Tissue Engineering
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Lecture - 20
Cell Differentiation

We had looked at tissue dynamics, and while we talked about tissue dynamics, we talked about what the cells would usually be doing when they are residing in the tissue, and the first thing we talked about would be the cell division, which is cells dividing and multiplying. You have cell differentiation as a next step if you have a stem cell, which is where most of the tissues are going to originate from. And the cells would all come from stem cells, rather than matured cells multiplying to create a large number of cells. Usually, for creating new tissues, stem cells are recruited, and you have stem cells multiplying to form the desired tissue; for that to happen, differentiation is a crucial factor.

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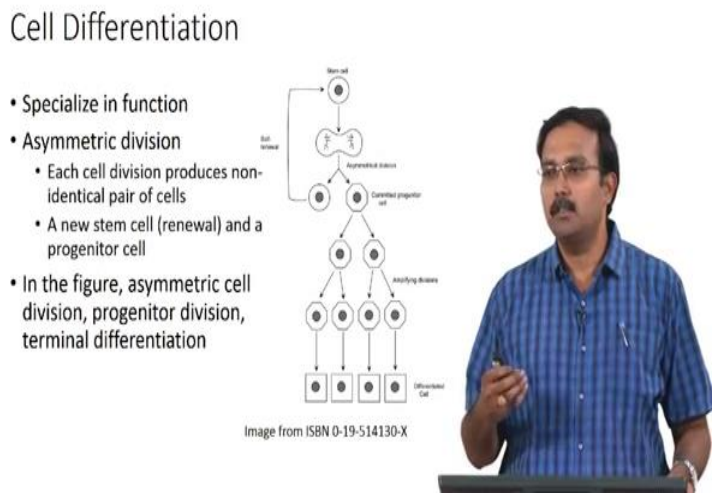

We will talk about cell differentiation. Differentiation is the process of cells specializing in a function. Stem cells, as you all know, do not have any specialized function; they can mature to form any type of cell.

The differentiation is the process that results in the formation of a specific cell type with the desired functionality. There can be cell differentiation because of the process called

asymmetric division. Asymmetric cell division is basically where you have two non-identical cells which are formed; the daughter cells are not the same. In regular mitosis, you would have two cells, which are exactly the same.

Here, in asymmetric cell division, one is basically for the self-renewal property of the stem cell. It basically divides and retains its stemness; Other goes into the progenitor cells. These progenitor cells can then become more and more differentiated to form the final differentiated cell lines. Usually, these progenitor cells multiply through just regular cell division to form more number of progenitor cells, which then get differentiated. So, this is because, as I already mentioned, progenitor cells divide at a much faster rate compared to fully differentiated cells.

What you see in the figure is the first step is the asymmetric cell division where the cells are dividing, one is forming stem cell, which is self-renewing, the other one is a committed progenitor cell. The committed progenitor cell then multiplies and amplifies. This is the progenitor division so that you have more number of cells that can be differentiated. And the final step is the terminal differentiation to form the fully differentiated cells.

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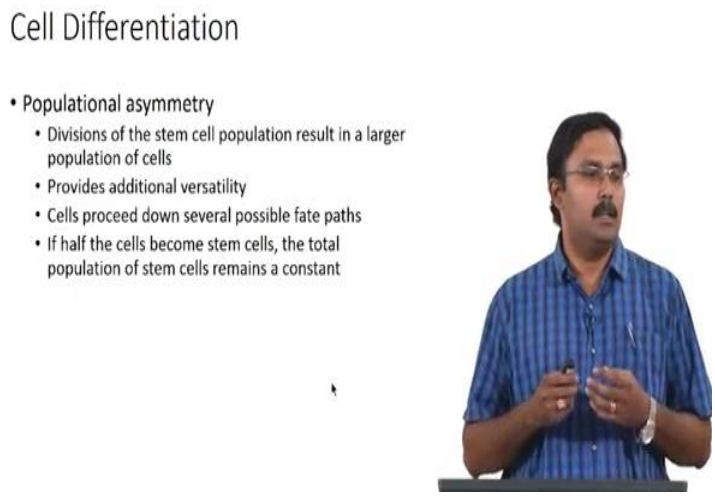

During cell differentiation, you would experience something called population asymmetry. Divisions of stem cells population result in a large number of cells, and these can form different types of cells, and this provides additional versatility. Cells can

proceed down several possible fates depending on the environment they are in, conditions that are provided to them, and so on.

If half the cells become stem cells, the total population of the stem cells remains constant. So, that way, you have the self-renewing property of the stem cells taken care of.

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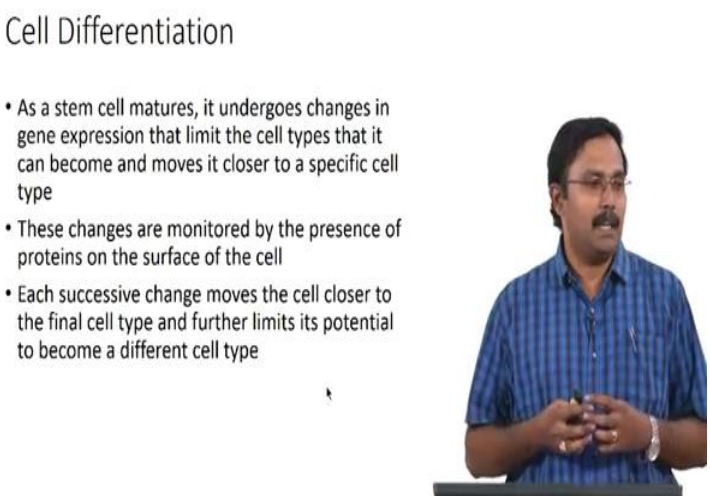

What happens in a differentiation process is, as a stem cell matures, it undergoes changes in its gene expression that limit the cell types that it can become. There are changes inside the cell, which make sure that it is beginning to get some of the functionalities of the differentiated cells. Because this happens, it starts moving closer towards the final cell type.

