Tissue Engineering
Prof. Vignesh Muthuvijayan
Department of Biotechnology
Indian Institute of Technology, Madras

Lecture - 22
Cell Migration

Cell Migration plays a crucial role in developmental biology and tissue engineering. We will try to understand what cell migration is, why cells migrate, what are the roles of the cell migration, and so on.

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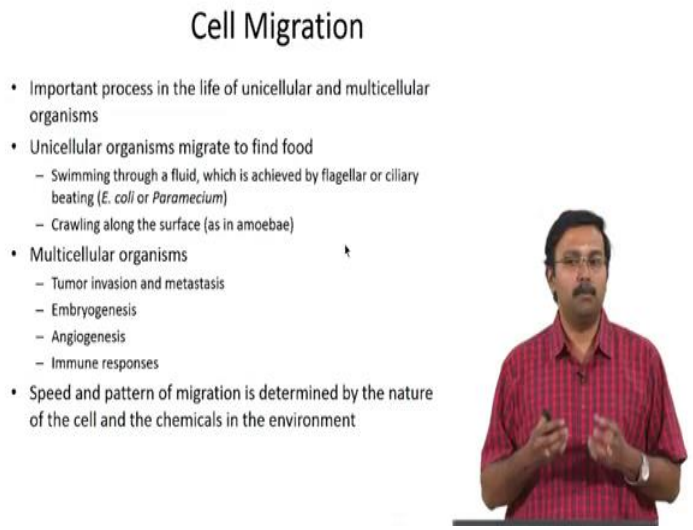

This is, in general, a very important process for both unicellular and multicellular organisms. For unicellular organisms, it is usually for finding food. The cells migrate; they usually swim through a fluid, try to find if there are nutrients that they can use, or they can also crawl on a surface as an amoeba would do. The multicellular organism also does cell migration, but for a more complicated process, it is not just for finding food. It could be for tumor invasion, metastasis, embryogenesis, angiogenesis or immune responses, and so on.

If you remember when we looked at wound healing; so, the aspect which we talked about was the migration of inflammatory cells, phagocytes, macrophages, and so on to see how they actually come to the site and so on. So, migration plays a crucial role in some of these aspects. The speed and the pattern of migration are determined by the type of cell and also

the chemicals present in the environment. So, some of these molecules can act as triggers or signals for directing cell migration.

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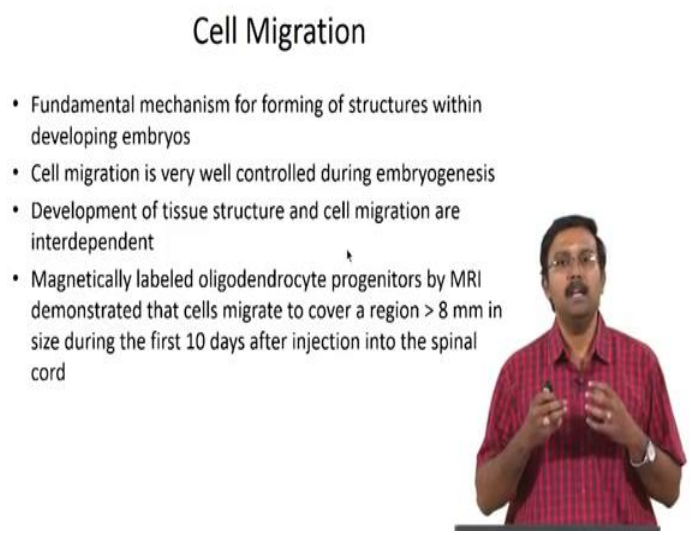

The fundamental mechanism for forming structures within an embryo is cell migration. Think about it; you only have one cell to start with, and it starts dividing. If the cell just keeps dividing, you just have a blob of cells. You are not going to get defined shapes. So, what is going to happen is these cells are going to produce matrices, and they are going to migrate to form different organs and tissues. Because of this, during embryogenesis, cell migration is very crucial. And, it is also very, very well controlled, and that is why you end up with two eyes on the place where they are and nose on where it is.

It is a very regulated process, and this requires cell migration to be controlled. The development of tissue structure and cell migration are interdependent. Only when you have the cells migrating to a particular area, the tissues can start developing. People have used magnetically labeled oligodendrocyte progenitors and monitor them using MRI to show that the cells can cover a region of greater than 8 mm in size during the first 10 days when you inject it to the spinal cord.

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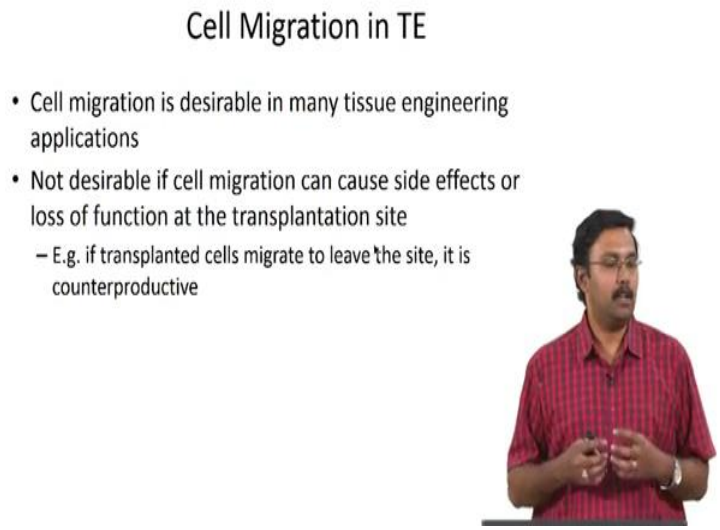

When you are talking about tissue engineering applications, cell migration can be very important. Because, if cell migration is going to be there, you might have host cells coming to that tissue construct and integrating very nicely with the cells. And, also in the case of angiogenesis, cell migration is required for vascularization to happen. However, it is not desirable if cell migration can cause side effects such as loss of function at the transplantation site.

If you have a cell-seeded scaffold and these cells are just going to migrate away, then you would have a problem, right. You would want to ensure that cell migration is optimized. Can you think of a way you would minimize this type of loss of cells from a cell-seeded scaffold?

Student: If they adhere properly.

If they adhere more strongly, if they prefer to adhere to the scaffold compared to what is present surrounding it, then they will probably not migrate away. So, optimizing the scaffold for cell addition can help in controlling cell migration.

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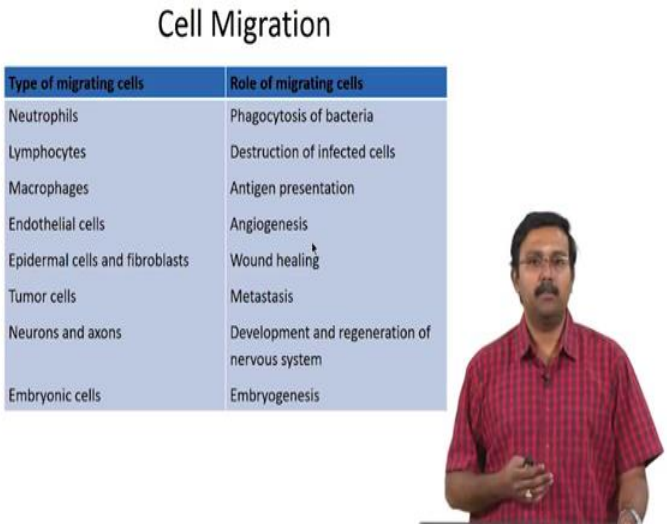

These are some of the cells which migrate very commonly, and these are the roles when it comes to migration. So, neutrophils migrate for phagocytosis; lymphocytes for the destruction of infected cells; macrophages in response to antigen presentation; endothelial cells to create angiogenesis; epidermal cells, and fibroblast during the process of wound healing; tumor cells during metastasis they will migrate; neurons and axons during the development and regeneration of nervous tissues, and embryonic cells migrate during embryogenesis.

