Tissue Engineering
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Lecture - 19
Tissue Dynamics

Today, we will be talking about Tissue Dynamics. We have been talking about cell culture and looked at how cells initially had to be harvested, isolated, and cultured and even differentiated. I hope the assignment gave you an idea of what differentiation is and how complicated directed differentiation can be. Because achieving differentiation is not too difficult, actually. If you are talking about just differentiation, then all you do is expose them to some stress condition and it will just differentiate to some random cells.

And mostly it will just differentiate to something very prevalent like a fibroblast or something. But if you want some specific directed differentiation, then it is quite complicated, and it can be quite tricky. So, having discussed some of those things, let us move on to tissue dynamics.

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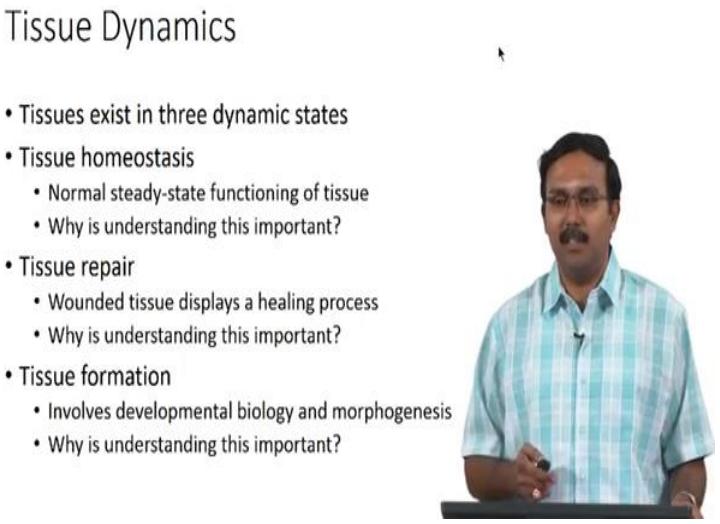

Tissues actually can exist in three dynamic states; tissues are not static; they can change in their status. And do you know what the dynamic states are? You might have studied it in cell biology, I hope. So, it is homeostatic, which is when it is normal steady-state functioning. The tissue is doing what it is supposed to do, and it is just existing in the

system. So, why do you think understanding this tissue homeostasis is important from a tissue engineering perspective?

Student: Generally, we prefer the tissues in this condition.

Ok, ultimately, the tissue has to exist in this condition after implantation. It is important to understand what its role is, what exactly it is doing, how the cells should behave, and so on, so that we can try to achieve it in our tissues. The next step is tissue repair. So, which is where any wounded tissue displays the healing process. And why do you think understanding this is important from a tissue engineering perspective?

Student: An implant could get damage.

The implant could get damage that is one thing, but you would actually be creating a wound during implantation. There are things like in situ forming hydrogels or injectable hydrogels; those things are not exactly going to cause wounds, but in many cases, you are going to cause wounds, and the healing can influence how the tissue integrates with your engineered tissue. So, it is important to understand this process, as well.

The last one is the formation or development. Here it is the initial formation of the original tissue, which can be understood by studying developmental biology and morphogenesis. Why do you think this is important? Not just how to grow the tissue; Like how to create the tissue.

How they will behave in general is unknown; with respect to tissue formation, whatever you did in the assignment for differentiation that comes from developmental biology. People do not just say I will throw these growth factors and see what happens. You try to understand how the tissue develops when an embryo is developing into a whole organism. And during that process, they try to understand what are all the signaling pathways that are involved in tissue development, what are the molecules which are involved and try to use these molecules to direct the differentiation.

Understanding this can will help you to create tissues, especially if we are going to be using stem cells, and we are going to direct differentiation. So, these are the three dynamic states.

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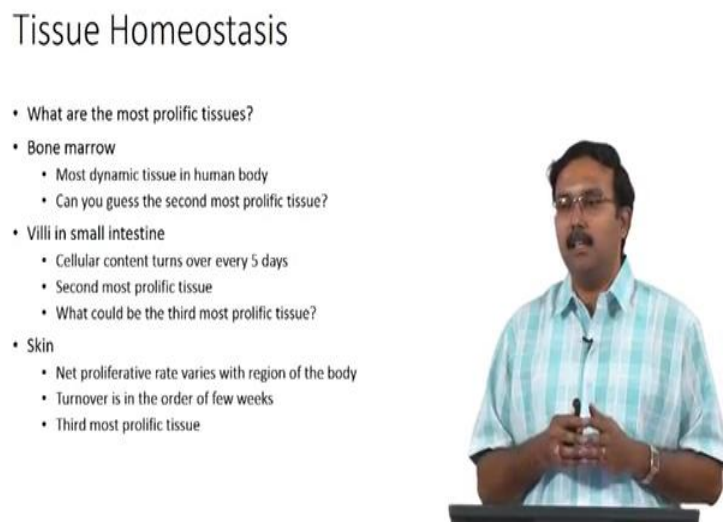

If you are going to talk about tissue homeostasis, some tissues would be in their homeostatic state for a long period, whereas some tissues are quite prolific, and they keep getting replenish regularly. So, can you guess what are the most prolific tissues in your body?

Student: Skin.

Skin is one of the most prolific tissues, yes. Anything else?

Student: Liver.

The liver can regenerate, but it is not that it keeps replenishing itself again and again.

Student: Mucosal layer.

The mucosal layer, the intestine layer, is one that gets replenished regularly. Anything else, what would you think the most prolific?

Student: Blood cells, blood.

Not exactly blood cells, but.