These changes can be monitored by the presence of proteins on the surface. These surface markers and receptors which are expressed can be used in identifying whether the cell is remaining as a stem cell or if it is moving closer towards a differentiated cell. With each successive change, the cell moves closer to the final differentiated cell.

And its potential for becoming a different cell is actually becoming lesser. So, it becomes more and more committed towards one particular cell type as more and more changes happened to the cell. It is not a one-step process; it is not like the stem cells form a committed progenitor, which multiplies, and then miraculously, it just changes to one

particular cell type; it does not happen that way. It is a sequential process that takes a significant amount of time, and as time goes on, it gets more and more committed towards a particular cell type.

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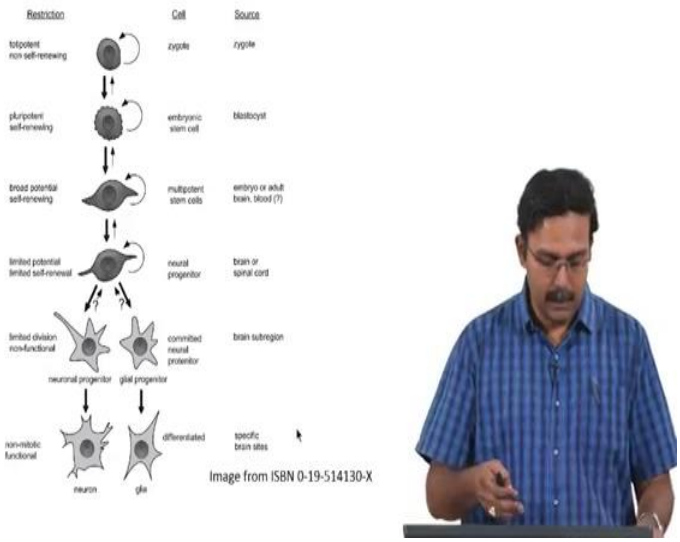

This is an example of how it could happen. What you see here is a totipotent cell, which is non self-renewing like a zygote. Then you have the pluripotent stem cells, which are self-renewing, and embryonic stem cells would be an example of the pluripotent stem cells. Then, this forms a broad potential self-renewing cell.

These are still self-renewing cells; these are multipotent stem cells that come from here, and then it becomes a limited potential, limited self-renewal cell. Until this process, what you would see is there is a possibility of the cells going back. So, it can actually go back to a stage because it is not fully committed yet. So, it can still go back, and here it forms some kind of a progenitor. Here, the example given is for neural cells. So, it forms a neural progenitor, and here it is more committed towards one particular lineage.

From here, it then divides where it is even more specific, where it can become a neuronal progenitor or a glial progenitor, and by this point, it becomes almost irreversible. After this, it is now committed to a particular lineage, and it cannot go back. Finally, it forms a neuron or glia based on how the conditions are. The cells can probably die at any of these stages, but they cannot go back after it commits itself to a particular lineage. So, this would be how a general process would look like.

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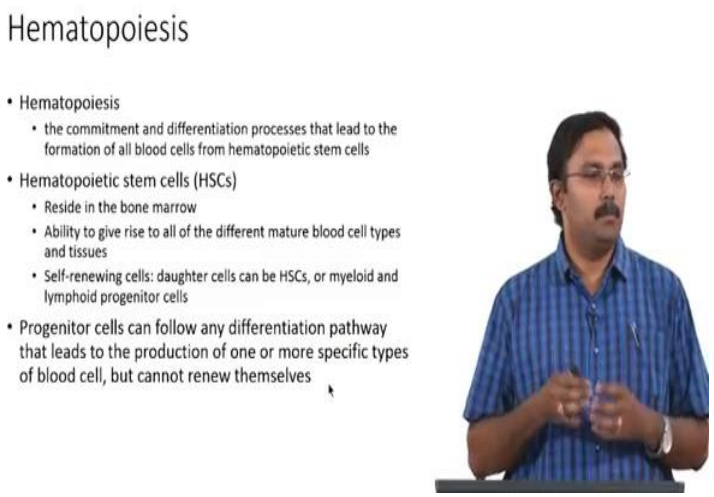

I will talk about a couple of things that are very popular when we discuss stem cells and cell differentiation. One process which is very well studied and has been very well established is the hematopoietic stem cell.

The process of hematopoiesis is the process where hematopoietic stem cells commit and differentiate to form different blood cells. That is basically the formation of all the types of blood cells is from a type of cell called hematopoietic stem cell. These reside inside the bone marrow, and they have the ability to give rise to various types of matured blood cells and tissues.

These are self-renewing cells where the daughter cells can either be a hematopoietic stem cell or a myeloid or lymphoid progenitor cell. Once it commits towards a myeloid or lymphoid progenitor, it will then go through the differentiation pathway and to form different types of cells.