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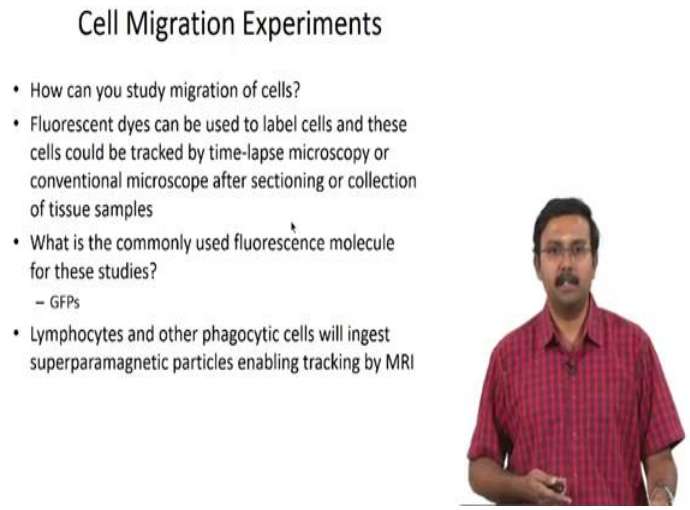

If you are looking to study the migration of cells. How would you go about designing an experiment to study cell migration?

Student: Culture cells.

No, migration does not always happen through the blood vessels.

It is not that the cells which are adhered to a surface actually enter into the blood vessel, and they are carried to someplace. It can happen during metastasis in some cases, but its migration. For it to move so, it has to come from somewhere to the blood vessel, if it is even going to do that, right.

Cell migration can happen on the surface, as well. See if any time cells are proliferating, they also have to migrate; otherwise, everything will just be forming a blog in that.

What you basically do is, you will need fluorescent label the cells, and these cells will be tracked using time-lapse microscopy or conventional microscopy. Confocal can be used, but confocal is usually more complicated. You can actually use it if you tag it with fluorescence, you can use simple microscopy techniques. You will see where the cells you seeded are and how far it has gone. You can use sectioning to see how far it has gone and so on. What is the commonly used fluorescence molecule for these studies?

Student: Can methylene blue be used?

Not really. What is the one common fluorescence molecule which you know for biological studies?

Student: Something of fluorescein.

Fluorescein diacetate; fluorescein diacetate is to stain live cells; you may be able to use it. But, the most common molecule which is used is GFPs.

Student: GFPs.

Lymphocytes and other phagocytic cells can also be tracked using MRI because they will ingest these superparamagnetic particles. These are phagocytic cells; they gobble up stuff. So, if you put a nanoparticle there, it will eat it up, and you can track them using MRI or other microscopy techniques.

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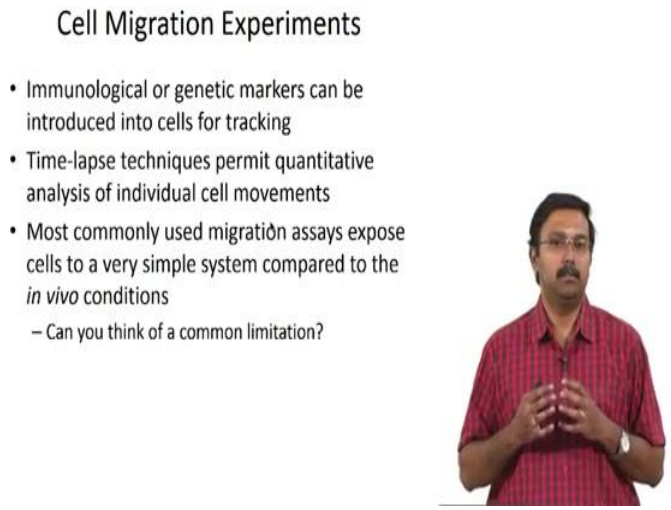

When we are talking about cell migration experiments, what you look at is to introduce some kind of genetic markers to the cells that can be tracked. Time-lapse techniques can permit quantitative analysis of individual cell movements. You might have seen these videos in some publications; they usually have these time-lapse videos. I do not know if you have. None of you might have taken my immunology right. Did anybody take immunology here? Ok. So, you did ok.

So, I am pretty sure Dr. Vani would have shown that video where a neutrophil runs behind a bacteria. It is a time-lapse video actually; it is not that it is going at that pace; it is about 30 seconds to 60 seconds video. And, you would be able to see a neutrophil chasing bacteria to eat it. You can look up such videos; these are time-lapse videos that were taken over a long period of time. Many a time you would see a clock which is running much faster than the actual time to show you what is the real time frame.

The most common migration assays which are used, expose cells to very simple systems compared to in vivo setup. In in vivo, the tissue is much more complex, whereas, a migration assay, if you are going to culture it on a plate and look at the migration, it is going to be very simple. Can you think of a common limitation which you would experience with such systems?

Student: While doing it on a plate can be of 2 dimensional versus 3D as you are putting down.

Yeah, that is usually the major problem. So most of the cell culture which is performed is done in the 2D setup; like only when people work on tissues, they start looking at 3D setups. So, that is important because when you have a 3D environment that the migration directions also increase. So, you might have to observe things from different angles.

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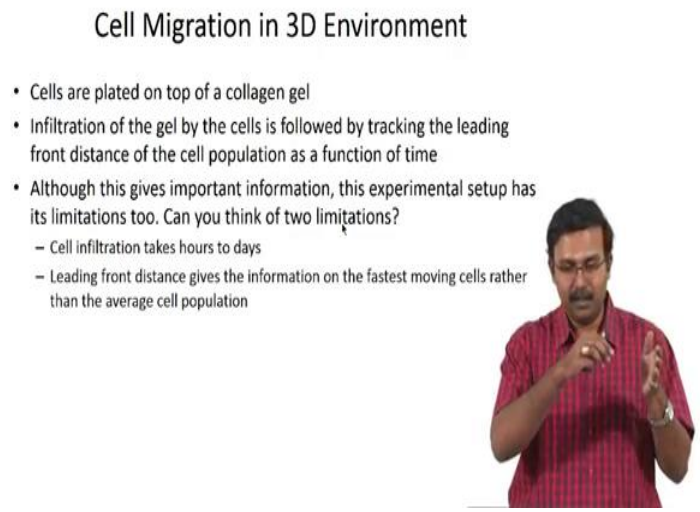

When you are talking about the 3D environment for migration, basically, what you do is similar to what Karan was alluding to. You have a collagen gel on which the cells are seeded, and you follow the cell infiltration into the gel by following the leading front of the cell population. See, not all cells are going to migrate at the same rate. You might have a lead cell, which is what you would track to see how it is moving. This gives important information about cell migration; however, this experimental system would have an important limitation. Can you think of any two limitations of migrating cells?

Student: Because of its 3D motion, the cell goes out of the focus.

Not necessarily, I am not talking about observational problems; I am talking about a design problem in the experiment.

Student: The scaffolds design will impede how it moves, migration.

But, that is what is supposed to happen right; the ECM is going to have its own barrier.