Student: white blood cells.

Bone marrow. So, bone marrow is one of the most dynamic tissues in your body. So, it might get replaced very quickly. And the second most prolific tissue is the villi of the small intestine, which is the mucosal layer, which is present on the small intestine. Here, the cellular content can turn over every 5 days.

And third most prolific is the skin. And so, the net proliferation rate can vary based on the region of your body, and the turnover is in the order of a few weeks, so that is homeostasis. And every tissue has its own homeostasis. Will not get into individual homeostasis because it is different for the type of tissue you are going to work with.

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However, the repair process is more uniform. It is common for any tissue which you are talking about. When a tissue is injured, the healing process that usually varies with age starts. So, there are two types of healing. You have fetal wound healing, which is rapid and leads to restoration of scarless tissue, and you have postnatal healing, which is slower, and it also leads to scarring.

That is why many of us would not have like scars from when we were a very little unless it was a really bad gash you had, you are probably not going to have scars from whatever injuries you faced when you are 1 year old, and all of us go through that right. But none of us have scars because the healing process changes as you age. And if you are in your teens or young adulthood, whatever wound you have created, these scars are going to be much more lasting.

The postnatal tissue healing is slower, and it leads to scarring. Wound healing follows a sequence of events; it is a very sequential process; it is very well controlled, and that make sure that the damaged tissue gets repaired to form healthy tissue.

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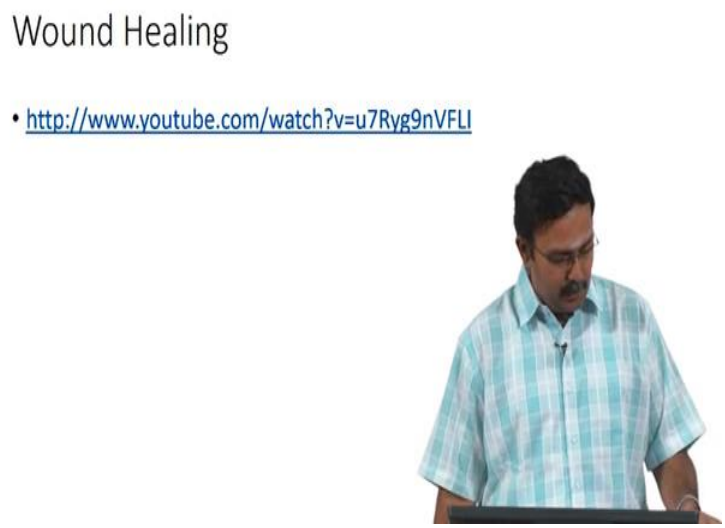

This is a YouTube video. I would like you to watch; it is about a 4-minute video; it explains the healing process with good animation. It is better to learn from this than for me to explain it. So, just have a look at the process.

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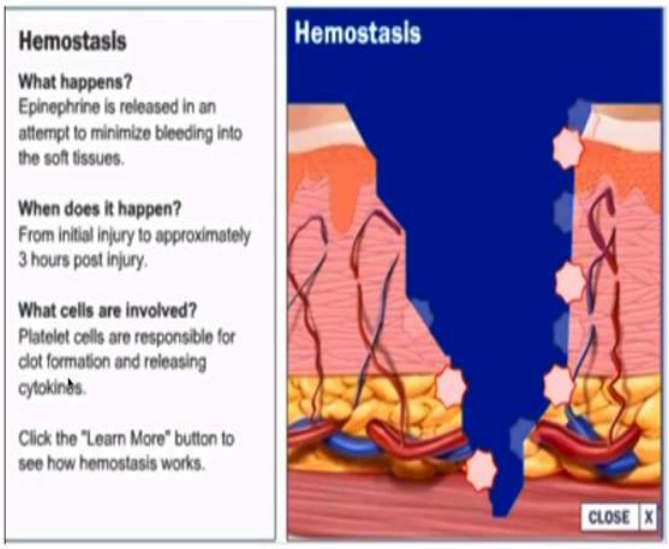

Hemostasis occurs right after initial injury. At the time of injury, epinephrine a chemical that constricts peripheral blood vessels, is released in an attempt to minimize bleeding into the soft tissues. The key cell responsible for this function is the platelet, which causes the body to form a clot to prevent further bleeding. There is an increased aggregation of platelets to enable the wounded vessel to complete the clotting process. Platelets also release key cytokines such as platelet-derived growth factor that calls in cells to participate in later phases of healing. The objective of the hemostasis phase of wound healing is to control bleeding. Following hemostasis, the inflammatory phase begins. The local signs and symptoms that occur during the inflammatory phase are swelling, increased fluid, perfusion of blood, redness release of epinephrine estimate heat histamine response and pain.

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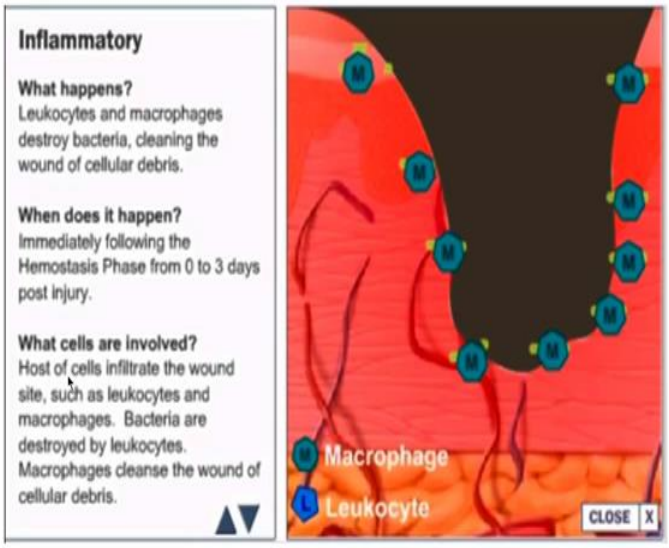