But it goes through asymmetric cell division first so that the self-renewing property of the hematopoietic stem cells is taken care of. The progenitor cells can follow any differentiation pathway. So, these myeloid or lymphoid progenitor cells are not going to form one type of cell. Myeloid and lymphoid progenitor will have its own variety of cells that can be formed. They can follow any of these pathways leading to the production of one or more cell types, but these cell types cannot renew themselves. So, these progenitor cells cannot renew themselves as they are already committed.

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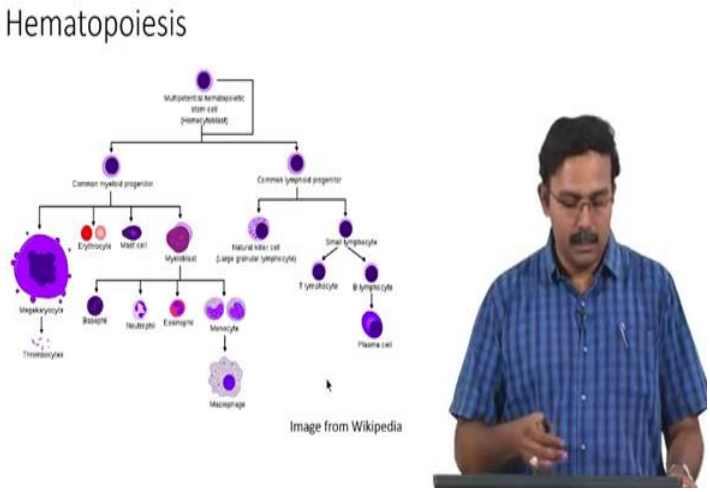

This is a simplistic representation of what hematopoiesis is. What you see here is the multipotential hematopoietic stem cell, which has the self-renewing property, then multiplying to form a common myeloid progenitor or a common lymphoid progenitor. This is formed through asymmetric division. You either have another HSC formed or a common myeloid progenitor or common lymphoid progenitor, which is formed.

From the myeloid progenitor, you can get megakaryocytes that form thrombocytes, erythrocytes, mast cells, and myeloblast. Myeloblast can then form basophils, neutrophils, eosinophils, monocytes, and macrophages. Common lymphoid progenitor cells can form the natural killer cells, or it could form small lymphocyte, which should then further be differentiated to form T cells and B cells. So, this is a very simplistic representation of what a hematopoiesis process is.

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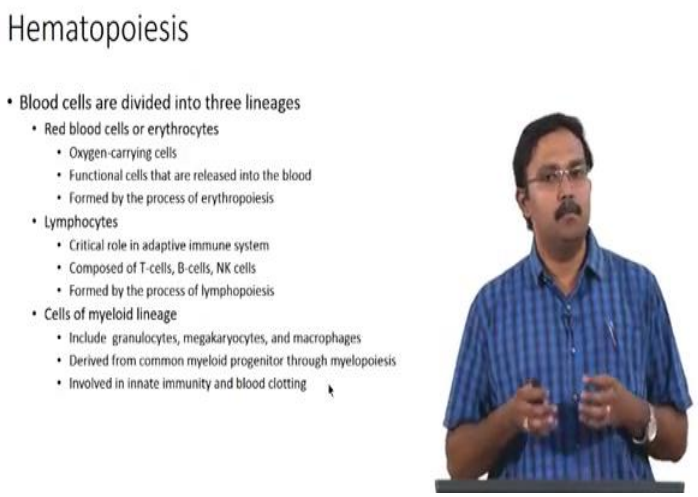

In general, blood cells are divided into three lineages. You have the red blood cells or the erythrocytes; these are the oxygen-carrying cells they have the hemoglobin. So, they bind to oxygen, and they can carry oxygen to the site. So, these are functional cells that are released into the blood. Once differentiation happens, it will get released into the blood.

They are formed by the process called erythropoiesis. Within hematopoiesis, the pathway which is taken for differentiation of hematopoietic stem cells to form erythrocytes is called erythropoiesis. You also have lymphocytes that have a critical role in immune response and especially in adaptive immune systems. So, these are composed of the T cells, B cells, and the NK cells or the natural killer cells.

These are formed by the process of lymphopoiesis, and cells from a myeloid lineage include granulocytes, megakaryocytes, and macrophages. These are derived from a common myeloid progenitor through myelopoiesis. These cells are also involved in innate immunity and blood clotting and many other mechanisms; they have a versatile functionality in your body.

So, these are the three major lineages for blood cells.

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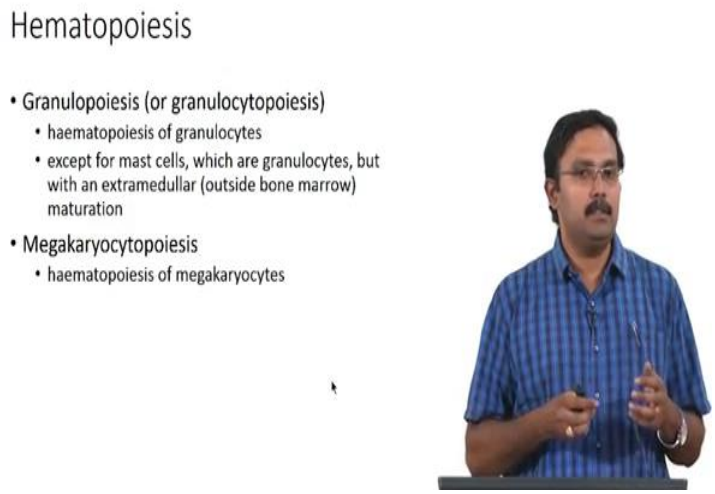

The process of forming granulocytes is called as granulopoiesis or granulocytopoiesis. This is done inside the bone marrow except for mast cells, where the mast cells are produced, and the differentiation happens outside the bone marrow, which is called this extramedullar maturation.