Ok, I will tell you. One thing is it is a very long process. So, it can take days for it to infiltrate and monitoring it, and maintaining the correct environment, making sure the cells do not die are all big challenges when it comes to performing these experiments. And, the other issue is more of a proper design problem, which is the leading front distance only gives information about the fastest moving cell. It is not the average cell population that you are looking at. So, whenever you are looking to collect data, you are trying to get something representative of the sample. This will not be truly representative of the sample; this will only represent the fastest cell.

Student: Sir, how are these fastest cells chosen likely?

No, because that is the leading edge you would be able to observe. When you observe it in a microscope, you will just be seeing the leading front as its moving.

Student: Ok.

That is all. See, if you were to have a fluorescence tagged thing, and you are observing in a microscope; you just look at the how far the cell from the starting point, that is all you are looking for.

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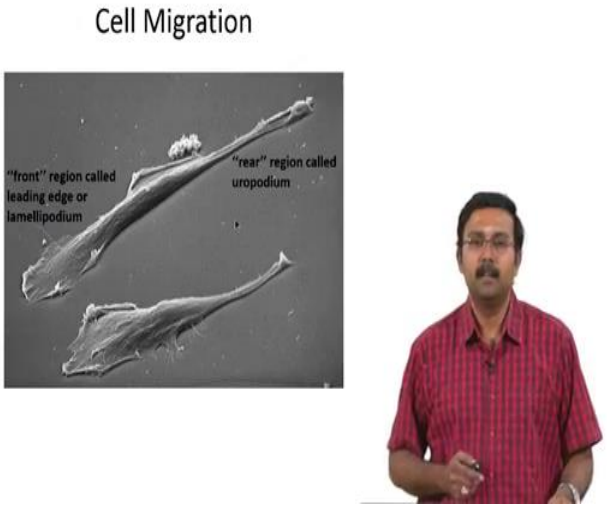

This is how a cell that is migrating would look like. So, what you see here is the front region, which is called the lamellipodium or the leading edge, and the rear region is the uropodium, which is seen in the back.

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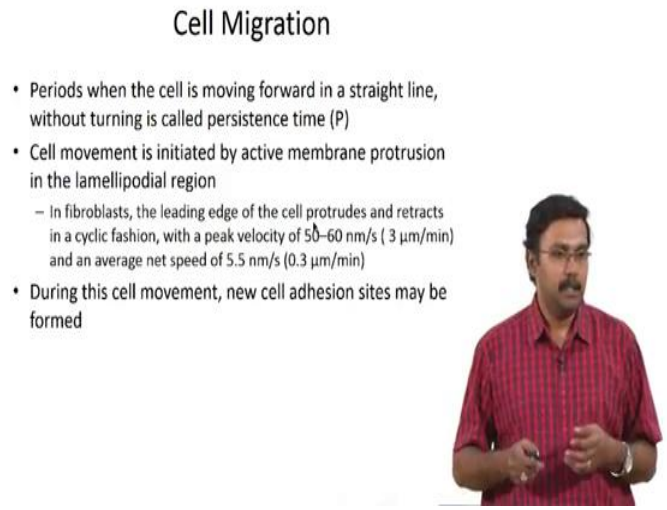

What happens during this time is, the cells which are adhered, starts moving, and they go and adhere to some other surface, and then they get detached from the previous site. So, once they have done that, they have moved one position. The periods when the cell is moving forward in a straight line without turning is called the persistence time. When you are talking about a cell migrating, the cells are not going to always move in random directions. So, they will continue in one direction for a while, and then they may change directions as well. This time frame in which it moves in one direction is called the persistence time, and this persistence time can be altered based on having chemoattractants and so on. If you have a chemoattractant, then the cells are going to try and migrate towards that attractant, and in that case, the persistence time will be longer. Otherwise, if it is going to be a uniform distribution of the chemoattractant, the persistence time will be much shorter. The cell movement itself is initiated by active membrane protrusion in the lamellipodial region.

In the case of fibroblasts, the leading edge of the cell protrudes and retracts in a cyclic fashion. The peak velocity, it can reach about 50 to 60 nm/s, and it averages a net speed of 5.5 nm/s; that is the rate at which they usually move. During this cell movement, new cell addition sites have to be formed; only then the cells can leave the previous adhesion site and go to the new adhesion site.

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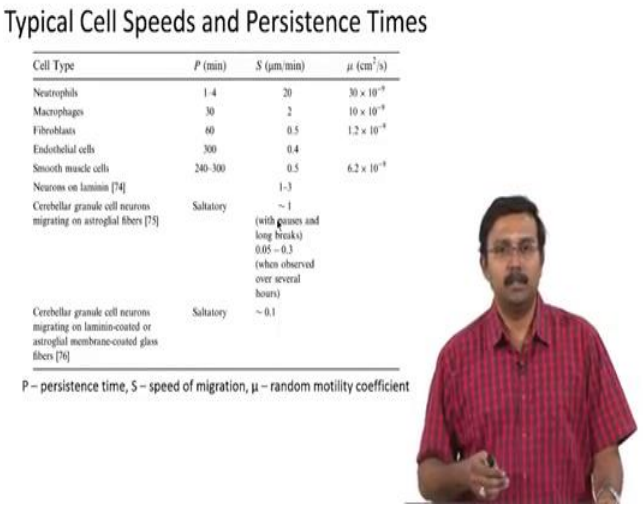

These are some of the cells, their persistence time, speeds, and their motility coefficients. Some of the cells, which are very motile, would have lesser persistence time, like your neutrophils or macrophages have lesser persistence times. Especially neutrophils have significantly lesser persistence times because they just have to figure things out in that region where they are. So, they cannot just keep moving in one direction, whereas, if you were to look at endothelial cells, they are not as migratory as these cells. They will have a much higher persistence time and whereas if you are looking at speeds, obviously, the cells with lesser persistence times end up having higher speeds and so on.

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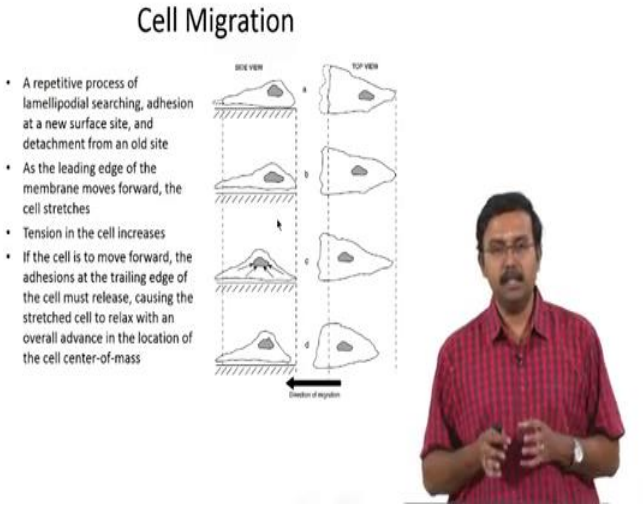

This is the process that describes cell migration. This is a repetitive process of lamellipodial searching for a new adhesion site. The new front edge, which is coming out or forming, will have to go and adhere to a new surface. Once it adheres to a new surface, the uropodial region has to get detached, and it moves to the new site. For this to happen, the cell would have to strongly adhere to the new site. So, the initial step is for the cell to stretch to figure out what is the right place to adhere. Once it adheres there, there is now tension in the cell; this is because the cell is stretched. This tension could cause it to either move forward or move backward, depending on whichever site has higher affinity. If the cells have a higher affinity to the new site, it will move forward; if it has a much higher affinity to the old site, it will move backward. So, this is something which you can even simply test as well. Like I do not know what are these jelly kind of clay which is called like now they are.