The inflammatory phase is characterized by our host of cells leukocytes and macrophages infiltrating the wound sites. Bleeding is controlled by hemostasis. Any bacteria that is present is destroyed by leukocytes, particularly the polymorphonuclear neutrophil. About 4 days after the injury, macrophages work to destroy bacteria, cleansing the wound of cellular debris. Macrophages, replace the leukocytes and produce a host of cytokines and growth factors. These act as chemoattractants to other cells needed for tissue repair.

Macrophages also convert macromolecules into the amino acids and sugars necessary for wound healing. It is thought that the macrophage also attracts contractual cells to the

wound to encourage wound contraction, vasodilation with resultant edema warmth and rubber are the result of factors secreted from the macrophage and other leukocytes present at the wound site in response to the inflammatory process. The objectives of the inflammatory phase at wound healing to clean debris and bacteria and prevent infection.

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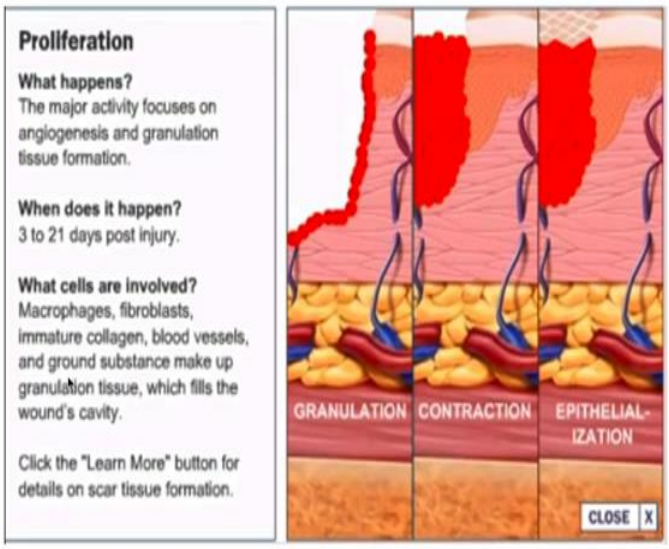

Scar tissue formation is characterized by three distinct phases, granulation, contraction epithelialization. Click on one of the phases to learn more. In an open wound, granulation tissue is generated, producing red beefy shiny tissue with a granular appearance. This tissue consists of fibroblasts, capillaries, and neutrophils as this type of tissue proliferate. Fibroblasts stimulate the production of collagen, which gives tissue its tensile strength and structure.

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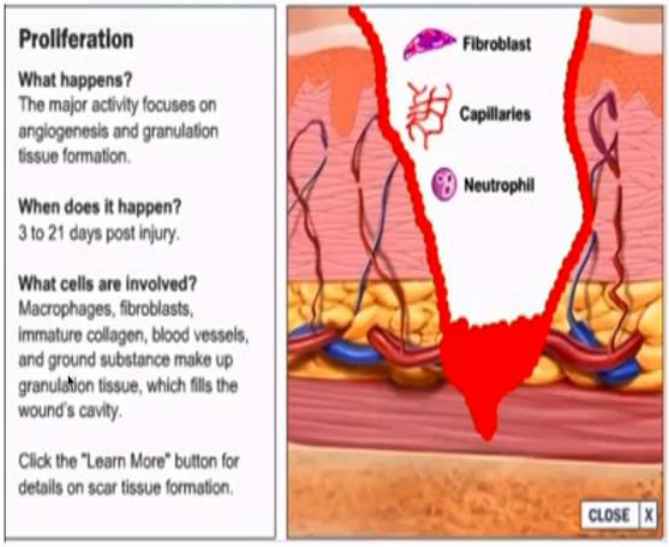

As the wound site fills with granulation tissue gives margins contract or pull together, decreasing the size of the wound. The extent of contraction is dependent upon the mobility of the surrounding tissue. During the epithelialization, the final step of this phase cells migrate from the wound margins, divide and ultimately touch one another, sealing the wound.

Epithelialization can only occur in the presence of viable vascular tissue. Epithelial cells will not migrate across a dry surface or necrotic tissue. During the maturation phase, the collagen fibers reorganize, remodel, and mature, gaining tensile strength. Collagen fibers, proteoglycans, and fibronectin are rearranged and redistributed.

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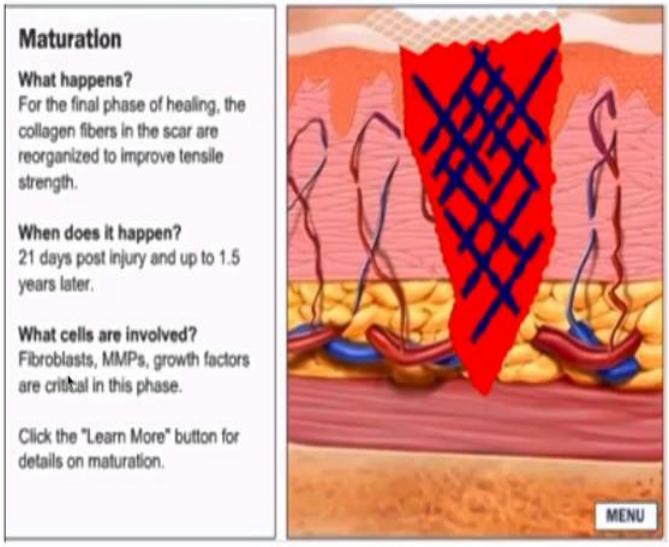

The scar becomes less cellular and gains tensile strength. However, this tissue will always be at risk for breakdown, because its tensile strength is less than that of uninjured skin. Collagen synthesis begins with the fibroblasts, which secrete procollagen. The growth of procollagen fibers is a complicated process. Macrophage and platelets are key growth factors in the development of procollagen. Procollagen fibers mature into collagen fibril.

