Introduction to Mechanobiology
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Week – 05
Lecture – 23
Collective Cell Migration

Hello and welcome to our today's lecture of Introduction to Mechanobiology. So, in the last two lectures I had spoken about two different modes of migration and introduced amoeboid migration right. So, you have mesenchymal migration or adhesion dependent, you have amoeboid migration which requires weak adhesions or no adhesions and towards the end of last lecture I had introduced another mode of migration which is collective cell migration.

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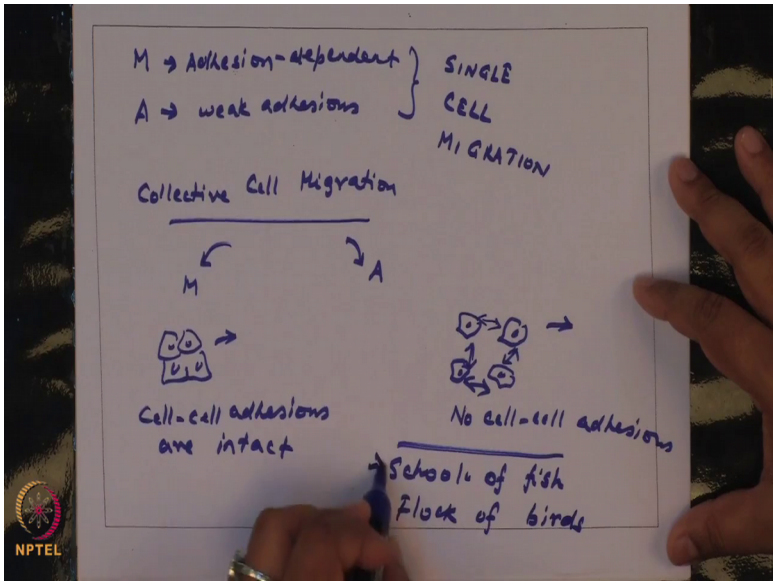

So, both these modes in both these mesenchymal and amoeboid modes of migration I was essentially talking about single cell migration. So, there are two different modes of single cell migration in collective cell migration or in collective cell migration can be mesenchymal or can be amoeboid.

In collective cell migration you have groups of cells, you have multiple cells which moves together. So, in this case my cell-cell adhesions are intact, but you might also have a situation and you have multiple cells which are moving coherently in a given

direction, but they are not attached to each other. So, there are no cell-cell adhesions, but still you have a directional to the migration in this case there are soluble cues which coordinate the interaction between these cells. At the macro scale if you look at school of fish or a flock of birds, so you again you have multiple entities which migrate together yet they do not clash into each other. So, there is some special signaling which regulates the spacing between each other.

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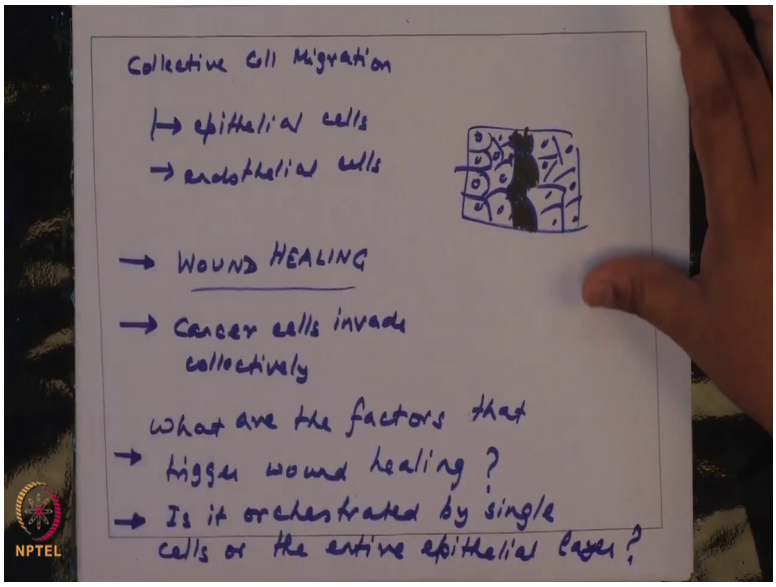

So, in today's lecture we will talk more about this kind of migration or cell where cell-cell adhesions are intact their collective cell migration in the case of epithelial cells or endothelial cells which migrated together with their cell-cell adhesion intact.