Student: Slime.

Slime yeah. You can play with that, and you will be able to emulate something like this. So, if you stick it strongly at one site and you stretch it out and place it on another site, it will either come to this site or go to the other site based on how strong your adhesion is.

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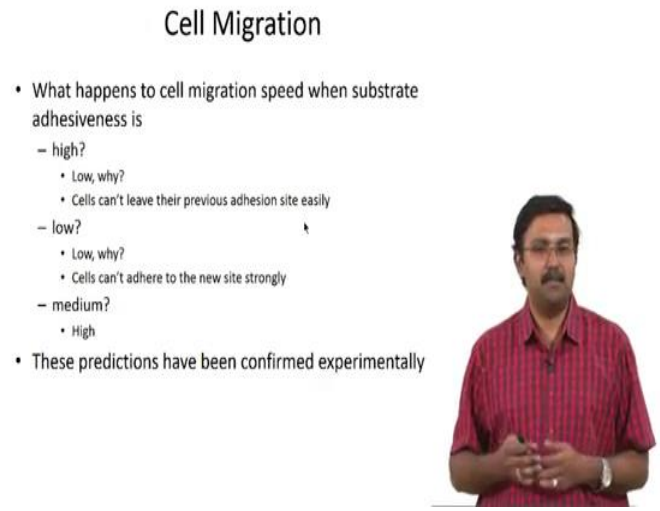

Let us look at some of the different adhesion properties of the surfaces and how that would affect your cell migration. So, what would happen to the cell migration speed, if your substrate has very high adhesive properties?

Student: The initial site or the?

The surface, in general, has very high adhesion. It is not like; I am not going to look at one cell. If I am preparing a scaffold with very good cell adhesion properties, then how do I expect the migration speeds to be?

Student: If it is high, it will be slow.

It will be slow because it has already adhered strongly; it might not want to go to another site. What if it is very low?

Student: Easier for it to migrate, but that does not necessarily mean it might want to.

If it has not adhered to the site, it means that it does not like the site. So, it might want to migrate as well.

Student: It is not getting the right grip to pull itself.

So, that would be the problem. In the first case, it will not be able to detach itself from the first site, whereas, in the second case, it will not have a position where it can attach and move. In both cases, the migration rate will be very low. When it is medium, that is when you would have a high migration rate, and as I have always said, biology always has some optimal values. So, high, low are never good; something in between is where you are looking for. These predictions have been confirmed experimentally as well.

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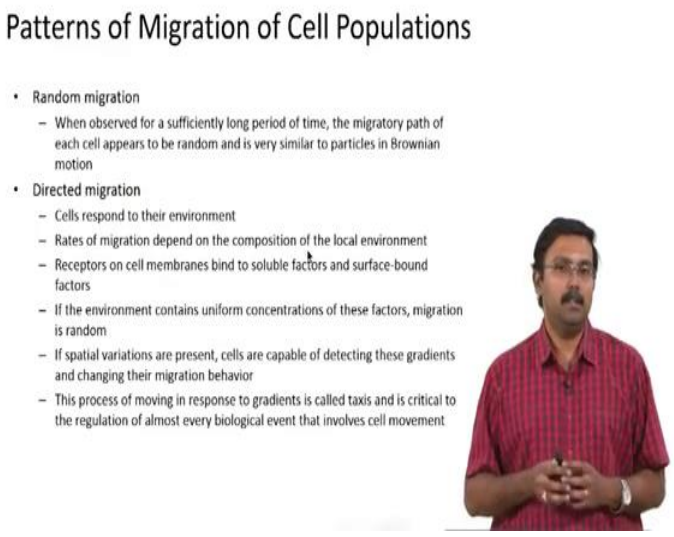

When you are talking about cell migration, there are two major types of migration. You have a random migration. This is observed when you look at it for a sufficiently long period of time. See, we looked at the persistence times, right? So, we said that the persistence time for endothelial cells is 300 minutes based on the table. If my period of observation is only 150 minutes, then I am going to think that the cells are moving only in one direction. And, if I am going to observe only one cell as well, like a smaller number of cells that is going to happen. If I were to observe a very large number of cells for 3000 minutes, then what is going to happen is different cells are going to keep moving in different directions. So, it will look clear that it is a random migration that is happening, assuming there is no signal which is directing the migration.

Directed migrations happen when it comes to cells responding to their environment. The rate of migration will depend on the composition of the local environment, whether the receptors are present, and what soluble factors or surface-bound factors are present. If the ligands are present on the surface, then the cells will migrate towards that, and so on. If the environment contains uniform concentration, then the migration becomes random. If you have one region which is rich in these ligands, then you might have a directed migration. If spatial variations are present, the cells are capable of detecting these gradients and changing their migration patterns towards these attractants.

This process of moving in response to some gradient is called taxis, and it is critical for the regulation of any biological event that involves cell movement. Otherwise, if cell movement is always going to be random, then you would have very little control over tissue development or anything. So, this directed migration ensures that this is happening. In case of the random migration, I had also put some term called Brownian motion. So, what is Brownian motion?

Student: Movement of particles in a random direction.

Ok. Why would it happen?

Student: Kinetic energy.

It has its own energy kinetic and internal energies, which make it move, and as it collides, they change directions. So, that is why it is random motion. If you observe random

migration of cells for a long period of time and you track the cells, it will look like it is a Brownian motion because it will be quite random.

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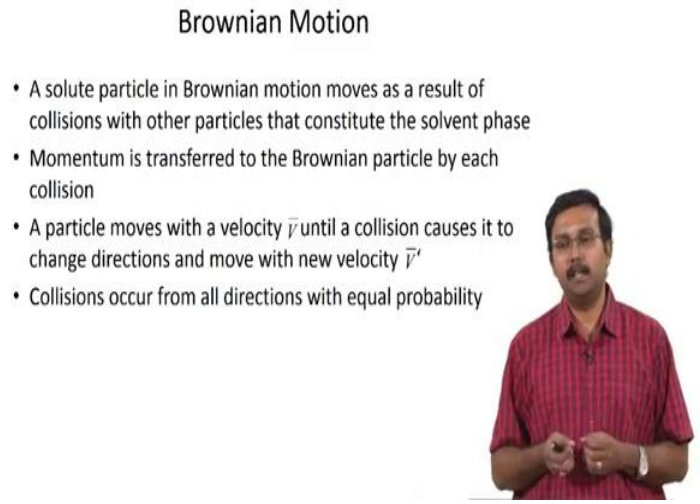

Brownian motion is a solute particle that moves as the results of collisions and their own energies. So, the momentum is transferred from one particle to the another during the collisions, particle move with the velocity; So, they can move with the velocity of $\overline{V}$ until the collision causes that to change directions and then move with the new velocity $\overline{V}'$. Otherwise, it will keep persisting in that. That is your persistence time.

Only when there is a collision there is going to be a change in direction, so that will affect your speed and velocity as well. Either could be momentum, which is lost or momentum gained based on the velocities and mass of these two particles, which are colliding. Collisions occur from all directions with equal probability; that is what causes this kind of random motion. If it is going to be in one particular direction, then you are not going to have a random moment.

