Introduction to Mechanobiology
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Week - 03
Lecture - 14
Focal adhesion organization

Hello, and welcome to our lecture of Introduction to Mechanobiology. In the last class I describe some of the focal adhesions proteins which are most prominent in dictating the function of focal adhesions. So, focal adhesions if I draw cell you can have these as focal adhesions.

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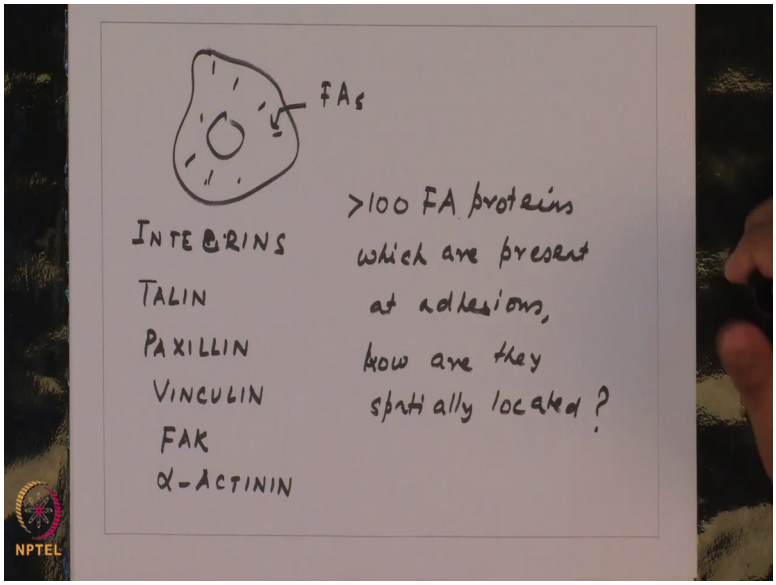

So, these are your focal adhesions I will write FAS and some of the major focal adhesions that we discussed in last class include Integrins, Talin, Paxillin, Venculin focal adhesion kinase or FAK and alpha actinin. So, all these focal adhesion proteins play various roles in dictating the behavior of these adhesions. So, and what has been found was. So, given that there are greater than 100 proteins which localized which are present adhesions.

So, the question is how are they spatially located ok.

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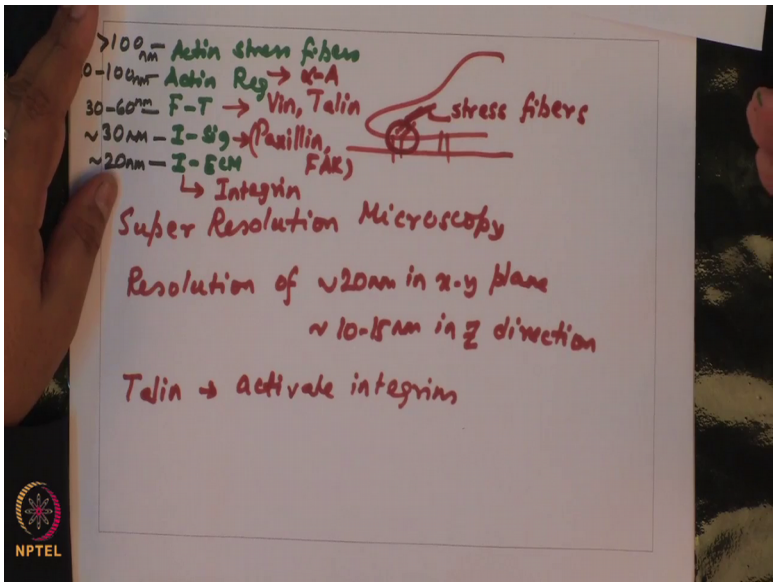

So, this question was answered by Clare waterman Storer. So, she used super resolution microscopy. So, she used super resolution microscopy. So, super resolution microscopy techniques have resolution of order 20 nanometers in xy plane, and order 10 to 15 nanometers in Z direction. So, using this she determined the spatial localization of individual focal adhesion proteins within focal adhesions. So, what she saw was. So, I is short form for integrin.