So, collective cell migration is applicable to let us say wound healing or even in pathological processes where cancer cells invade collectively. So, we will focus more on this wound healing. So, what is wound healing imagine you have a patch of skin and you have cells, each of these is a cell and when you have an wound. So, you imagine you have a wound in the center so the center cells die, in the way that I have drawn imagine these center cells are dead.

So, the question, you know wound healing occurs when we have a cut in our skin you have the cells which migrate inward. So, the question is that what drives this wound healing what are the factors that trigger wound healing. So, is it that some factors are released which triggered the migration of these cells and or is it that it is just the

availability of space is the signal which triggers migrations of cells and the second question is. So, this is the first question the second question is, is it orchestrated by single individual cells single cells or the entire endothelium or epithelium right because you can have the space filling. So, you can look at this problem, imagine again I have a patch and imagine this is the space which is available by creating a wound.

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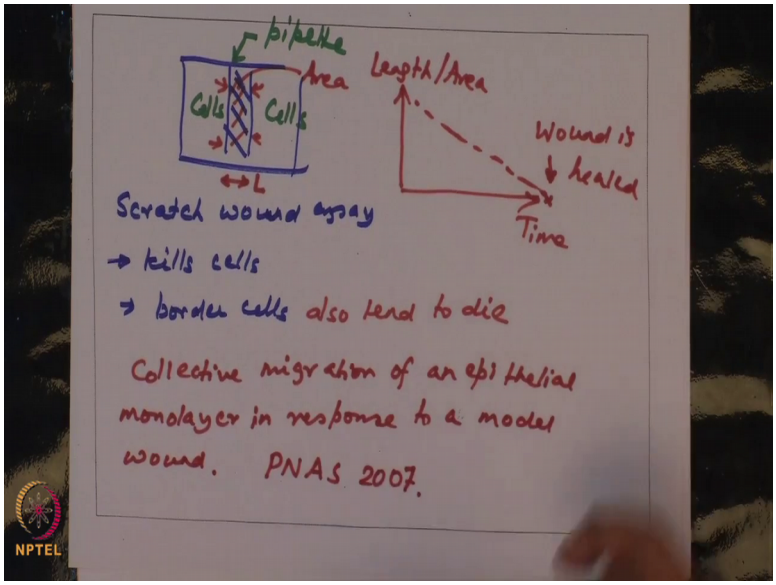

Now, typically in most experiments people use the scratch wound assay. So, you have these cells here you have cells on this side you have cells on this side and you scratch it you introduce this model wound with a pipette. Now what it does it of course, kills cells, but many of the border cells. So, many of the bordered cells are also disrupted, so which are the border cells? The border cells are those which connect which are in contact with the edge that you have created by scratching the surface.

So, many of the border cells are also tend to die. So, though this assay is used a lot still there are question some issues about this acid. So, how do you track wound healing what people would typically do is the track this length as a function of time - this is my time axis this is my length or the entire area or you can look at the entire area slash area and you should ideally have decrease with time and eventually when you strike zero; that means, that at this point your wound is healed. So, what I am going to do is to discuss one paper. So, the title of the paper is collective migration of and epithelial mono layer in response to a model wound it was published in PNAS in 2007.

So, let me first describe the setup.