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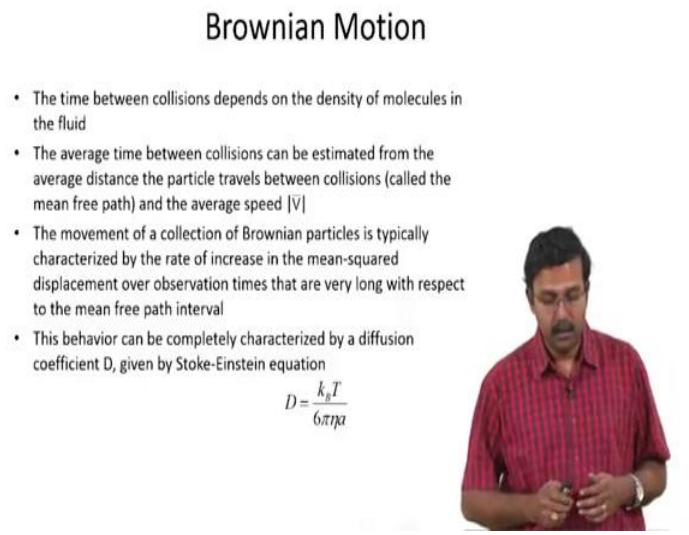

In the case of Brownian motion, the time between these collisions will depend on the density of the molecules. If there is going to be a higher concentration of the solute molecule, there is going to be a higher chance of collision and so on. The average time between collisions can be estimated from the average distance the particle travels between collisions, which is called the mean free path and the average speed at which these are moving. The movement of a collection of Brownian particles is typically characterized by the rate of increase in the mean-squared displacement over a period of observation.

Usually, this timeframe for observation is much larger than the mean free path interval. Similarly, if you were to draw an analogy to our system, your period of observation has to be much larger than the persistence time. That is why I was saying, if you are looking at 300 minutes and have observed it only for 150 minutes, it is not going to work. The persistence time has to be much smaller than the period of observation for you to understand random migration. This behavior can be characterized by a diffusion coefficient D, which can be given by this Stoke-Einstein equation.

$$D = \frac{k_B T}{6\pi\eta a}$$

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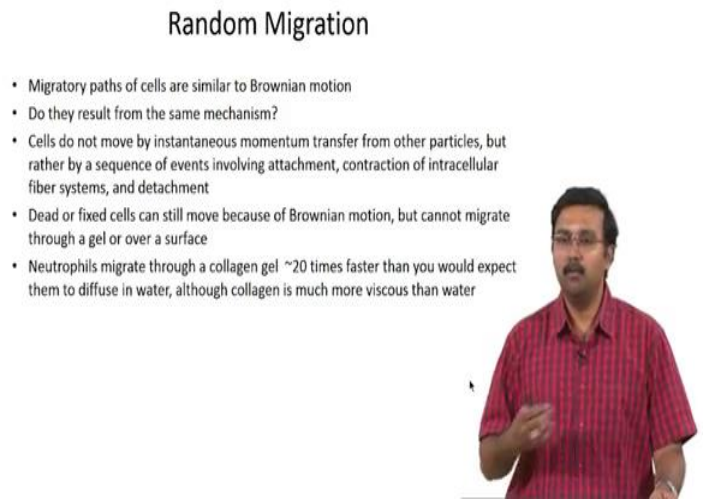

We talked about how random migration can look like Brownian motion and what Brownian motion is. But, are the mechanism similar?

Student: Random migration is different.

Ok. So what would be the difference?

Student: I do not think there could be a collision between.

Yeah. So, it is not that the momentum transfer is happening to cause the change in directions, but change in directions is happening because of adhesive properties and other things. And, the other thing is, this change in direction is not instantaneous. In the case of molecules colliding, you are going to keep coming then boom, and then go in different ways; it is not this way. In cell migration, the change in cell direction is going to be very, very slow.

It is not a transfer of momentum, but it is a sequence of a significant number of events, which include an attachment to a new surface, a contraction of the intracellular fiber system, and detachment from the previous surface. Dead or fixed cells can also move because of Brownian motion. So, they will not migrate on the surface. Those would just be particles right; those will be particles in a suspension.

Neutrophils can migrate through a collagen gel 20 times faster than you would expect them to diffuse in water. Although collagen is a much more viscous system than water, showing you that it is not going to be dependent on the momentums, but it has mechanisms to make sure it reaches things faster. So, neutrophils usually are trying to attack stuff when they have to migrate through the collagen matrix, so they move very very fast in that environment.

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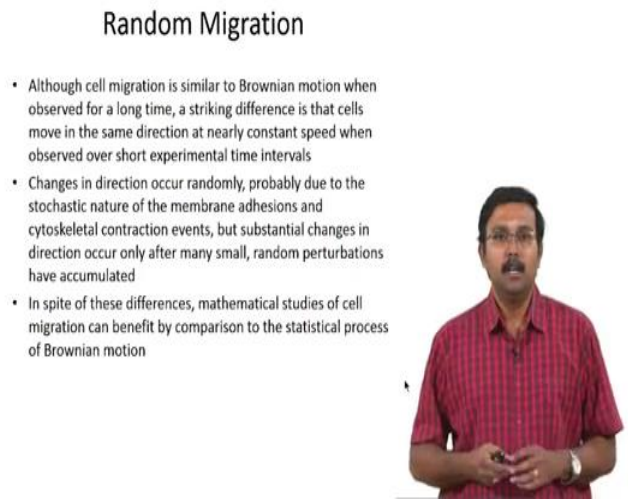

Although cell migration is similar to Brownian motion when you observe it for a long period of time. The striking difference would be that the cells can move in the same direction at a nearly constant speed when you observe it for relatively short intervals. The change in direction can occur randomly, and this is completely stochastic in nature, and it depends on the membrane adhesions and cytoskeletal contraction events. But, substantial changes in direction occur only after small random perturbations have been accumulated.

It is not like the cell which is moving this way, suddenly starts changing it in directions and moving the other way. It will then move slightly, slightly to another site, another site, another site, before it completely changes directions. So, in spite of these differences, you can use mathematical studies based on Brownian motion for understanding for random cell migration.

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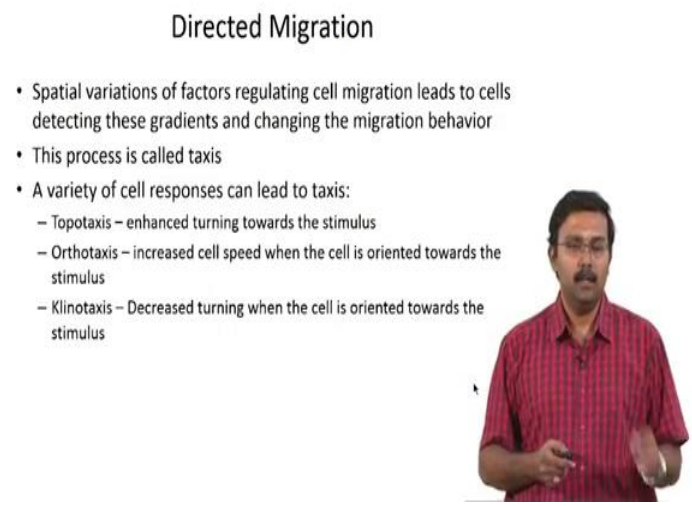

In our department, Professor Murugan works not exactly on cell migration but on aspects of protein migrations and protein folding using Brownian motion and so on. When you are talking about directed migration, that is spatial variations of factors that regulate cell migration, and this leads to cells detecting these gradients and changing their migration pattern. This process is called as taxis. Topotaxis is enhanced turning towards the stimulus. Orthotaxis is increased cell speed when the cell is already oriented towards the stimulus, and you have klinotaxis, which is decreased turning when the cell is oriented towards the stimulus. So, these are the four ways that cells can respond in the presence of a stimulus.