The medulla is the technical term for the bone marrow. So, its extramedullar maturation. Megakaryocytopoiesis is the hematopoiesis of megakaryocytes. So, this is the general hematopoiesis process.

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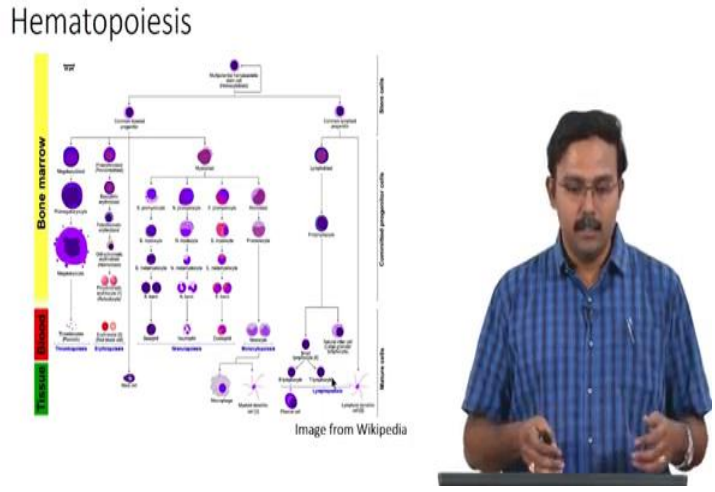

If you were to look at in a more detailed step by step fashion, this is what it would look like. The multipotential hematopoietic stem cells have the self-renewing property, and they form common myeloid progenitors or common lymphoid progenitors, as I had already mentioned.

The common myeloid progenitor can basically come out of the bone marrow and then mature into a mast cell, which is the extramedullar maturation to form mast cells. Inside the bone marrow, this myeloid progenitor can then become megakaryoblast, which then becomes promegakaryocyte and a megakaryocyte. So, this megakaryocyte then comes to the blood where it forms thrombocytes or platelets.

This process is the thrombopoiesis, where you end up forming the platelets. So, the formation of megakaryocyte is megakaryocytopoiesis. The other pathway for erythropoiesis would be, it forms proerythroblasts, then basophilic erythroblast, and then polychromatic erythroblast, then orthochromatic erythroblast or a normoblast and from where it forms a reticulocyte or a polychromatic erythrocyte. This enters into the bloodstream where in the form of erythrocytes where it gets matured. So, this pathway is erythropoiesis.

As you see, these are all coming into the blood, whereas mast cells enter into the tissue. From the common myeloid progenitor, you could also have the myeloblast, which can then differentiate into different granulocytes like basophils, neutrophils, eosinophils, and it can also form monocytes which can then differentiate to form dendritic cells and macrophages.

So, as you see, there are steps for each of them. It all goes through the promyelocyte then to myelocyte, then metamyelocyte, then a band cell, finally forming either a basophil or a neutrophil or an eosinophil and so on. And what you need to notice here is, where these differentiations happen. Until the last step, everything happens inside the bone marrow, and the final step is where it happens in the blood; the final maturation happens in the blood, or it gets released into the blood.

The common lymphoid progenitor, however, forms these lymphocytes, which go through either a lymphoblast, prolymphocyte and then form small lymphocytes, which would then develop into B cells and T cells, or it could go into forming natural killer cells which are the larger granular lymphocytes. You could also have this common lymphoid

progenitor forming the lymphoid dendritic cells directly. As you see, there is a significant number of steps that are involved in each of these processes. Unlike the previous thing which we saw. So, myeloid to erythrocyte is what a simple representation is, but in reality, it has so many different steps; the cells exist in four or five different levels of differentiation before it finally forms the fully differentiated cell.

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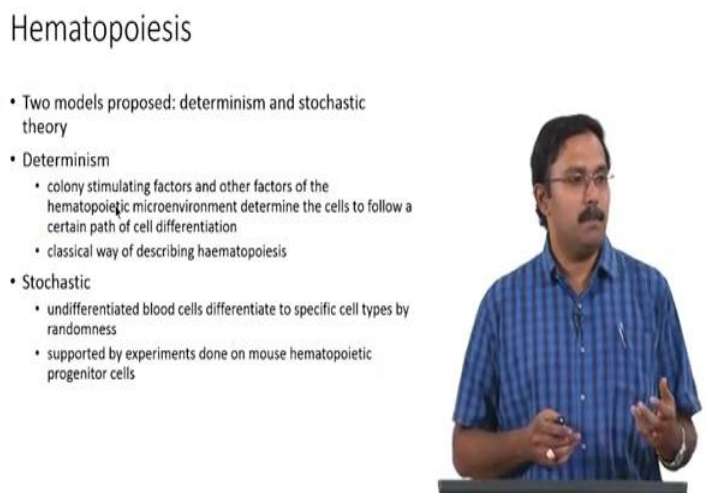

Hematopoiesis can be modeled, and there are two theories for modeling it; one is the deterministic model, and another is a stochastic model. A deterministic model is the classical way of describing hematopoiesis; what we kind of saw is the deterministic model where we say it goes into this, this environment will provide this particular condition where it goes into everything, and it goes into particular pathway, and there is no real randomness to this process. It just depends on the factors, the colony-stimulating factors, and other factors present in the microenvironment. This determines the path for cell differentiation. So, this is what we kind of have always believed in, and this is a classical pathway.