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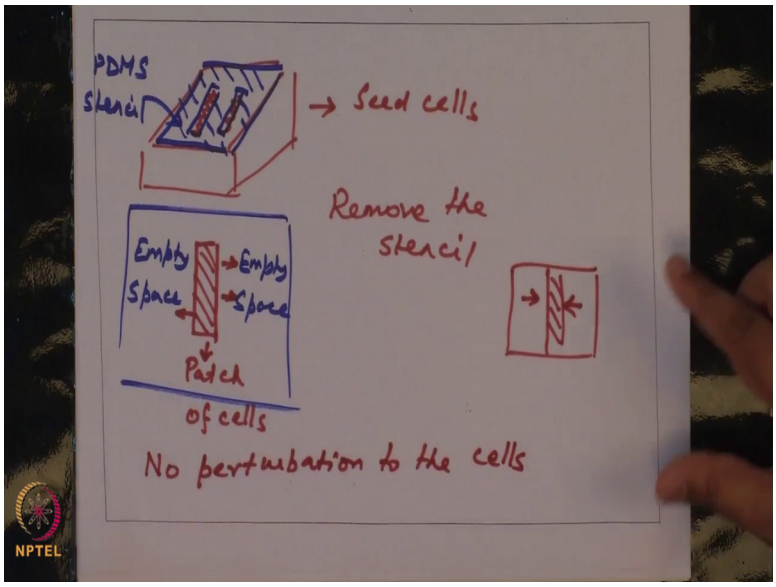

If you have the top surface let say this is your glass coverslip top surface. So, what the author state was designed a stencil they designed the stencil. So, this is a stencil PDMS made of PDMS in last words stencil. So, what you do is you plate cells you seed cells on this surface. So, this entire hashed region in blue cells cannot sit. So, what the cells instead do is they populate this base available. So, what you essentially have is you have created a patch of cells this is a patch of cells and around it is area which is not accessible by the cells, but let us say you have the stencil around it, but, this is the entire surface. So, if you remove the stencil so what you have is empty space on all sides of these stencil of these patches. So, you have these rectangular patches and you have created empty spaces when you remove the stencil. So, what will happen is the cells will then migrate in all directions they will migrate outward.

So, compared to the wound healing assay, compared to the wound healing assay where this is your wound and the cells migrate inward this is similar in this case the cells migrate outward, but you have no perturbation to the cells, cells are not perturbed. So, not a single cell has died in this particular assay that is one of the stencil of assay.

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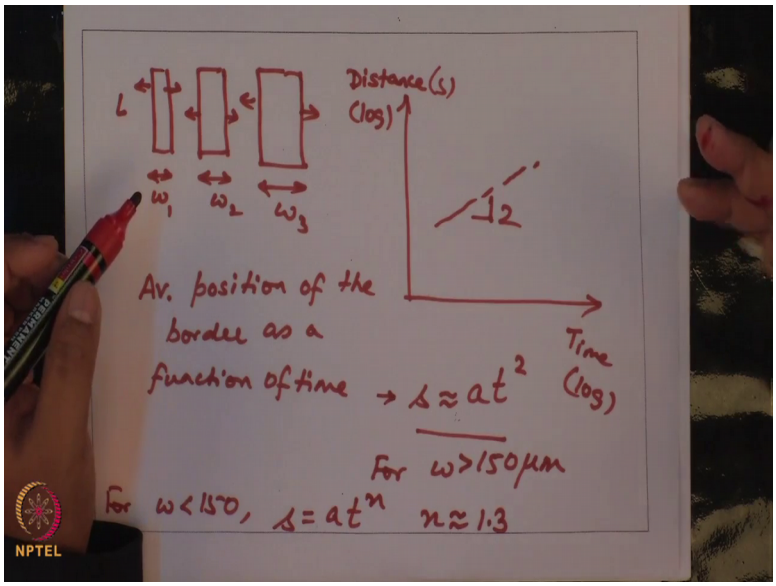

So, what the authors did was the first asked that if they made these patterns of various widths, they varied the width of the pattern keeping the same length and they asked that how was the outward movement of cells from this patterns how would how did the vary depending on the width of these patterns. So, what the track was essentially for each edge which track moved outwards they tracked the average distance moved or average position of the border. So, you have the border you track the average position of the border as a function of time. So, what they found. So, let us say this is some distance moved and this is time.