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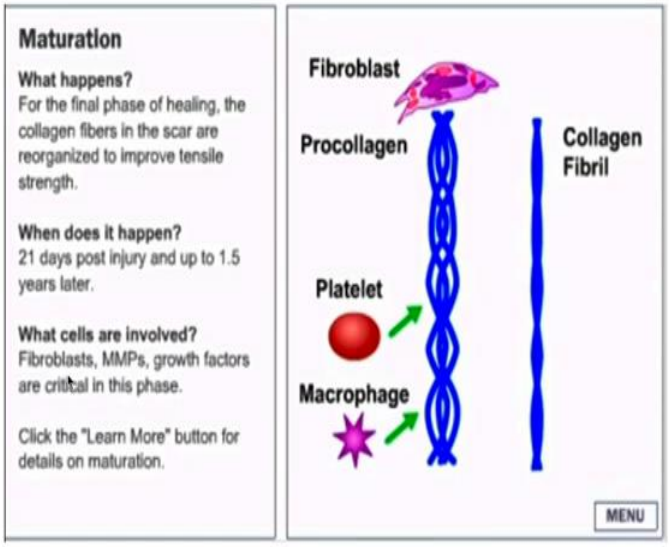

The fibrils then link together into a very strong rope-like collagen fiber. There are about 10,000 fibrils interconnect within a single collagen fiber. Research is currently underway to determine the chemical details associated with collagen synthesis.

As you saw, the general thing is, it is quite logical right first stop the blood, and then there is an inflammation phase which is a phase where you feel the pain and so on, and then you have proliferation phase where there are granulation tissue formation and collagen deposition and so on. And then finally, epithelialization and the healing process. So, this makes sure that any tissue can be healed. In impart conditions, one of these phases can get prolonged.

For example, in diabetic wound healing, the inflammation phases are prolonged; that is why it ends up becoming a chronic wound rather than healing within a period of time. So, trying to treat these aspects can help you. Even if you are going to develop an engineered tissue, you might have to consider having some growth factors which will help in the healing process, so that there will be a better host integration and so on.

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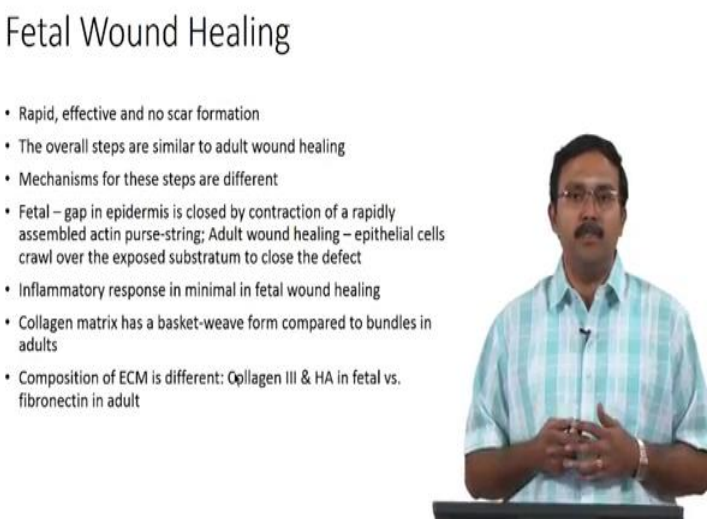

In the case of fetal wound healing, the process is much faster, and it is quite effective, and there is no scar formation. The overall four steps that are involved are similar to that of adult wound healing. However, individual mechanisms are different. What happens is in the fetus; the gap in the epidermis is closed by contraction of a rapidly assembled actin purse-string. Basically, it is like a zipping up of the wound. Whereas, in the case of adult

wound healing, the epithelial cells crawl over the exposed substratum and then close the defect. So, this is a slower process to do that.

The inflammatory response in the case of fetal wound healing is minimal. So, the inflammatory phase is quite short, and the process goes on quickly. The collagen matrix has a basket-weave form compared to the bundles which you see in adults, so that is one of the reasons you have a lesser scaring when it comes to fetal wound healing. The composition of the ECM is also different; you have collagen III and hyaluronic acid in fetal wound healing, whereas, it is primarily fibronectin in adults. So, those are the differences in fetal wound healing versus adult wound healing.

