but when they plotted the retrograde flow again as before what you had from the sides? From the sides you had flow inward like this and inward like this as if there is a contraction from the sides. Along line 2, so along line 2 you have a contraction.

So, there then asked are integrin adhesions required for this kind of migration? So, what they found was integrins were present.

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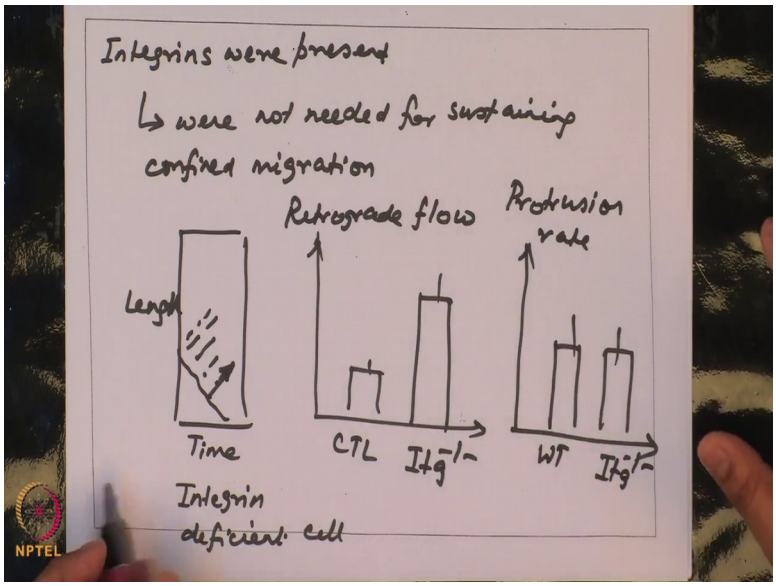

However, integrins were not needed for sustaining confine migration. So, this can be measured, this can be tracked once again by doing by tracking the retrograde flow. And to their surprise what they found was. So, if I draw a kymograph; this is the kymograph in an integrin deficient cell.

So, this is the membrane which is moving forward. So, again once again, this is my time axis, this is my length axis what they found was? They found again these streaks indicating retrograde flow to the magnitude of the retrograde flow increase significantly. So, if this was the average retrograde flow in control cells, in integrin deficient cells integrin null cells the retrograde flow was significantly increased.

So, they then asked whether is it because so this higher retrograde flow whatever the protrusion rate? Surprisingly the protrusion rate between control and integrin null cells was same. So, this is your wild type of control and this is your integrin null cells; so same protrusion rate. So, what this suggests is that when you remove the integrins over all the actin polymerization rate.

So, what is actin polymerization rate? I can write down the actin polymerization rate equal to protrusion rate plus retrograde flow.

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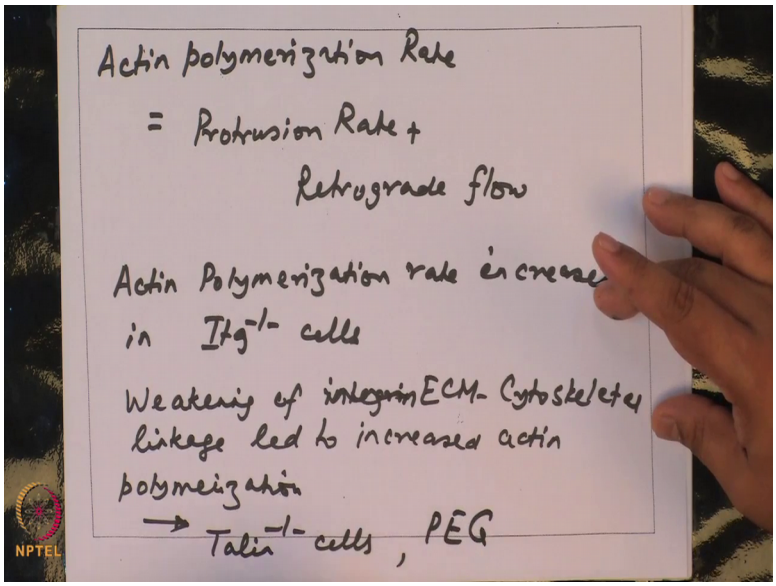

So, if you do this then what you would find this actin polymerization rate increases in integrin null cells.