So, you have this cell you have the matrix. So, closest to the substrate is of course, the integrins because this is what engages the outside of the cell to the inside of the cell. Just on top of integrin you have integrin signaling layer and here you have proteins like Paxillin and FAK. Both of these proteins can directly bind to integrins. In force transaction layer you have Vinculin and more important Talin which exists which exhibits a angular configuration, which means that you are n terminal domain of Talin would be present close to integrins because Talin has been associated with integrin activation ok.

So, Talin is known to activate integrins, you have Vinculin which also binds to Talin. So, both of these proteins are present in the force transaction layer on top of which you have actin in regulatory layer and this is where alpha actin in is present. And at the exact top you have. So, if this was in whole was in adhesion at the exact top we have stress fibers

which emanate from these adhesions. So, these are your stress fibers. So, this shows you the organization the special organization of proteins within the adhesions.

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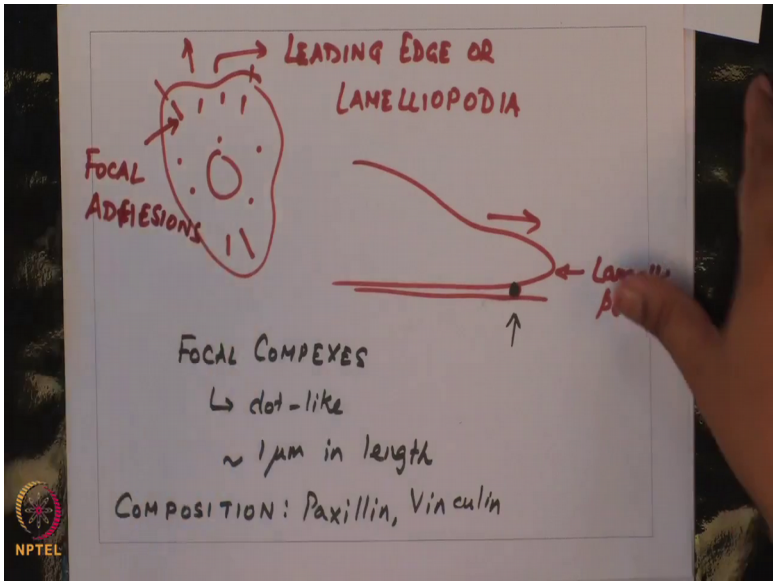

So, I also mentioned that in the same cell you have different types of adhesions, their sizes are different the dynamics is different. So, at the edge of the; so if the cell is migrating this way, then this edge is called the leading edge or Lamelliopodia ok.

So, in side view if I want to draw the same picture in side view, so, a cell is migrating this way just. So, this is where you have your lamelliopodia right underneath these you have these two different colors, these small dots. So, these are called focal complexes. So, these are more dot like. So, they are roughly order one micron in size in length and the typical. So, morphologically also the adhesions differ morphologically they differ and compositionally also they differ. So, in terms of composition these are enriched in Paxillin and Vinculin and. So, these are the smallest adhesions that you can have at the periphery of the cells. So, these larger adhesions that I had drawn, so, these are called focal adhesions ok.

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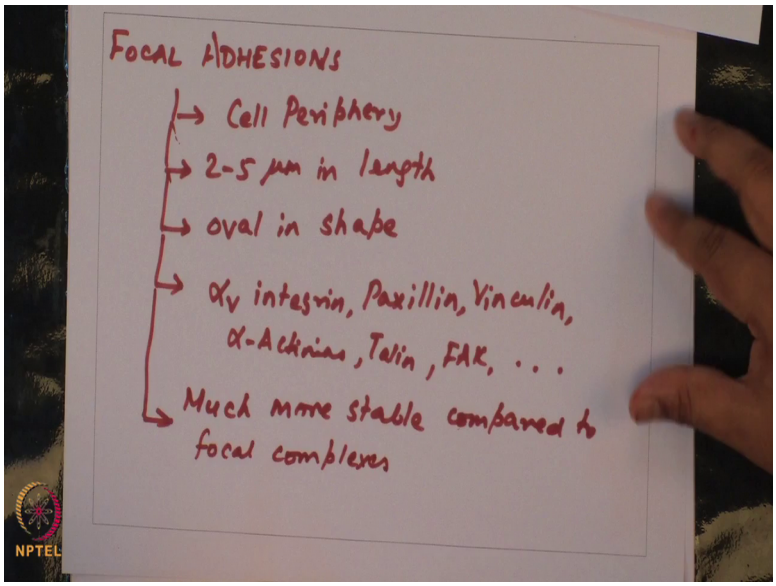

So, the size of the focal adhesions, so focal adhesions are present at the cell periphery can we 2 to 5 microns in length, and these are also more oval in shape and these in terms of compositional you have integrals like alpha v, Paxillin, Vinculin, alpha Actinin, Talin FAK and many others. So, the average time scale of this focal adhesion. So, these are much more stable compared to focal complexes on top of this you might have fibrillar adhesions.