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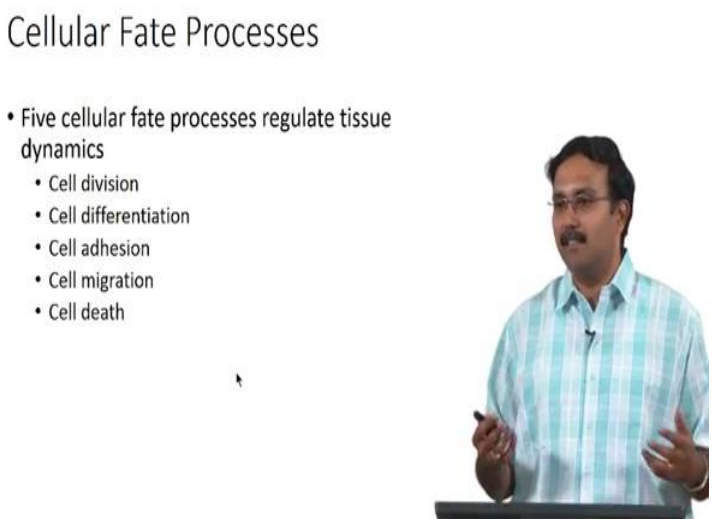

Whenever we talk about tissues, the tissues are basically made of cells, right? So, tissue dynamics depend on what the cells are doing. Five cellular processes regulate tissue dynamics. So, can you again guess what those five could be? What could the cells be doing?

Student: Growth function.

Grow. Sorry, what was it, differentiation, yes.

Student: Proliferation.

Proliferation or growth ok. Where is cell death?

Student: death.

So, those are the biological aspects of cell death, but we have cell proliferation or growth, you have cell death. You said cell differentiation. What else would a cell do when it is present in a tissue? So, it basically can divide, differentiate, adhere, migrate, or die.

And based on what it does, the tissue dynamics will change. And you are going to have cell populations which are doing different things in the same tissue at varying stages. There will always be some cells which adhered to, some which are migrating, some which are dividing and some dying and so on.

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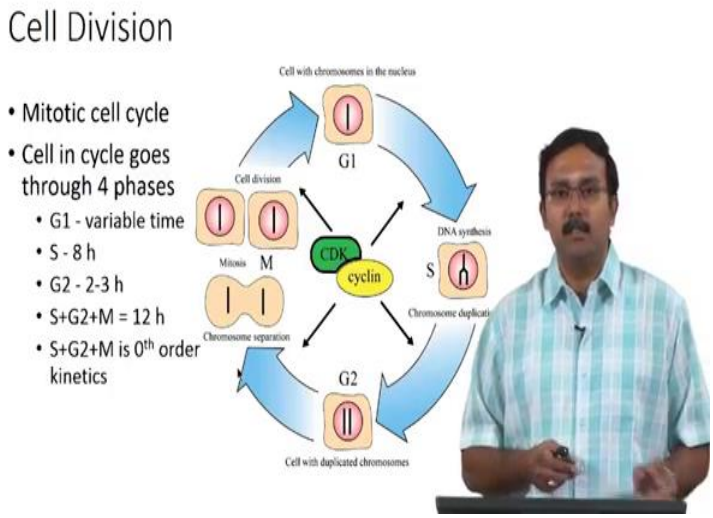

We will go through quickly about fundamentals related to division and death because that is something most of us would be familiar with. You would have studied at either as part of cell biology when it comes to mammalian cells or as part of microbiology and biochemistry when you are talking about bacterial cells. The principals are still the same. So, we will just quickly go through them.

Cell division basically, we talk about mitotic cell division, so meiotic is not relevant for tissue engineering, so that is just the formation of gametes, which has nothing to do with the creation of tissues, at least from this perspective. In a mitotic cell cycle, you have four phases. So, you have the G1 phase, which is where the cells are present and doing their job, and this can have a variable time. And you have the S-phase, which takes about 8

hours, which is a period in which the DNA synthesis happens and here the chromosome duplication and everything is done. And then you have the G2 phase, which takes about 2 to 3 hours.

In the G2 phase, you have the cells which have the duplicated chromosome; they have two sets of chromosomes present. And it moves to the M-phase, which is where the mitosis or cell division happens, the chromosome is separated, and the cells are divided to form two daughter cells.

And the time taken for S, G2, and M phases together is about 12 hours; and S, G2, and M are 0th order kinetics. It does not depend on the number of cells which is present; it is going to just depend on the process itself. Although overall cell division can be depended on the cell number. These particular phases are not depended on the cell number; it is dependent on the processes which have to take place for the cell division to happen. This is what cell division is; I am pretty sure that you have studied this in cell biology.

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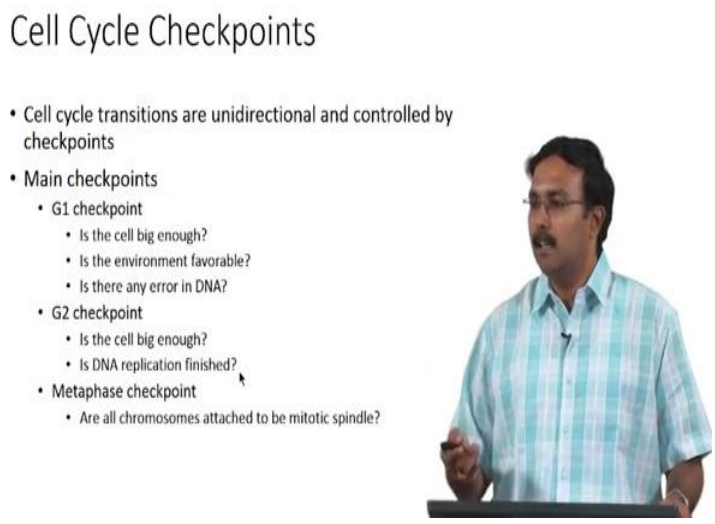

And when we talk about these phases, what happens is the cell moves from one phase to another in a unidirectional fashion, which is controlled by checkpoints. After it duplicates its chromosomes, it cannot say no, I will go back to G1, and it is not possible. It has to go to the M phase, or it has to die; those are the only options; that is because you have a checkpoint. So, do you know what the checkpoints are?