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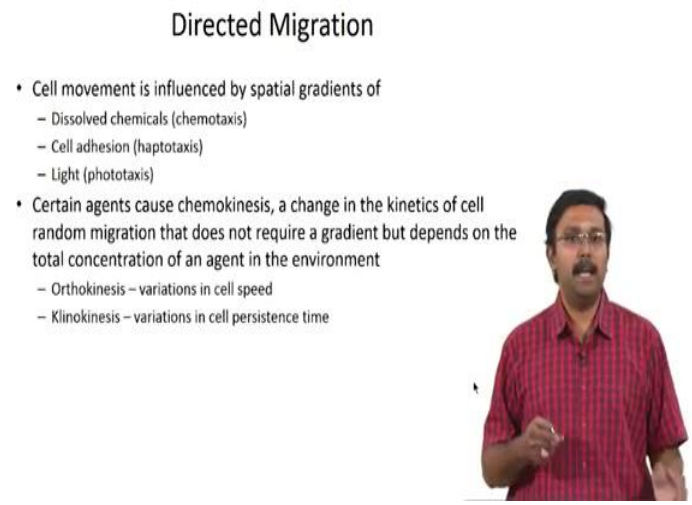

Based on what stimuli are causing the migration, the process can be labeled as chemotaxis, haptotaxis, or phototaxis. Chemotaxis is when you have dissolved chemicals, some chemicals which are acting as signaling molecules. Cell adhesion would cause haptotaxis, and light could be the trigger for phototaxis. Certain agents can cause chemokinesis, which is a change in the kinetics of cells random migration that does not require a gradient, but it will depend on the total concentration of an agent itself.

This is in response to a particular concentration the cells will move, and again, we use the same terms like orthokinesis and klinokinesis. Orthokinesis is variations in cell speed, and klinokinesis is variations in cell persistence time.

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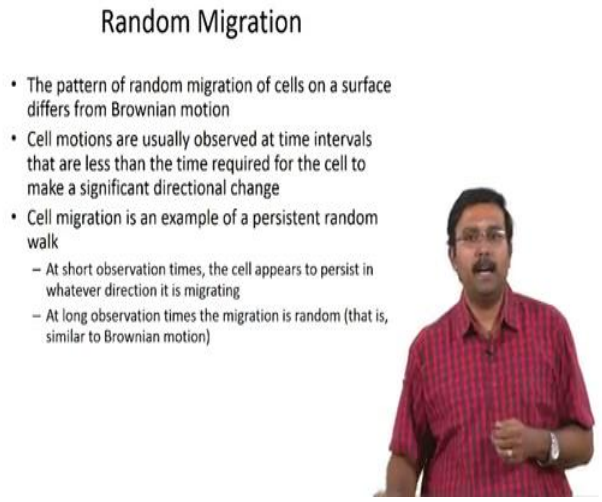

The pattern for the random migration of cells, as we said, can be similar to Brownian motion if you observe it for a long period of time; however, if you look at it only for a short period of time, it will not be. You need to make sure that there are significant directional changes so that you can look at it as Brownian motion.

However, cell migration is actually a very good example of something called a persistent random walk. So, what happens is at short observation times, the cells seem to be persisting towards one direction, and for long observation times, the cells are migrating in random. So, that is what is a persistent random walk; it represents both sites.

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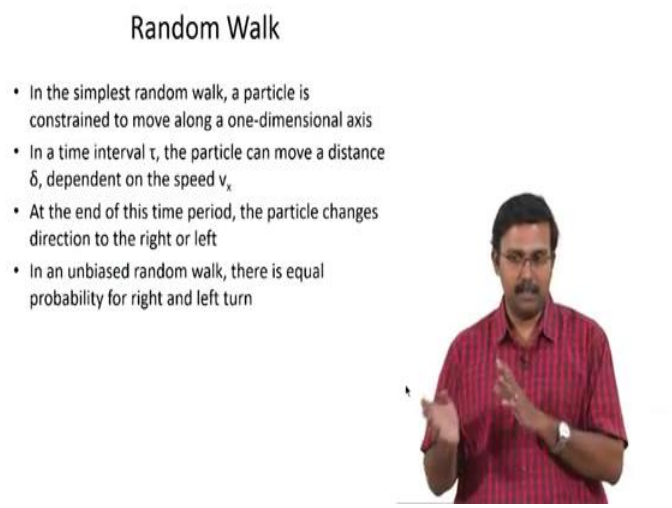

In the simplest random walk, what happens is that a particle is constrained to move along a one-dimensional axis, and in a time interval τ, the particle can move a distance of delta depending on the speed in which it is moving. So, that is quite logical. At the end of this time period, the particle will change its direction. So, this is the persistence time. At the end of the persistence time, it will change directions and go into something else. The particle can go into left or right.

In an unbiased random walk, there is an equal probability that it will go to either left or right. Here, I am walking towards a certain direction. Say from here, let us say I am walking like this, and if 3 steps are my persistence to walk and then I can turn left or right and either way is fine. So, I do not have a preference, and that would be an unbiased random walk.

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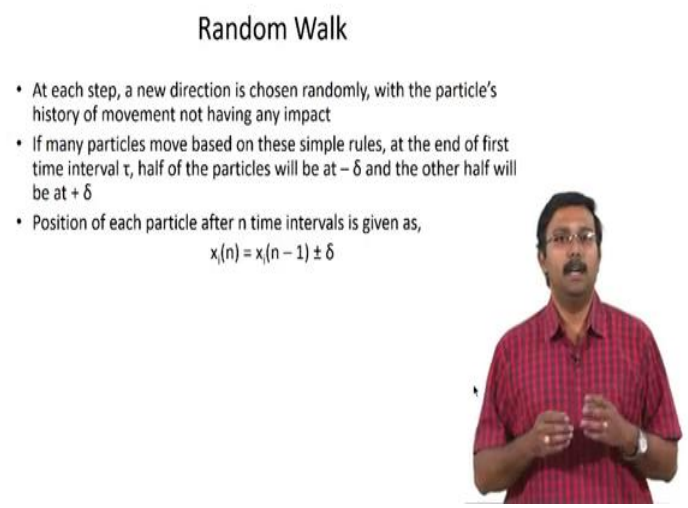

At each step, a new direction is chosen randomly and with the particle's history of the moment having no impact; that is quite logical, right. If you are to think about the cell, once it gets detached from the previous site; the previous site has no role to play in where the cells are moving towards. If many particles move based on these simple rules at the end of the first time interval τ, half the particles will be at -δ, and half the particles will be at +δ.

If all cells have exactly equal probability of moving left or right, then half would have moved left, half would have moved right. So, the center is 0; it would have moved to -δ, another would have moved to +δ. The position of each particle after n time intervals can be given as

$$X_i(n) = X_i(n-1) \pm \delta$$

That would basically mean at (n-1) this is my position; it could either be this or this, right. So, those are the positions I can be in after one step.

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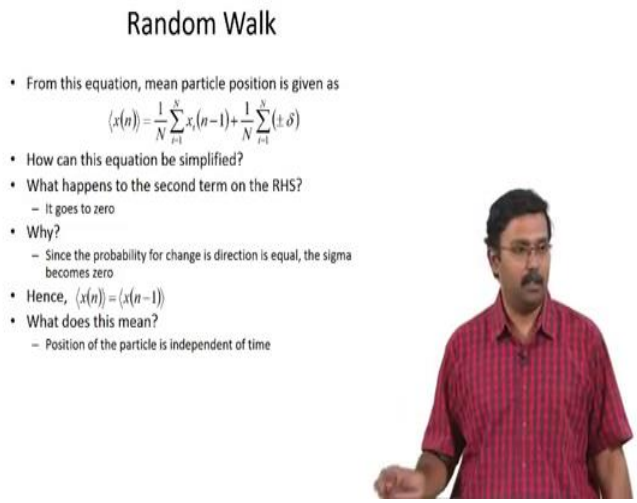

From this equation, you can calculate the mean particle position using this. Basically, you just divided it by the number of particles; like the summation of all the positions divided by the number of particles. So now, you need to simplify this equation. If you were to simplify this equation, can you think of what would happen to the second term?