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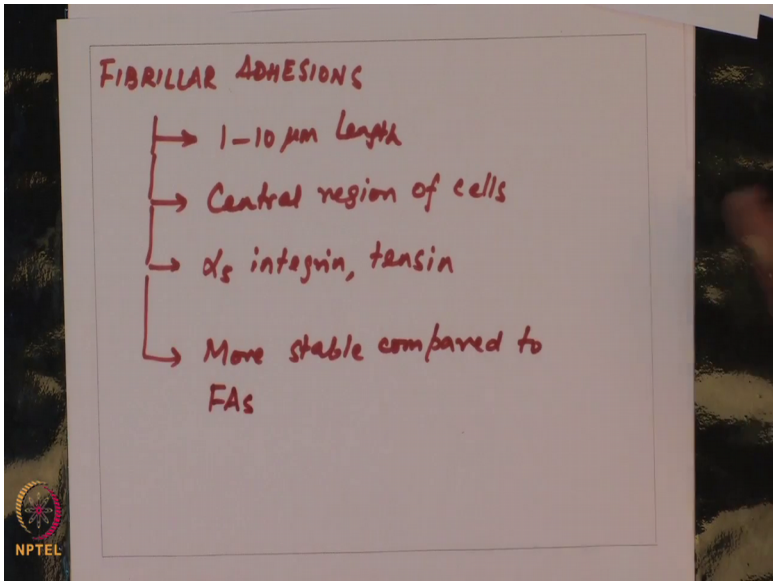

These are 1 to 10 microns in length, and they are present more centrally. So, central region of cells and they have alpha 5 integrin and the small call tensin in and these are also more stable compared to FAS ok.

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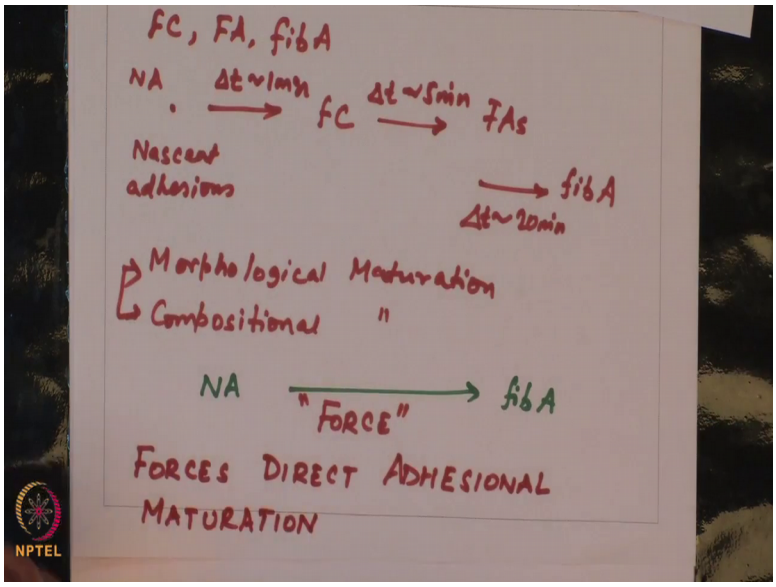

So, if I were to draw these if I were to write down this one. So, let us say focal complexes you have focal adhesions and let us say fibrillar adhesions. So, when cells first engage adhesions you might have these appear as small dots, these are even called nascent adhesions. So, if you follow the time span. So, delta t is order one minute you can begin to see focal complexes FCS. So, this is right nascent adhesions 2 FCS, delta t is order 5 minutes, you have FAS and then order 20 minute you have fibrillar adhesions. So, along with the size maturation see you have morphological maturation, go hand in hand with composition or compositional maturation. So, these two happen at the same time, and what has been found as the transition, the agent which converts immature to mature on nascent adhesions all the way to fib A depends on force ok.