Student: After G1.

After G1 ok.

Student: After S, after S.

After S ok.

Student: basically, after S, it checks that the DNA is here synthesised properly.

Ok.

Student: After G2, it checks about their organelles.

Ok.

Student: And after G1 its check, all it like raw materials for some reason available.

Whether this cell is ready for division, ok, the G1 checkpoint is basically to verify if the cell is large enough whether the environment is favorable, whether the cell already has any error in its DNA. If it is a cell with some DNA error, it's not going to get divided. G2 cell again, it verifies whether the cell has become is big enough for accommodating all these and whether the duplication of DNA has happened and whether this is fully complete, and you have two proper sets of DNA.

And then, the final checkpoint is your M checkpoint or metaphase checkpoint, where you verify if all the chromosomes are attached to the mitotic spindle, whether all the organelles are in proper shape. And to make sure that this happens before the division happens. These are the three checkpoints to ensure that the cell division happens properly. And when one of these checkpoints fails, you end up with damaged cells, which can end up being cancerous also.

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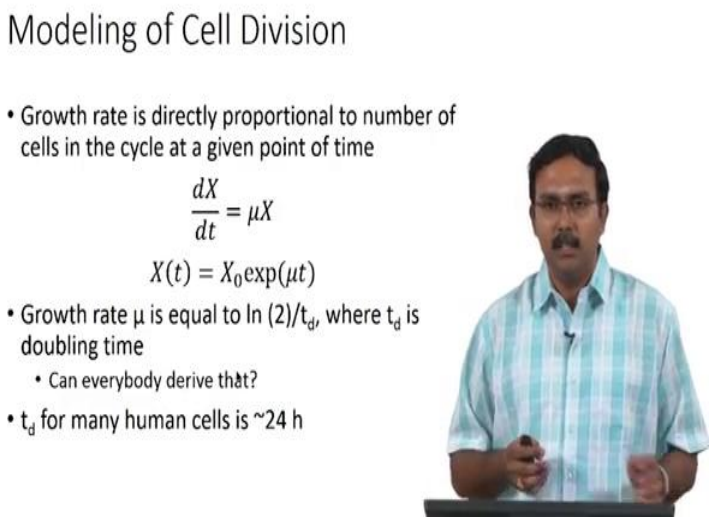

When you are talking about cell division, this again comes back to the simple growth rate, and growth kinetics. So, cell division can be modeled, and the growth rate is directly proportional to the number of cells present in the cycle at a given point of time.

$$\frac{dX}{dt} = \mu X$$

Where μ is the specific growth rate, and dX/dt is the growth rate, and you have X.

This can be solved to get

$$X(t) = X_0 \exp(\mu t)$$

$$\mu = \frac{\ln(2)}{t_d}$$

Where, t_d is the doubling time.

So, I hope everybody can derive that. So, the doubling time for most of the cells which are present in humans is about 24 hours. So, the cells grow very slowly; mammalian cells are not like E.coli right. What is the doubling time for E.coli?

Student: It is 20 minutes, almost 30 minutes.

20 minutes.

Student: Right.

It is roughly 20 minutes. So, it doubles very very rapidly. Whereas mammalian cells are going to grow at a very slow rate, because of this, we have one advantage with mammalian cell culture. Can you guess what it would be, compare to bacterial cell culture? One of the major limitations in bacterial cell culture is not a big problem in mammalian cell culture.

Student: They have to keep regenerating.

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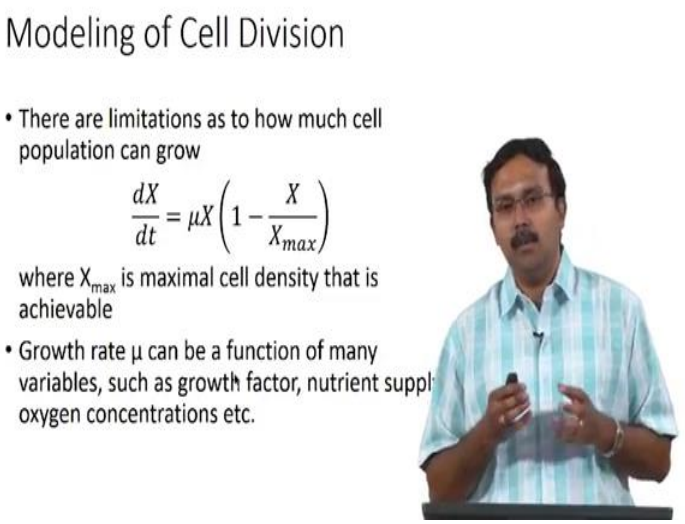

However, with respect to mammalian cells, it is not like the cells can keep dividing to get to very large numbers; in bacterial cell, you start with the very small inoculum, and it can multiply for hours, and you would be able to get very large numbers provided you maintain the nutrient concentration and so on. Here, that is not possible because even if you have nutrients supply, there is only limited space for the cells to grow.

There can always be limitations with respect to how much cell population can be achieved. Because of that, the equation actually can get a little trickier when you are modeling cell division, which would become

$$\frac{dX}{dt} = \mu X\left(1 - \frac{X}{X_{max}}\right)$$

So, which gives you an upper limit, where X_{max} is the maximum cell density achievable for the conditions you have. The specific growth rate, μ can be a function of many variables; it could be dependent on growth factors, nutrients supply, oxygen concentration, and so on. In many cases, is it not like depending on one limiting substrate.