So, this is an adaptive response and what the Arthurs then went on to show that any weakening of the ECM cytoskeleton linkage. So, of integrin sorry ECM cytoskeleton linkage led to increased actin polymerization. So, this they proved by doing experiments with talin negative cells, talin null cells and by plating cells on PEG.

So, PEG being inert cells cannot form adhesions; however, there was one important difference. So, first of all, two things to be said one is, if your overall Actin polymerization rate in talin null cells was this much. In peg the actin polymerization rate was not to the same extent.

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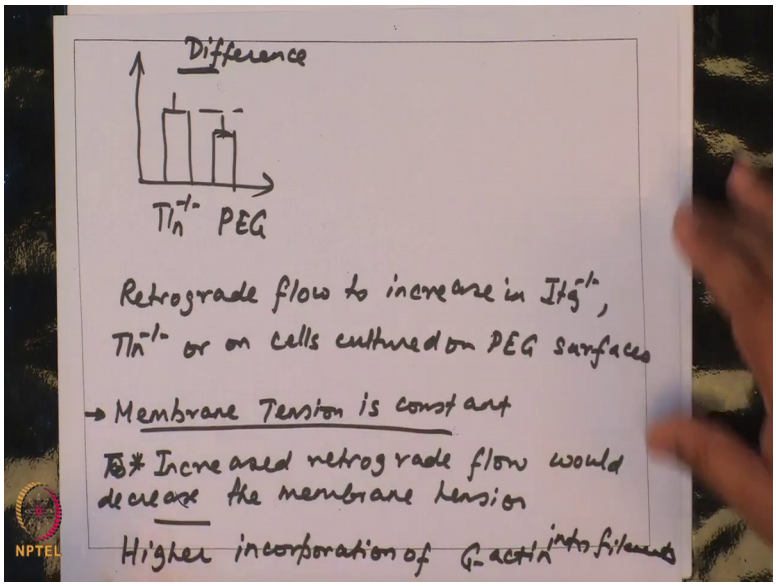

So, this difference there was a difference; between talin null and on PEG surfaces and this difference can be partly attributed.

So, first of all there are two questions that, how does retrograde flow increased? So, one possible reasons, so how is it that you can have retrograde flow to increase? In integrin null or talin null or on cells cultured, on PEG surfaces. So, what there is a possibility that since so if we assume that membrane tension is constant, so that means, that to maintain if this want to be constant.

Now in case of higher retrograde flow. So, increase retrograde flow would decrease the membrane tension. So, how can you maintain the membrane tension cost? By adding more amount of monomers or by increasing the rate of polymerization; so for this to be constant, since retrograde flow is high for this to be constant what you can do is, you can have higher incorporation of G actin into filaments.

So, thus far two things we found; for dendritic cells, so under adherent conditions, you have retrograde flow.

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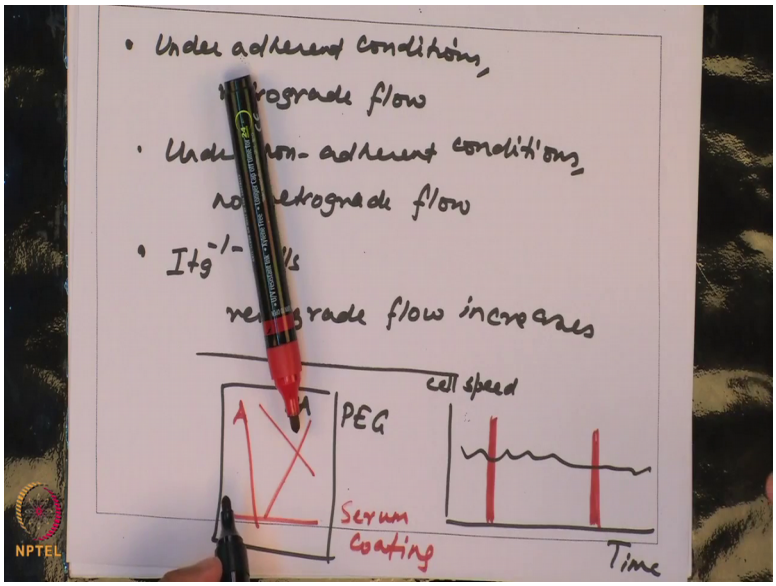