So, it is not that they just assemble together; it is force which drives the conversion of small adhesions to bigger ones suggesting that there might be some signaling associated with protein and folding which leads to recruitment, leads to positive cycle which recruits more adhesion proteins and that is how you get maturation of these focal adhesions. So, this kind of suggests that forces direct adhesional maturation.

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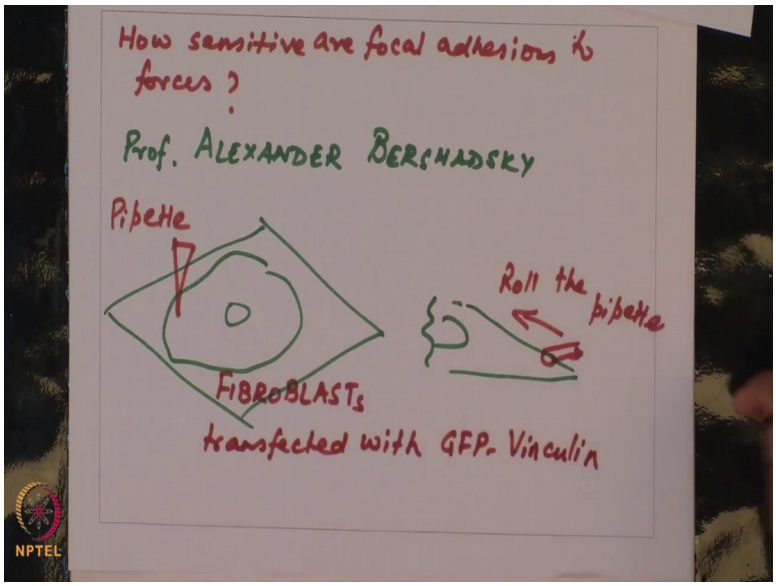

So, one of the first studies one of the first similar works which showed this that to ask the question that, how sensitive are focal adhesions to forces, you have Alexander Bershadsky, you have professor Alexander Bershadsky do this experiment, in we what we did was you take you take a cell on a substrate you come down with a pipette.

So, this is a pipette and so, in side view you would have something like this. So, side view you have this cell with the nucleus, you came down with the pipette. So, your pipette is you roll it roll the pipette. So, what essentially we have done is we have exerted some local forces on these cells. So, these cells were fibroblasts which were transfected with GFP Vinculin. So, what is showed was if you have a cell which has you know your may be this is not the right way (Refer Time: 14:23) redraw it (Refer Time: 14:24) ok.

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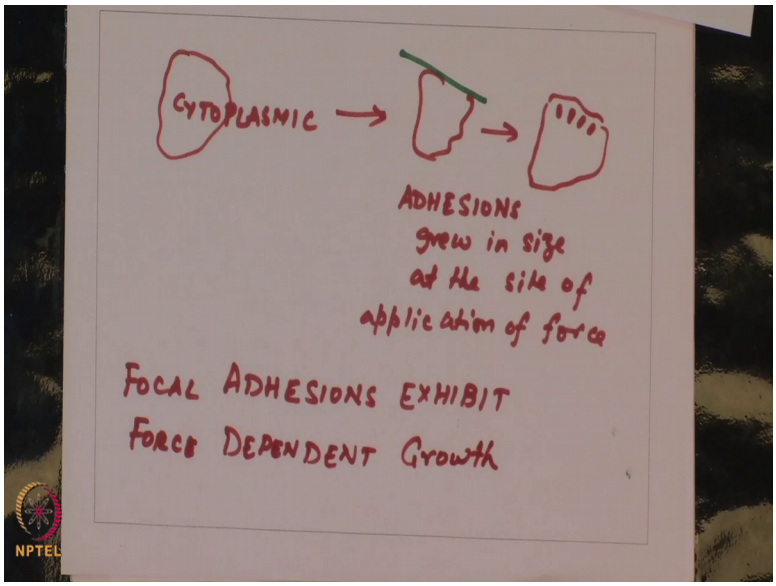

So, you had cytoplasmic localization, and then what you did you came down with this pipette and rolled it on the cell. And what he found was immediately after removing that recruitment of these adhesions at the site in which force was exerted. So, you have adhesions grew in size at the site of application of force.

So, this was the first demonstration that cells were focal adhesions exhibit force dependent growth. So, this was the first proof that focal adhesions exhibit force dependent. So, this was the first proof that forces influence or lead to growth of focal adhesions ok.