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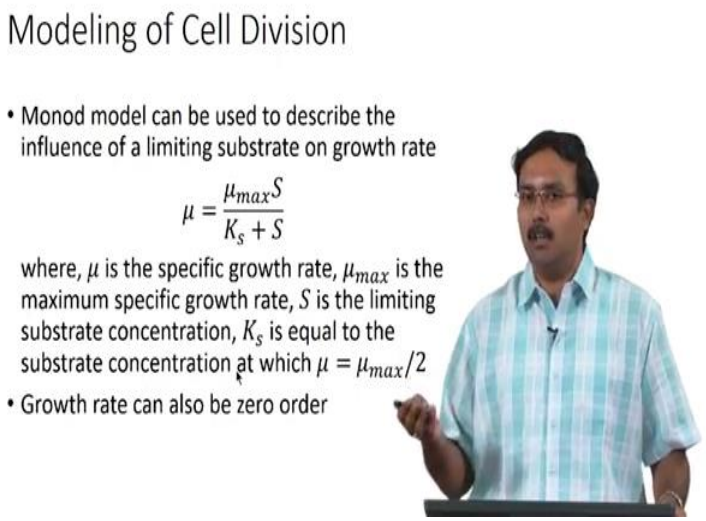

If it is influenced by one limiting substrate, then you can use the Monod model to model it. However, in many cases, there might be multiple thinks which are limiting the rate at which the mammalian cells have growing. So, this is the Monod model.

$$\mu = \frac{\mu_{max} S}{K_s + S}$$

And the Monod model is the equation similar to enzyme kinetics. Growth rate with respect to mammalians cells does not always have to be like first-order like what we started with, the growth rate can also be 0th order, and you might have to model it appropriately.

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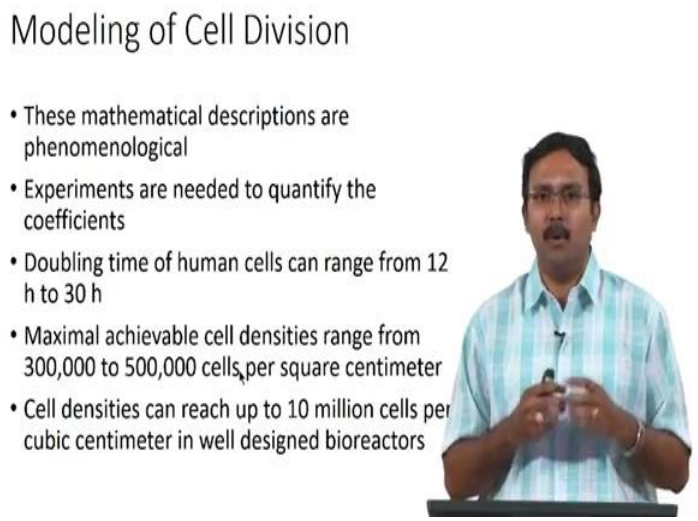

Whatever mathematical description we have is completely phenomenological, and you would need experiments to quantify these coefficients. In general, the doubling time of the human cells can range anywhere from 12 hours to 30 hours. So, 12 hours would be for progenitor cells, and, as you have cells that are less proliferative, you will have increased doubling time.

There can also be cells with much larger doubling time, but those are exceptions. In general, the maximum achievable cell density is somewhere between 3 to 5 lakhs cells per square centimeter, so that is dependent on the size of each of these cells when they are adhering to the surface. The cell density can reach up to 10 million cells per cubic centimeter if the reactor system is very well designed. So, that is why you have the spinneret flasks and things where you can have a very high concentration of cells that are growing.

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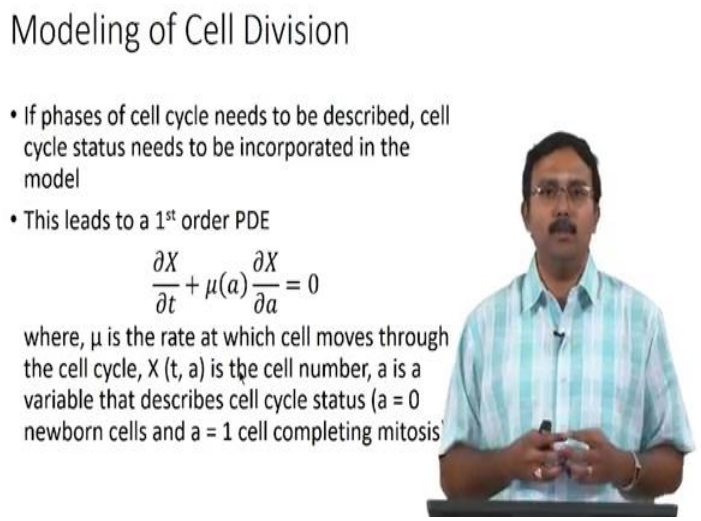

In some cases, the cell division can only be modeled if we describe the status of the cells itself because not all cells are going to divide. Even when you do bacterial cell culture, you make sure that everything starts at the log phase; you make sure cells in the log phase are the ones you are studying for understanding growth kinetics. But in mammalian cells, people do that as well. People can actually stop cell cycle, arrest it at one phase and then start them proliferating, so that all of them are uniform or you could also have things where they are in different phases.

So, if you have to account for that, then the model would also have to account for that. Then you end up with the partial differential equation, which would take the cell density changing with respect to time and cell density, also varying with respect to the cell cycle status.

$$\frac{\partial X}{\partial t} + \mu(a)\frac{\partial X}{\partial a} = 0$$

So, a is the cell cycle status, and if you have X(t, a), then a is the variable that describes the cell cycle status. Where a = 0 would be a newborn cell and, a = 1 would be the cell that is right about completing mitosis. So, it can range anywhere in between depending on which phase the cell is in.