So, when they plotted in log log scale log log scale they got this data which approximately had a slope of 2. So, which means that the distance let us say s is approximated by the expression a t square. So, what they found was s scaled as t square. So, this for w greater than 150 microns, beyond 150 microns whatever with the width you have a scaling relationship where the border moved it depending in an exhibited a parabolic profile on the time t. However, for w less than 150, so if I were to plot it as s is a to the power n then this n value was approximately 1.3 for widths which first smaller.

So, what is possible why is do why did the authors observe this difference? One possibility is when you have a bigger it cells from bigger patch migrating then the cells at this edge do not know of what happens what is happening to the cells at the opposite edge. So, you have that you can have these two edges which are behaving independently

of each other; however, when you make the patches smaller and smaller there is some level of confusion for cells at the center whether they want to migrate left or migrate free right that might be the cause why you have a slower migration when the width was smaller what they also found. So, what they also found, so if they zoom into one segment of the pattern.

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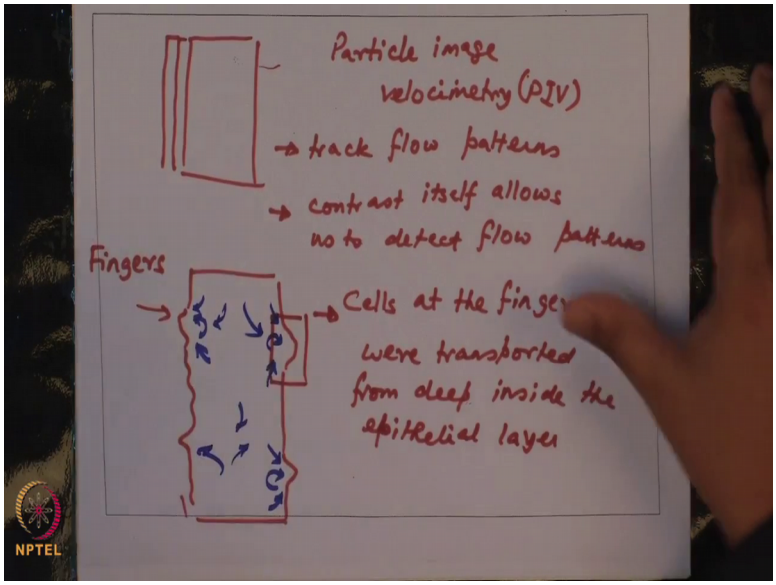

So, they asked that were cells all migrating in a line or what they were flows in other words where the active was the active reorganization of cells within the epithelial layer and what they use. So, they used a technique called particle image velocimetry or PIV that allows us to track flow patterns and for an epithelial layer because there is inherent contrast the contrast itself allows us to detect flow patterns.

So, what they found was, so often at the edge. So, the edge did not progress as straight lines and one would imagine, but rather the edge was exhibited these kind of extensions. So, these extensions are called fingers and what they found when we are looking at the flow patterns you could see that there was flow towards the fingers so on and so forth and this. So, you can have various flow patterns deep inside the tissue as well. So, even though this is the leading edge you can detect flow very far from the leading edge its suggesting that many of the cells at the fingers were transported from deep inside they epithelial layer. So, this flow allowed cells to migrate from the center towards the edges or towards the location of the fingers.

So, if you look zoom in into the fingers and ask what is happening why are these very local protrusions being formed as opposed to gradual expansion of the epithelial layer what the authors found. So, if let us say if I zoom into a finger let us assume you have this is a finger and you have multiple cells of the fingers so on and so forth these other cells.