Under non adherent conditions, you have no retrograde flow, but when you in case of integrin null cells, retrograde flow is very high. So, the Arthurs next asked that how fast. So, if you want to subject the cells to a different situation.

So, for example, let us say this is your background of PEG surface and on these PEG surface, you randomly draw these lines of serum coating. So, this is serum coating or adherent. So, your background is non adherent. So, this area is non adherent, but this is the only adherence stripes that cell sees. In these cases does is the cell able to change itself adapt itself speed in real time?

So, what the tract was the cell speed the average cells speed as a function of time and so if I want to draw these zones let us say this is one zone in which you have an adherence spot, this is another zone where you have the adherence stripe. And what they found was when the cell was migrating on these zones your cell migration rate or cell speed was unchanged.

So, your cell speed remained unchanged. So, even if you provide the circumstance of adherent and non adherent zones mix together the cells can adapt that to it in real time. when they do the same experiment with fibroblast again you have this background.

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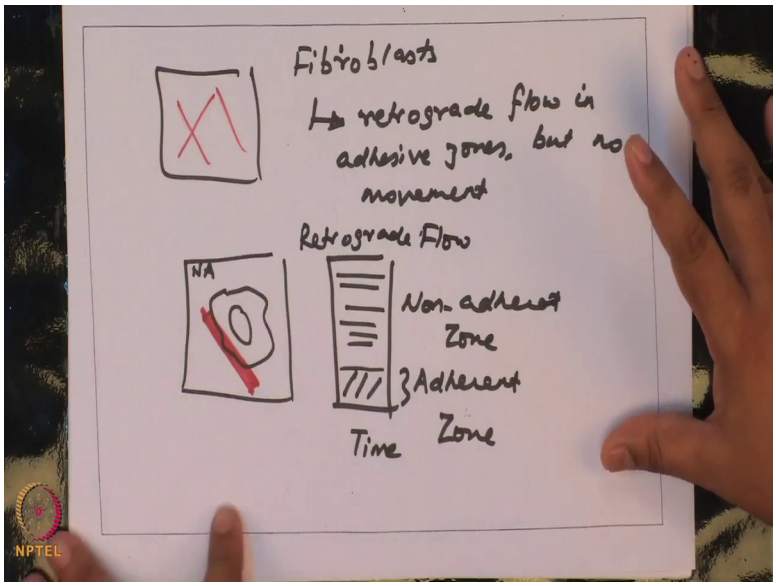

And you his red stripes and you did the experiment with fibroblasts what they found was there was retrograde flow in adhesive zones, but no movement.

So, how would the kymograph look for this kind of a situation? So, let us assume if I want to zoom in on a cell; let us say this is a cell and let us say this edge is a adherent spot and the background is non adherent. So, this is the cell. So, if you plot the kymograph what you will find is there is a zone in which you have retrograde flow, but in the other zones here retrograde flow is flat.

So, this is my again time axis. So, this is your adherent zone and this is non adherent zone. So, here retrograde flow is zero, in the non adherent zone, but it is positive in the adherent zone which suggests that the difference between fibroblast and these dendritic Celsius, for a dendritic cell let us say.