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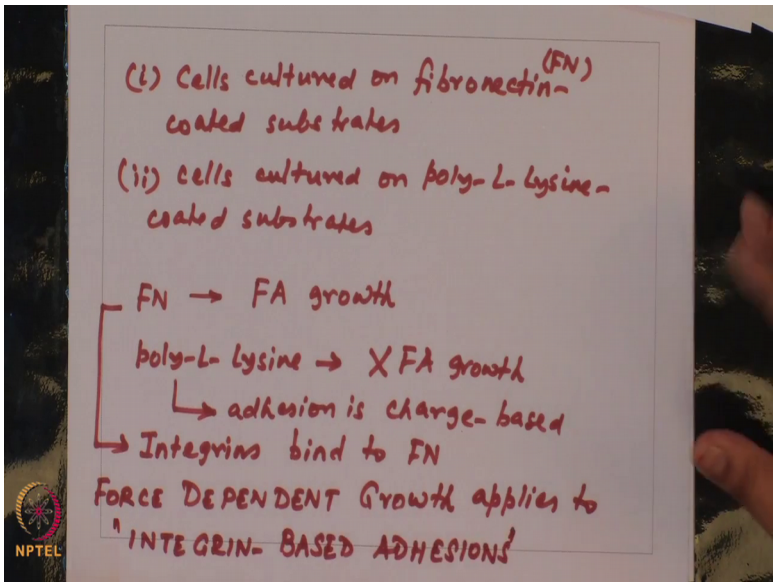

One more point he demonstrated. So, he repeated these experiment 2 conditions. So, one condition was cells cultured on fibronectin coated substrates and the other condition was cells cultured on poly l lysine coated substrates. So, in fibronectin surfaces he observed FA growth. So, focal adhesions growth when cells were exerted when cells were exposed to force exposed to pipette pushing on the cell, but on poly l lysing there was no FA growth ok.

So, this suggests. So, possibility in fibronectin integrins engage fibronectin, on poly l lysine in adhesion is well charged based because poly l lysine is positively charged, the plasma membrane is negatively charged. So, you just have charge mediated adhesion. So, this proved that this force dependent growth applies to integrin based adhesions. So, this was the seminal paper which first demonstrated the sensitivity of focal adhesions to forces and also that integral mediated adhesions are what are necessary in order to mediate this force dependent growth. So, one other study you have two groups Benny Geiger.

So, you can so on these focal adhesion growth the forces that are required. So, this experimental setup did not allow a way of quantifying the forces, which the cells were filling or how much forces the cells are capable of doing for that purpose ok.

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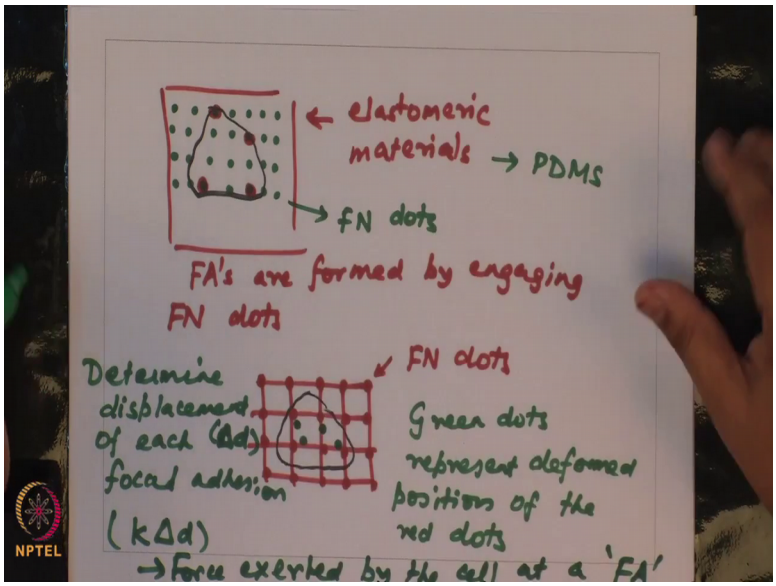

So, to actually be in a position to measure forces what people have done is they have taken elastomeric materials, and what you can do is you can pattern them. So, imagine these are fibronectin dots and the elastomeric material like let us say PDMS is by itself (Refer Time: 20:23) inert. So, cells cannot stick on this, but when you play it when you functionalize the substrate to these fibronectin dots and you fit cells they would happily spread on these and they will make focal adhesions at the site of contact. So, you will see focal adhesions being formed by the cells or formed by engaging fibronectin dots now what is setup allows. So, imagine you have a grid of fibronectin dots. So, imagine at each of these corners you have a fibronectin dot. So, let us say these are fibronectin dots and you are tracking the position of these dots.