However, recent experiments are supporting the theory of stochastic differentiation. Basically, undifferentiated blood cells can differentiate into specific cell types by randomness. There is no real control over how this happens, and there are factors that do control, but there have been studies that show that the cells can go back at each step.

There is a significant level of randomness that cannot be completely ignored; you cannot just say that it is deterministic saying this environment will lead to this; it is not that.

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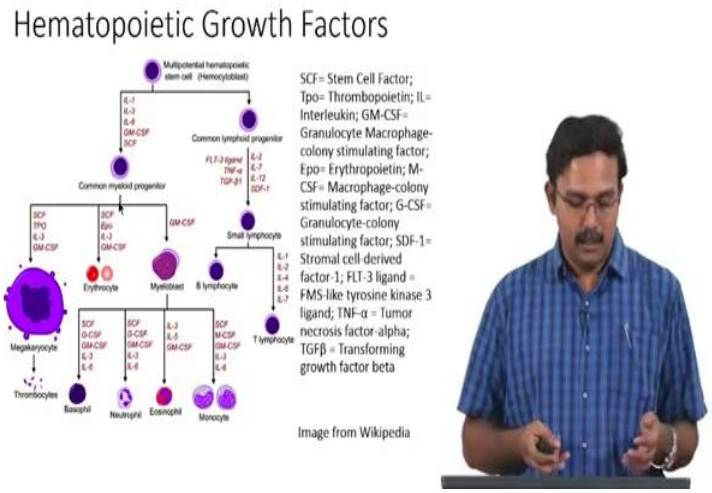

This is a representation of the growth factors and other molecules that can guide into specific differentiation. What you would see here is many of these factors are repetitive, which is probably one of the reasons for the stochastic model of differentiation. For example, this IL-3; it is present in the formation of the myeloid progenitor, which can then form megakaryocytes or erythrocytes. So, it is present in both. So, it could play a role in both these conditions.

Based on this, it is very difficult to say that one particular environment is going to force cells into dividing into forming particular things. But people are trying to use this understanding to design the cocktail in which the cells can be grown so that there can be differentiation, that is directed towards per specific cell lineage.

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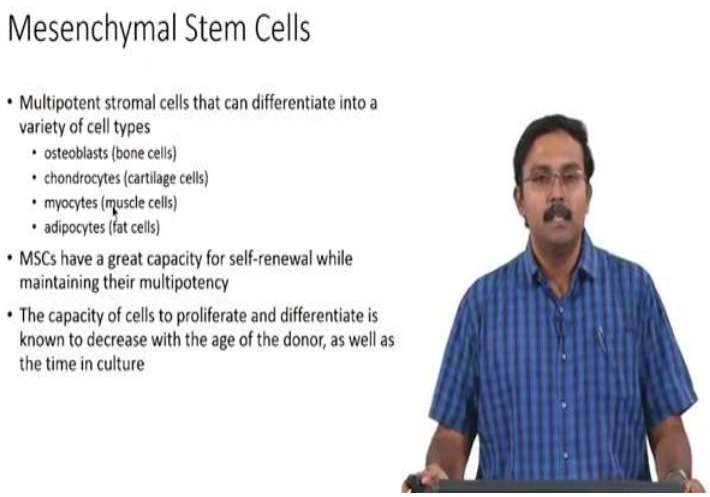

The next major type of stem cells which are extensively studied and extensively used in tissue engineering applications is the mesenchymal stem cells. These are multipotent stromal cells that can differentiate into a variety of cell types. They have been shown to form osteoblast, chondrocytes, myocytes, and adipocytes. So, osteoblast is bone cells, chondrocytes are cartilage cells, myocytes are muscle cells, and adipocytes are fat cells.

Mesenchymal stem cells have a very good capacity of self-renewing while maintaining their multipotency. The capacity of the cells to proliferate and differentiate is known to decrease with the age of the donor as well as the time in culture. As you have multiple passages, then you would see that the rate of proliferation would be lesser, and its ability to differentiate into different cell types comes down.