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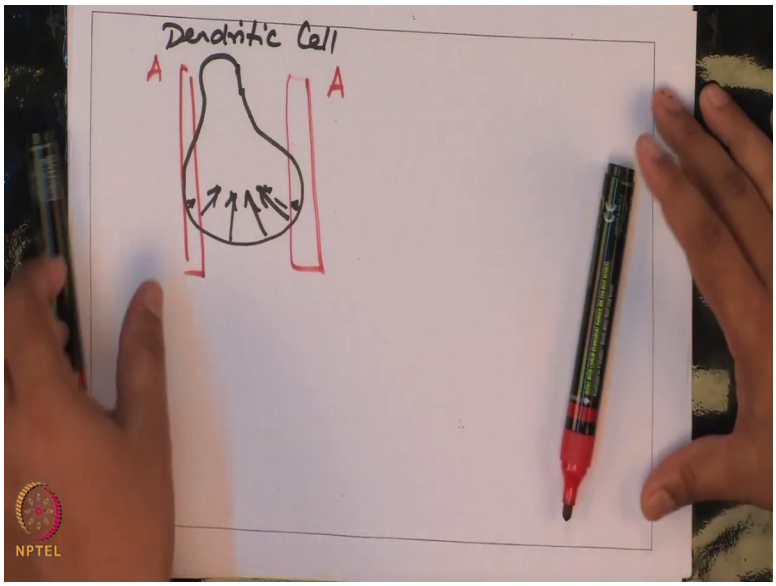

Let us assume, this is an adherent zone and this is an adherent zone this is adherent.

So, what the cell does is the retrograde flow is very negligible in an adherent zone, but what the cell does in the non adherent zone the retrograde flow increases. So, this is for a dendritic cell versus for a fibroblast this kind of accommodation is not possible. So, if I want to draw a picture similar to that of a molecular clutch what you have is as possible.

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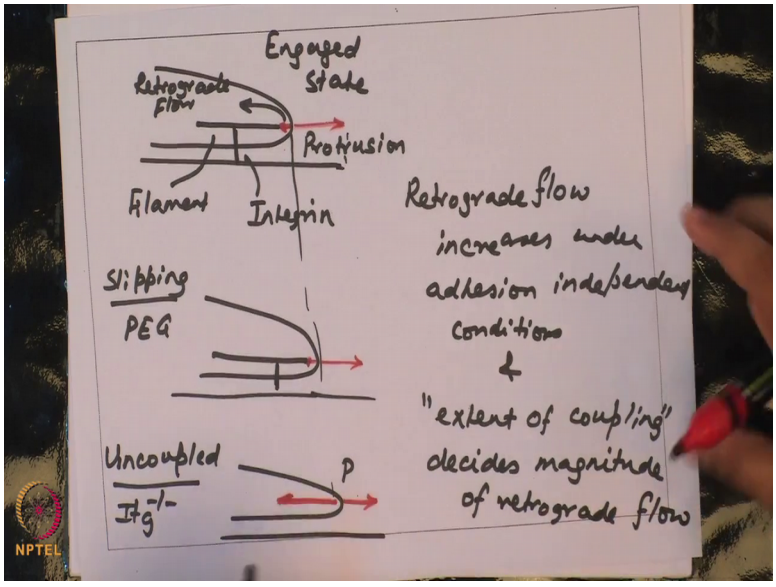

Like before, so this is in an engaged set, you have two things. So, this is the protrusion and this in is the retrograde flow. What I have drawn here is the filament and this is the

integrin connection. So, when you have a situation; a slipping kind, what is the slipping kind? Like you observe in PEG platted surface where your integrin is there, but it is not engaged to the surface.

So, you have this filament your retrograde flow is partially increased, but your protrusion remains the same. So, your retrograde flow is partially increase, in the other case you can have uncoupled. What is uncoupled? When there is integrin not present, in this case what the cell does is it increases its retrograde flow and its protrusion rate is this.

So, as a consequence what you find is, its protrusion rate is always maintained constant, but the retrograde flow. So, actually this length should be, the same the magnitude of the retrograde flow is extend. So, retrograde flow increases under independent conditions and this state by the extent of coupling decides. So, with that I end our discussion on amoe boidal migration.