Now, let us say that we draw the outline of a cell under some condition if you see. So, with the green dots I have shown the position deformed position of some of these red dots. So, and what you find the way I have drawn it is intentionally. So, the green dots represent deformed positions of the red dots. So, since this is a grid you can very easily capture in what direction these beads have been these points have been deformed. So, this would allow me to determine displacement of each focal adhesion. So, this allows you to find out how much has been the displacement of each of these positions, and because this material is as elastic. So, you can just multiply this delta d by the spring constant of the material. So, k delta d is the force exerted by the cell at a focal adhesion ok.

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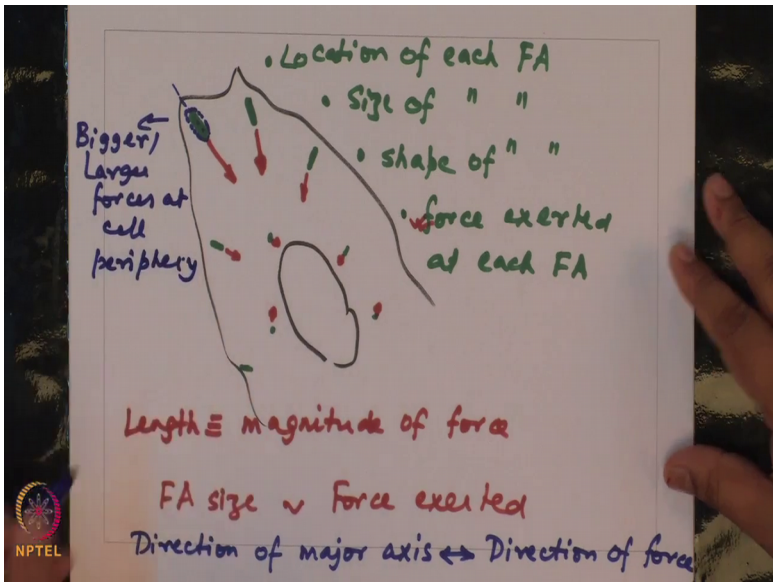

So, using this strategy you can actually quantify the force that the cell is exerting at the focal adhesion. Now what this experiment allowed, if I would to draw a cell. Let us say this is portion of the cell this is the nucleus and let us say. So, you can find out by doing these experiments, you can find out the location of the focal adhesion, the size of each focal adhesion, the shape of each focal adhesion and as well as the force exerted at each focal adhesion. So, what the authors found was that at each of these focal adhesions. So, I have drawn these red arrows, these red arrows depict the forces at each adhesion and the direction. So, length of each arrow is equivalent to the magnitude of the force that has been exerted, and the direction depicts the direction along which the cell has exerted in the force.

So, what you see from this picture is that the biggest adhesion has the highest force. So, there is a scaling between focal adhesion size force exerted. So, there is a scaling between the size of the focal adhesion and the force, also if you want to approximate each of these as an ellipse. So, what you see is the direction of major axis is same as direction of force suggesting that the forces of the focal adhesions are aligned in the direction of forces.

So, two things bigger the focal adhesion larger the force the long axis of the focal adhesion coincides with the direction in which the forces has been exerted at that particular direction and as you go inward towards the nucleus. So, bigger forces at the

periphery or larger forces at cell periphery and as you go inside the focal the size the magnitude of the forces. So, for example, right around the nucleus I have intentionally drawn small arrows in random direction. So, there is no constant behavior among these adhesions which are small in size and close localized close to the nucleus.

With that I stop here, but let me summarize by saying that focal adhesion. So, there is a very close relationship between force and focal adhesion assembly. So, cell exert contractile invert directed forces at focal adhesions, the magnitude of the force is proportional to the size of the focal adhesion and the direction of the force coincides with the long axis of the focal adhesions.

That is it for today I will look forward to next day's lecture.

Thank you.