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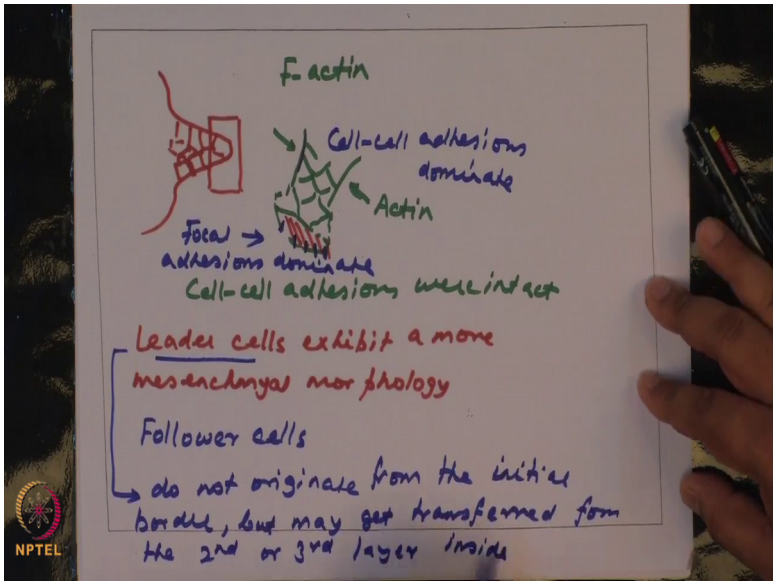

So, if the zoomed in to let us say few of the cells right at the edge of the finger what they found. So, I will draw the effect in image effect in was, you had these lines at the edges where the cells remained attached to each other, but you had this corner right the periphery of the right the periphery of this thing where you had, this kind of actin was completely different in organization at the edge. So, you did have, here you did have cell-cell adhesion, intact, but you can have these cells right at the periphery these were called the leader cells they exhibit a more mesenchymal morphology.

So, in contrast to sell swing attached to each other this guy actually forms lot more focal adhesions with the substrate. In these cases you had cell-cell adhesions dominate, but at the periphery you had focal adhesions dominate. So, what the focal adhesions are allowing is this leading cell is kind of trying to go outside while these other cells at the rear end are following the leader cell so, but the other thing is. So, you have leaders and followers. So, you have leader cells right at the leading edge and you have follower cells.

Now, the flow profile I discussed about suggests that leader cells, these leader cells do not originate from the initial border, but may get transferred from the second or third layer inside. Another thing was there was a notable difference there was a notable difference if you think the behavior , if you plot the displacement as a function of time you could see different behavior for the leader cells and the as opposed to the general cells which served as the followers.

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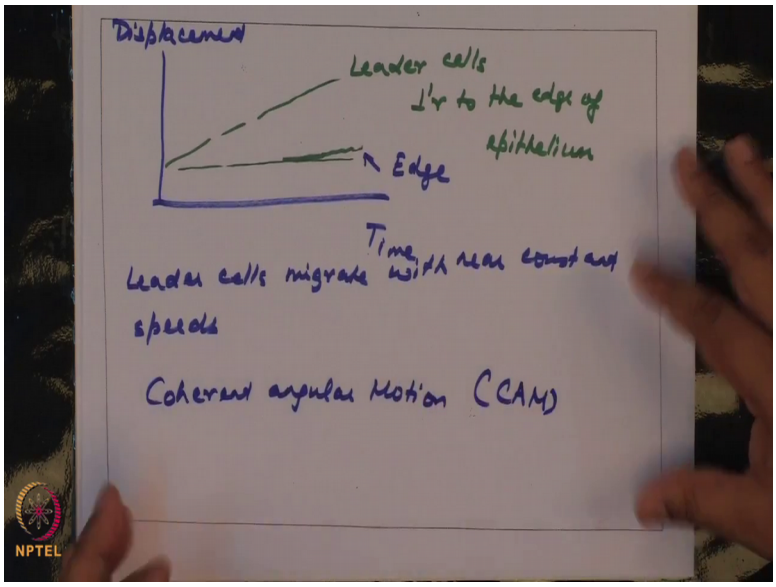

So, the leadeir cells the leader cells exhibited faster migration rate. So, leader cells were migrating almost perpendicular these are my leader cells and they migrated they were perpendicular to the edge of the epithelium in contrast the follower cells. So, these were. So, this is a displacement of the follower cells they were migrating much slowly as a function of time. So, these are the free edge which contain most of the follower cells.