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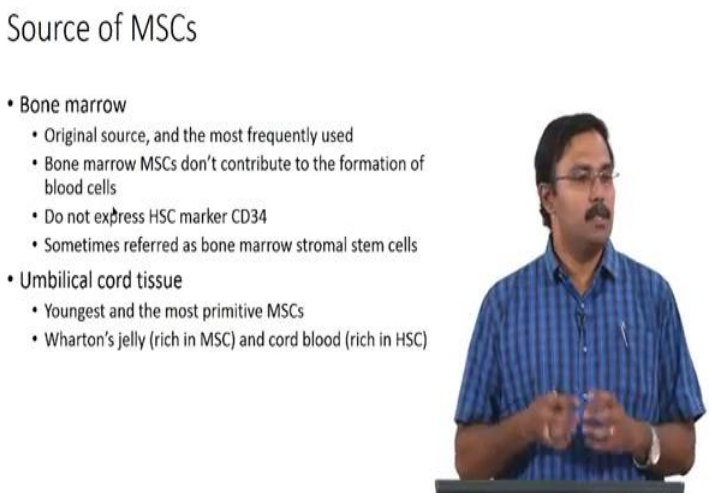

The original source for mesenchymal stem cells is bone marrow. Hematopoietic stem cells are also from bone marrow; however, these are different from the mesenchymal stem cells. The HSCs and MSCs are different, although they both reside in the bone marrow. Bone marrow mesenchymal stem cells do not contribute to the formation of blood cells, whereas hematopoietic stem cells are fully committed towards the formation of blood cells in your body.

Because they are not involved in the formation of blood cells, they do not express the HSC marker CD34. So, that is used to identify whether the cells you have isolated is a mesenchymal stem cell or a hematopoietic stem cell. These are also referred to as bone marrow stromal stem cells, and so these terms are used interchangeably in many cases.

Another source for getting mesenchymal stem cells is the umbilical cord tissue. This is the youngest and the most primitive mesenchymal stem cells. That is why there is a lot of interest in storing the cord blood and the cord placenta to hope that you would be able to use it for some regenerative medicine that might be developed in the future. This basically has two different tissues one is the Wharton's jelly, and the other is the cord blood.

So, the Wharton's jelly is a gelatinous material that is present in your placenta, which is very rich in mesenchymal stem cells, and the cord blood is rich in hematopoietic stem cells.

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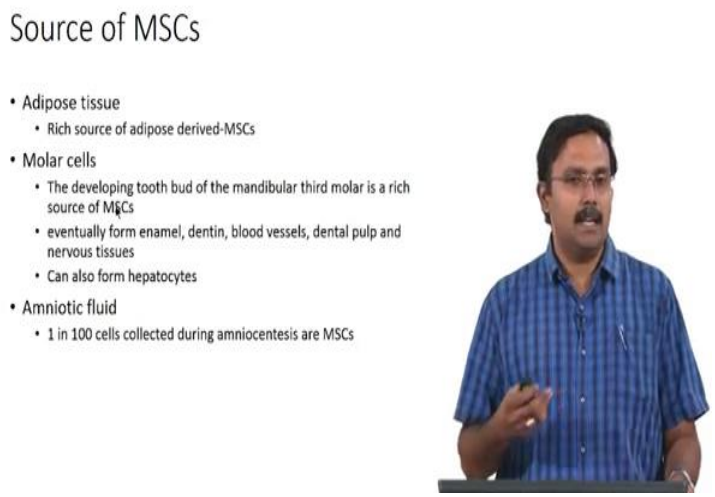

There are other sources, as well. Adipose tissue is a rich source of adipose-derived mesenchymal stem cells. Molar cells, which are basically the teeth tissue. If you have a developing tooth bud, then those are rich in mesenchymal stem cells. So, these mesenchymal stem cells are the dental stem cells that actually form enamel, dentin, blood vessels, dental pulp, and various nerve tissues.

People have also shown that these can form hepatocytes and many other types of cells. Amniotic fluid is also a source from where you can get mesenchymal stem cells. It is the fluid in which the fetus is present, and this can be harvested using a process called amniocentesis. Amniocentesis is nothing but taking a large gauge needle and injecting it into the uterus to draw the amniotic fluid. So, this is usually done for diagnostic purposes. This amniotic fluid has a lot of cells, and one in 100 cells which are collected using amniocentesis is a mesenchymal stem cell, which could also be used for tissue engineering applications.

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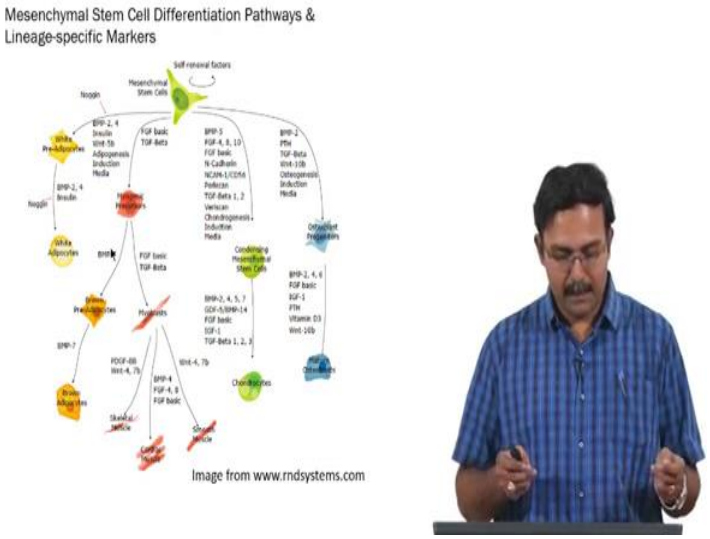

This is a representation of how mesenchymal stem cells can differentiate to form various lineage cells. So, I am not going to go into great detail. What I have here is for adipocyte, myocytes, chondrocytes, and osteoblast. Within that, we have classifications such as white adipocytes, brown adipocytes, skeletal muscle and cardiac muscle, smooth muscle and chondrocytes, and mature osteoblasts. So, this kind of gives what the different pathways these mesenchymal stem cells can take and what would be the growth factors and other molecules that can help direct the mesenchymal stem cells into these lineages.