So, you will have another reading assignment.

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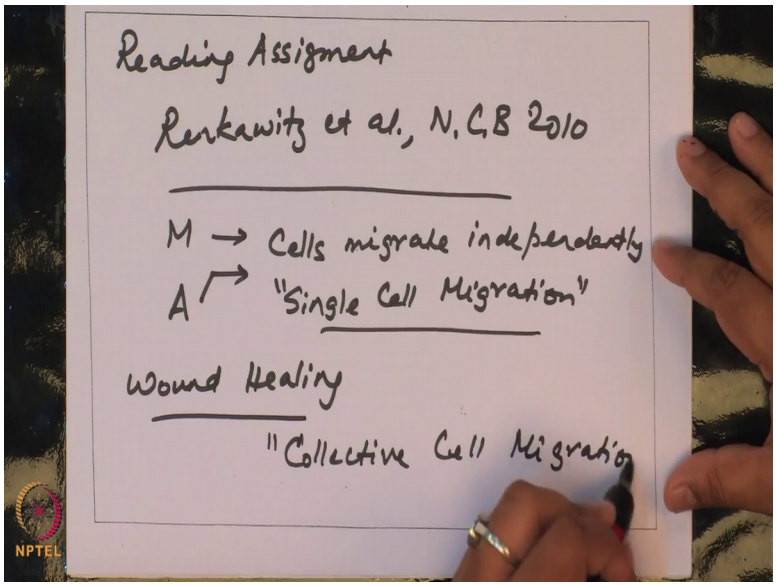

This is the Renkawitz Et Al, paper nature cell biology 21. So, I will just touch upon another type of migration. So, we have mesenchymal, we had amoe boidal. So, in both these cases, the cells migrate independently or in other words you can say this is an example of single cell migration.

So, just you understand that, if you want to think of wound healing. So, what you have? You have groups of cells which migrate. In the case of wound healing instead of single cell migration what you have is collective cell migration.

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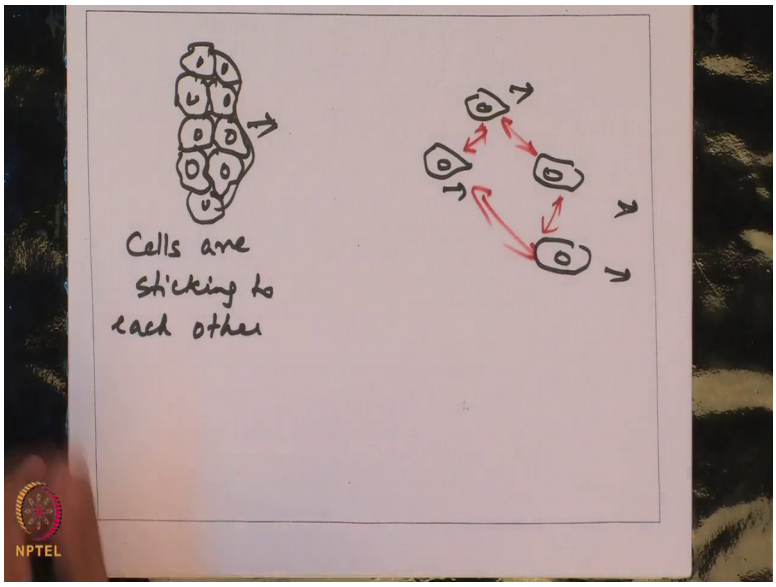

So by collective cell migration you have a group of cells. So, let us say this is a group of cells. So, each of these is a cell which is migrating, but it is not necessary. So, the way I have drawn this that cells are sticking to each other.

So, you can also have a situation when you have a group of cells migrating, all of them are effectively migrating in the same direction, but there is some other signaling between each of these cells such that their relative distance is maintained.

With that I stop here for today in the next class we will discuss about collective cell migration.

Thank you for your attention.