Student: Does it not get to cancel?.

Why?

Student: Because that is plus delta minus delta.

Yeah.

Half will be plus half will be minus so, it goes to 0. This happens only in an unbiased random walk. If there are biased, then you cannot do the simplification. So, basically what happens is

$$\langle x(n) \rangle = \langle x(n-1) \rangle$$

So, what does this mean?

Student: You mean the position of the cells remains the same.

Yeah, it is independent of time; the position of the particle is not dependent directly on time.

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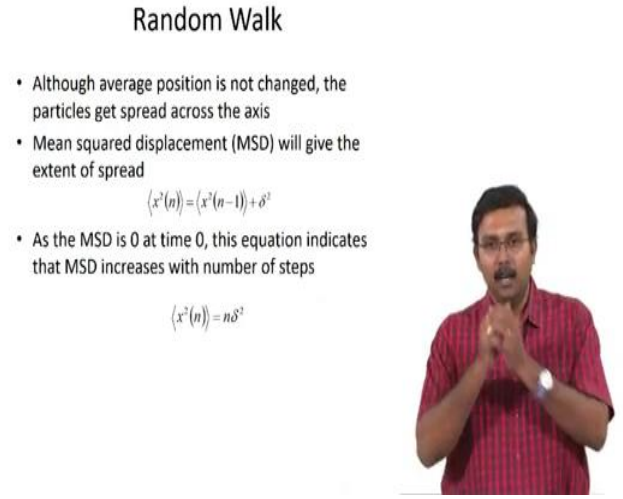

All the average position has not changed; the particles are spread across the axis; it is not that the cells are not migrating. For every cell which is moving to the right, there is another cell moving to the left. So, its mean distance from its origin is different, they have increased, but if you were to add all cells that would even out.

The mean squared displacement will give you the extent of spread. That is why you look at this squared value rather than just the actual value because that will give you a 0. As the means squared distance is 0 at times 0, the equation over here indicates that the mean squared distance increases with the number of steps; that is logical, right. As you have more steps, you would have more distance covered and more spread.

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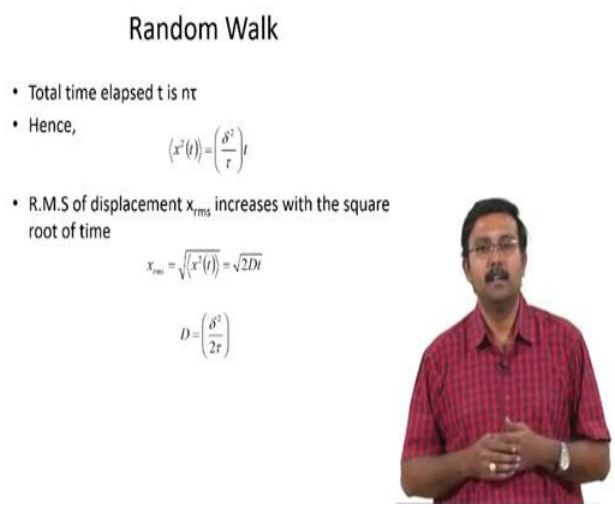

If the total time elapsed is nτ, then this equation would simplify to this.

$$\langle x^2(t)\rangle = \left(\frac{\delta^2}{\tau}\right)t$$

All I am doing is dividing it by nτ. The root mean square of the displacement, which is x_{rms} is given by this equation,

$$x_{rms} = \sqrt{\langle x^2(t)\rangle} = \sqrt{2Dt}$$

and you have D equals to

$$D = \left(\frac{\delta^2}{2\tau}\right)$$

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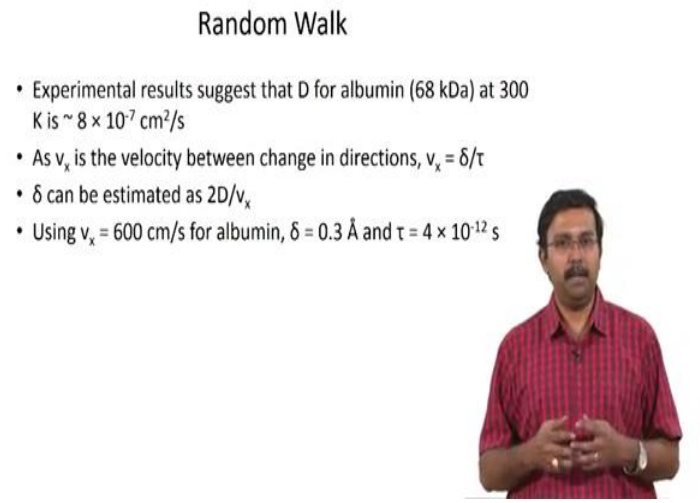

People have shown that experimental results suggest that D for albumin is about 8×10^{-7} cm^2/s, D is a diffusion coefficient. As v is the velocity between the change in directions, during the persistence time, they have a migration velocity, and that would be δ/τ. δ is the distance covered, τ is the persistence time, so that would be your velocity. So, δ can be estimated as $2D/v_x$. Using this v_x of 600 cm/s for albumin, δ is 0.3 Å, and τ is 4×10^{-12} s.

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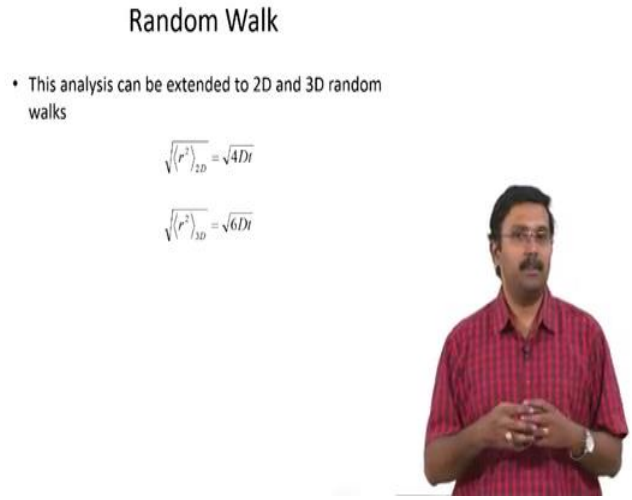