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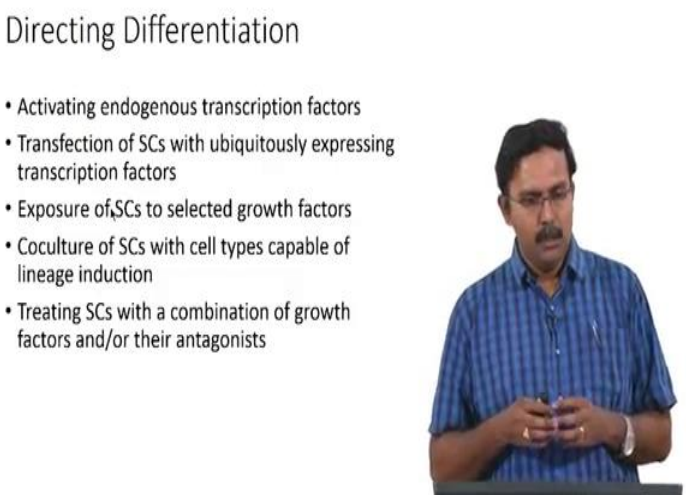

Whatever we looked at is the basic concept of differentiation that usually happens in your body. You want to try to use this to direct differentiation; ultimately, that is the aim. Because we are looking at a tissue engineering application, where we are going to use these stem cells, and we would want to differentiate these stem cells to produce specific cells with desirable functionality. So, the tissue we develop will be able to perform its functions.

This is basically done through activating endogenous transcription factors, or it can also be done by the transfection of stem cells with ubiquitously expressing transcription factors. You can also have the stem cells being exposed to selected growth factors, or you can culture the stem cells along with other cell types, which can induce lineage of the specific cell type.

You can also do combinations where you treat it with growth factors or antagonists and try to use this kind of a process for directing the differentiation of stem cells.

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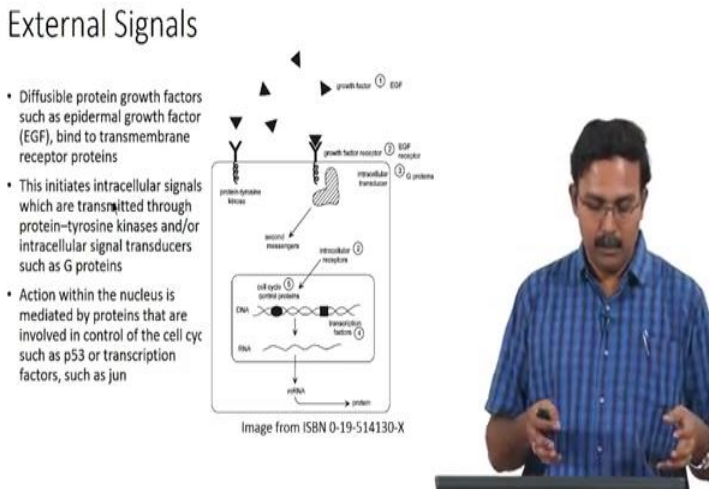

Basically, this is what happens, let us look at an example where you are talking about an external signal, which is the growth factor which you add. Let us say a growth factor is present in the media in which the cells are being cultured, what happens is, this diffusible protein growth factors will bind to the receptor of the protein; will have to diffuse and reach the receptor of the surface of the protein where it will bind to the receptor. Then it initiates an intracellular signal which is transmitted through protein tyrosine kinases or

other intracellular signal transducers like G-protein. Once that happens, there is an action within the nucleus, which causes the expression of certain transcription factors, which results in the cells getting differentiated. So, this is what you see in external signal stimuli.

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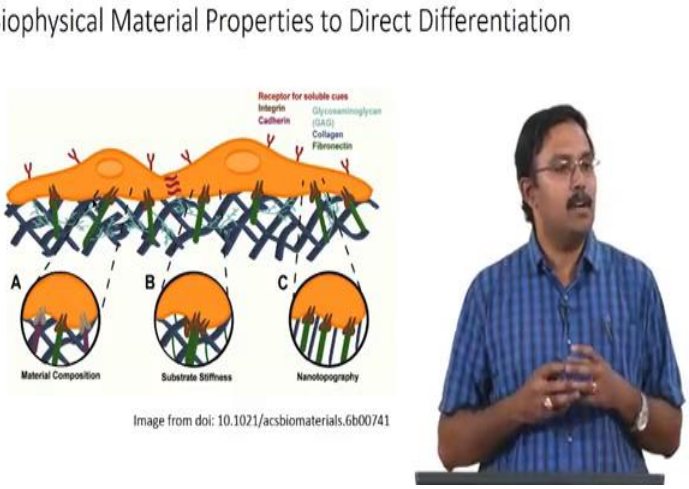

Other than this, you can also direct differentiation using biophysical properties, which would be the mechanical or the physical properties of the material on which the cells are being cultured. This is a brief representation of what are all the factors that can actually affect. You could have the material composition that could that play a role. Depending on the material composition, how the cells interact with the material will change, the integrins and cell adhesion could all change based on the composition of the material that you are using.