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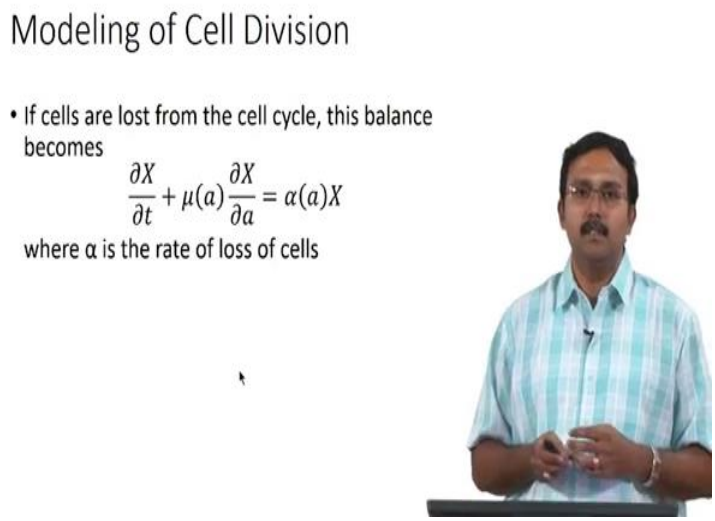

Cells can also be lost from the cell cycle. If you are going to have that, then you have to account for the balance, taking into account α(a) and X, where α(a) could be the rate of loss of cells in that particular state.

$$\frac{\partial X}{\partial t} + \mu(a)\frac{\partial X}{\partial a} = \alpha(a)X$$

So, this is not exactly cell death; this is more of weeding of cells from that particular state.

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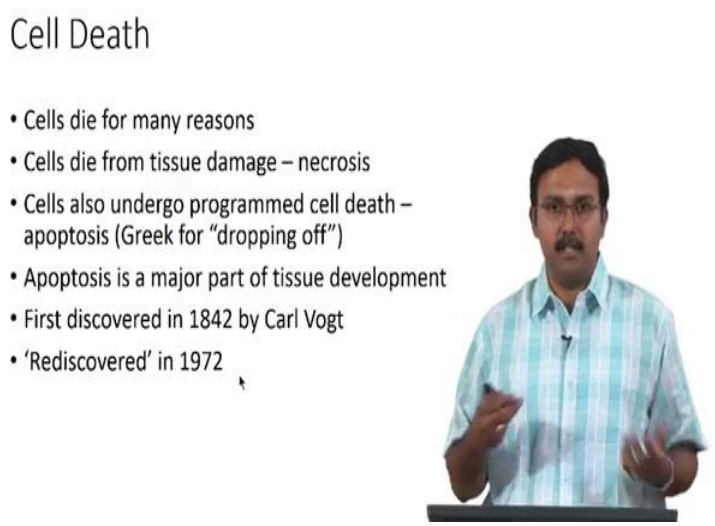

You could also have cell death; cells can die for different reasons tells. Cells die from tissue damage, which is necrosis. Cells also undergo programmed cell death, which is called apoptosis. Apoptosis is a major part of tissue development. Apoptosis itself was first discovered in 1842, but it was rediscovered in 1972. People did not bother to follow it up after that, and then they realized it was a crucial part of tissue development

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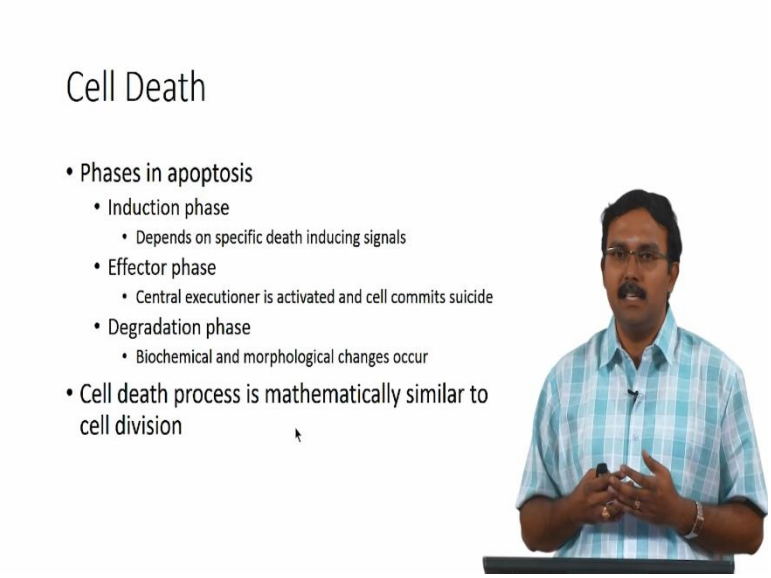

There are different phases in apoptosis. You have the induction phase; this depends on the specific death-inducing signals which are sent to the cells. Then you have the effector phase, where a central executioner is activated, and the cell basically commits suicide. You have the degradation phase where the biochemical and morphological changes are happening as the cell is getting degraded. The cell death process can also be mathematically modeled in a similar way to cell division. It is except that the rate will be negative compared to the positive of cell division.

These give us a brief introduction on a couple of processes. There were also other cellular processes which we will talk about. We will talk in detail about cell differentiation and also about cell adhesion and migration. We will talk about how these can also be mathematically modeled.

Thank you.