That just gives you a feel for what this would be. So, whatever we looked at right now was a 1-dimensional random walk. So, it could only move left or right; it could not move front

or back; from one point, it only did left or right. In a 2-dimensional random walk, you will also have front or back and other directions in the same thing. That would end up with an equation of

$$\sqrt{\langle r^2 \rangle_{2D}} = \sqrt{4Dt}$$

and 3-dimensional random walk will give you

$$\sqrt{\langle r^2 \rangle_{3D}} = \sqrt{6Dt}$$

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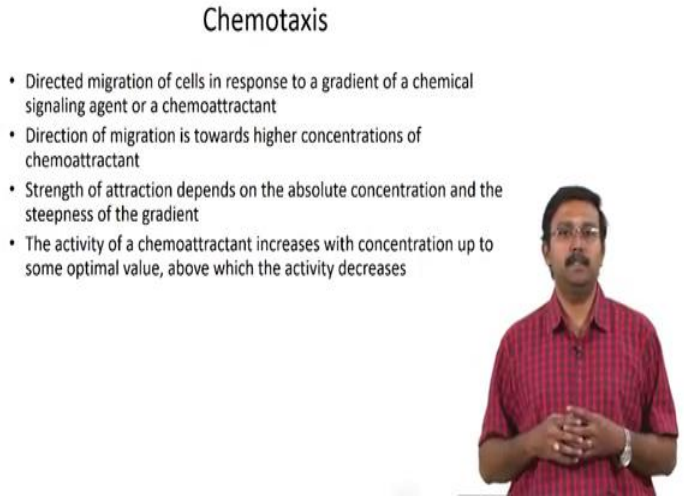

Directed migration of cells in response to a chemical attractant or chemical agent is called chemotaxis. So, this could be a chemical signaling molecule or a chemoattractant. The direction of migration is usually towards higher concentrations of chemoattractants. The strength of attraction will depend on the absolute concentration and the steepness of the gradient.

Your concentration has to be high and also the region where the cells are present if the concentration is low, then there is going to be this gradient which will make it move faster. The activity of the chemoattractant increases with concentrations up to an optimal value, and then the activity starts decreasing.

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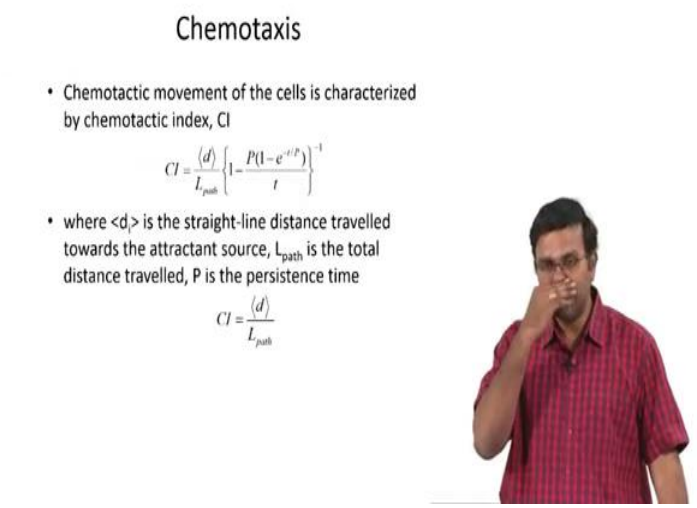

Again the chemotactic index is the term which is used to characterize the chemotactic moment. This is just an equation that describes it.

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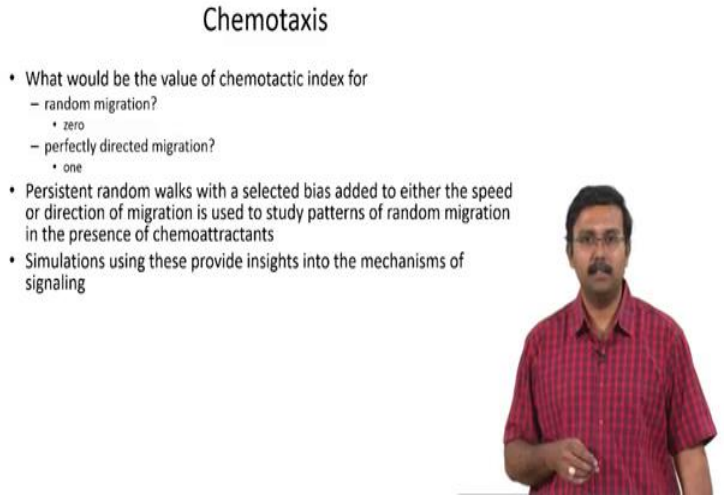

Basically this is given as d/L_{path}, where d is the straight-line distance, which is traveled by the cell towards the attractant. If there is a chemoattractant, the cells will be moving towards that, and the distance which it persists towards that is called as d. And, the L_{path} is the total distance that is traveled by the cells, and P is the persistence time, and t is the time, and this is the correlation. It can be simplified to get something like a CI equals

d/L_{path}. So, what do you think would be the chemotactic index for a random migration? There is no real attraction, and it will keep moving all over the place, whereof perfectly directed migration?

Student: one.

One, because your d will be L_{path}, right.

So, the persistent random walks with a selected bias added to either the speed or the direction can be useful for studying the patterns of random migration in the presence of chemoattractants. That would be the directed migration, and simulations have been done to understand this.

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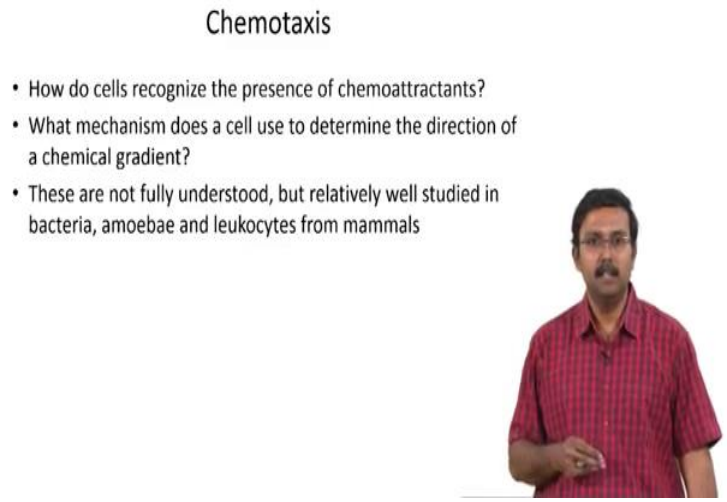

However, it is still not fully understood as to how cells recognize the presence of chemoattractants and what mechanism the cells use to determine the direction of the chemical gradient. This is not understood in mammalian cells; it is very well understood in bacteria and other unicellular organisms. So, not all mammalian cells have been studied well enough to understand these factors.

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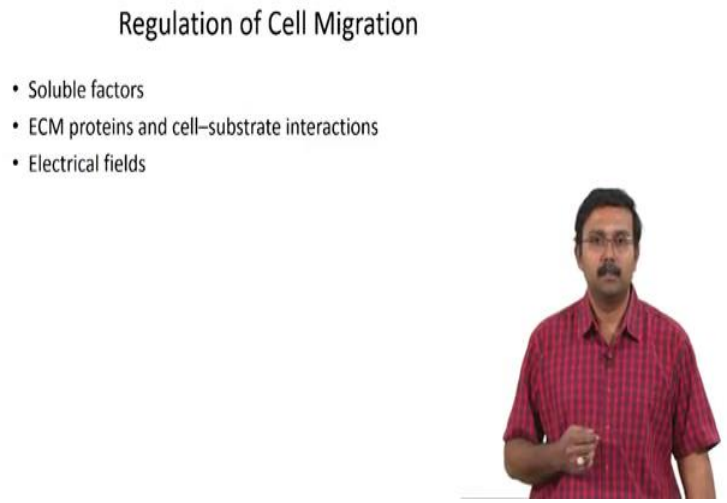

Some of the factors which can regulate cell migration are soluble factors, ECM proteins, cell-substrate interactions, even electrical fields have shown to impact cell migration. So, that gives an overview of what cell migration is, and the only aspects which we still have to talk about are reactors and how we use signals. And, with that, we will come to the conclusion of all the fundamentals related to tissue engineering.