So, one important thing for the leader cells was that these cells. So, this line was almost a straight line which meant that the slope is constant. So, leader cells migrate with near constant speeds. So, these are the two important things that you learn from it. So, first of all the importance the novelty of the technique where it allows you to study wound healing without introducing artifacts because of the experimental setup because you have scratch spoon as say the scratch that you create may not be uniform same size across multiple conditions you of course, kill many cells near the border that may have an effect in how the wound is getting healed. In contrast here there is no cell death, but you can

see expansion of the cells, expansion of the mono layer and you can also see the leader and followed one another aspect of collective migration that I wanted to discuss which is this phenomena of coherent angular motion or refer to as CAM.

So, when you confine, so the cells which I was I had discussed just now the experiment I discussed first now was performed with MDCK cells. So, these are in if I expand Madin-Darby Canine Kidney epithelial cells. So, this is the MDCK epithelial cells.

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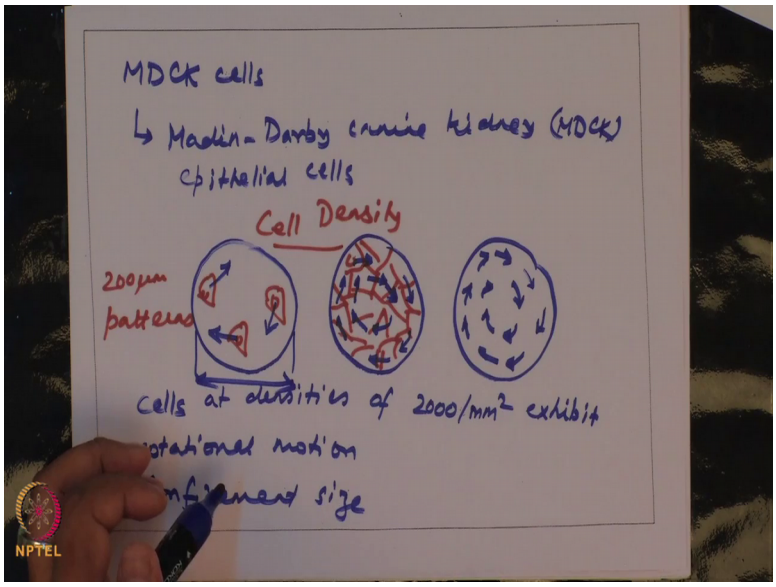

So, if you played these MDCK cells on a circular pattern. So, you played this cell some circular pattern and you can what you do is, these are patterns so let us say these are roughly two hundred micron patterns. So, patterns I mean that around it. So, on these patterns these are probably coated with fibronectin or some other ECM ligand. So, cells will attach to this, but not on the surrounding areas.

So, when you plate cells on these patterns and you increase play with the concentration of the cells. So, you play with cell density and you ask the question; that what is the average nature of migration exhibited by these cells on these patterns. So, what you see is once you rage once you reach confluency, once you reach confluency these cells exhibit what is called as CAM and; that means that they migrate they migrate in tangential direction in the pattern. So, it is almost like a group of cells they exhibit this rotation. So, if I were to draw at the directions of cell migration within these patterns at confluence you would see this complete rotation.

So, the cells at densities of 2000 per millimeter square exhibit rotational motion. So, this is called covalent angular mutation rotation and what you see, so this, cell density is one of the factors which regulates covalent and row mutation apart from cell density just this size of the pattern or confinement size is also one of the factors which regulates this kind of rotation. So, if you make your tissues bigger and bigger then this rotation will stop.

With that I stop here for today and I summarize that we discussed collective cell migration where with epithelial cells or cell-cell adhesions are intact, but you can also have corrective cell migrations where cells migrate without forming cell-cell adhesions, but they still they coherence in the direction. I discussed about two phenomena one is this aspect of leader versus follower cells and also this aspect of coherent angular rotation.

Thank you for your attention.