Substrate stiffness has also been shown to affect how the cells differentiate themselves, and the last one is the nanotopography were the surface topography of the material could actually direct cells. While the cells are cultured, they might have to be aligned in the presence of this nanotopography, and if that happens, the cells will differentiate to specific cell types and so on.

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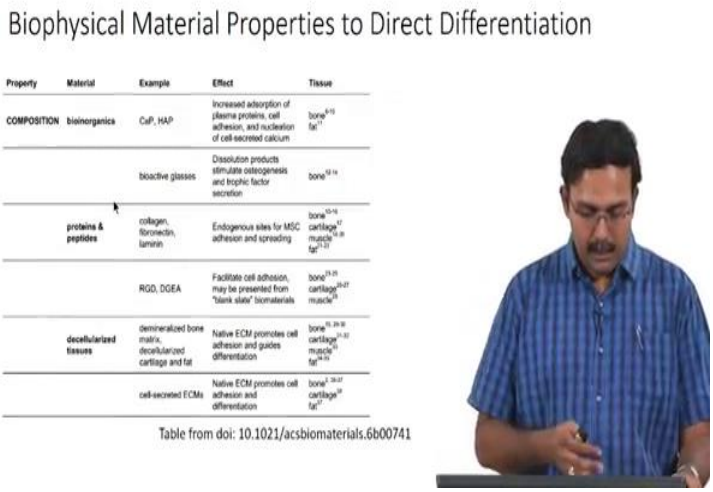

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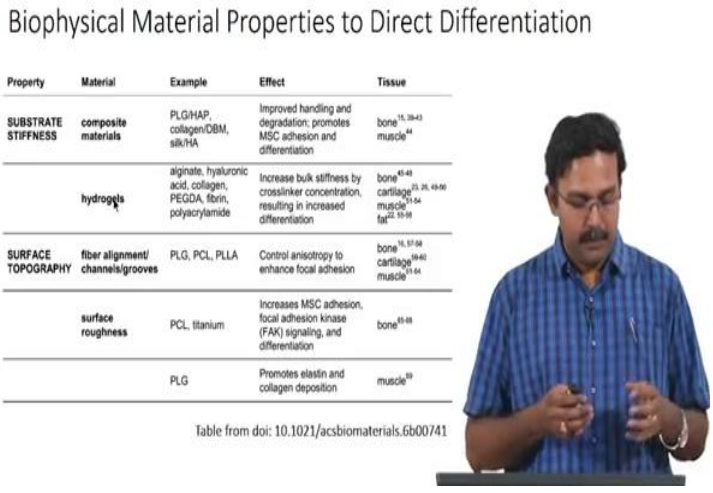

Here are some examples where composition and stiffness and topography have been used to get different types of cells. I am not going to go into each and everyone, but I hope you would go through this to try and understand what each of these materials and each of these properties can do.

So, this gives an overview of what people have tried and what people have been able to accomplish.

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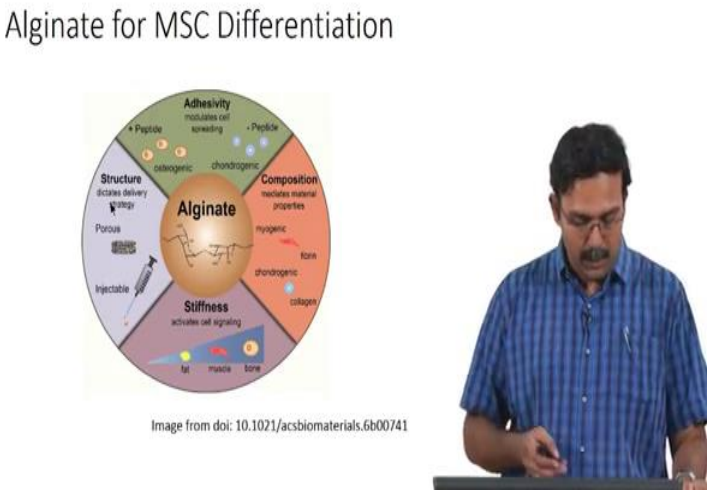

One last example would be alginate. Alginate is something that has been studied extensively for mesenchymal stem cell differentiation because they are very easy to work with, and you can change their properties very easily. People have shown that the structure of the alginate, whether it is an injectable solution or if you have it as a porous scaffold, can alter the way these molecules are delivered, thereby that can play a role in how the mesenchymal stem cells are differentiating.

Another factor that has been shown to modulate differentiation is the adhesiveness of the surface, adhesivity of alginate material. In the presence of a peptide, people have observed it to be differentiated to osteogenic, whereas when there is no peptide, it differentiates towards the chondrogenic cell line, even when every other property is the same. And composition can mediate the material properties, and it can be used to differentiate cells to form myogenic or chondrogenic depending on whether you have collagen along with this or fibrin along with this.

People have shown these kinds of differentiation are also possible. Mechanical property such as stiffness plays a role; as the stiffness is lesser, you would end up forming something like an adipocyte, and as the same stiffness increases, you would end up forming a muscle or a bone tissue. So, it is quite logical, right? It depends on how the ECM of the natural tissue is, and based on that, this differentiation happens. So, these

give a brief introduction on the biology of differentiation and how directed differentiation being explored for stem cells.

In the next lecture, we will talk about a little bit about the mathematical modeling aspects with respect to how these external factors interact with the surfaces and so on.

Thank you.