Tissue Engineering
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Lecture - 16
Cell Source

Until now, we have been talking about materials, which are one of the three different aspects of Tissue Engineering. We will now move on to the next aspect, which is Cells. Any tissue engineering application you want cells along with the scaffolds or many times cells are also used by themselves without a scaffold just for the regeneration of tissues.

So, we need to know what types of cells are being used, where we can get them, and how we can harvest them and whether we can culture them to get more number of cells. Because we might not be able to get enough numbers just by isolation from host tissue. So, we might have two cultures the cells. So, we need to understand all these concepts. We will first start with the source of cells.

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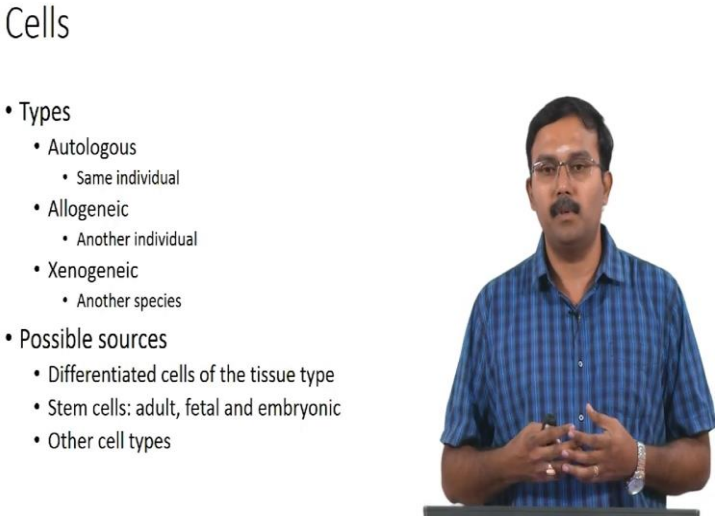

Cells are basically of three different types. We have already discussed this earlier. You have autologous cells, which are cells from the same individual. Allogeneic cells, which are cells from another individual of the same species and xenogeneic cells from another species altogether.

There are different sources for these types of cells. You could either have differentiated cells of the tissue type, or you could use stem cells and fetal cells or embryonic cells, or you could use cells that are of a different type. In the sense that you might be trying to engineer bone, but you would probably have osteoblast, which is the type of cell present in the bone along with endothelial cells, which are not specific for that bone or the tissue type, but it has its role in tissue engineering.

So, you could use cells of this kind of types. There are also studies where people have shown that one type of cell can be converted to another type of cell; even if it is a somatic cell, it means without actually taking them to the pluripotent state, you could just transdifferentiate them from one to another type. There are different things which people have worked on. So, we will try to understand some of the fundamentals and take it from there.

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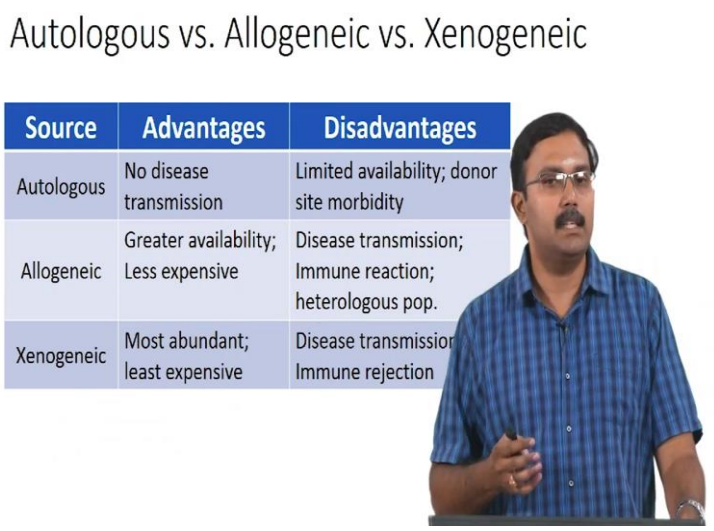

Autologous, allogeneic, and xenogeneic have their advantages and disadvantages. With respect to autologous cells, the biggest advantages, there is no risk of disease transmission or immune rejection. Whereas, the disadvantage is that there is only limited availability, and you would be injuring the site from where you are harvesting the cells. Allogeneic cells have greater availability and are less expensive; however, there is a risk of immune rejection and disease transmission, and you would also end up with the heterologous population when you use cells from another individual.

Xenogeneic cells are cells from other species, which means they are the most abundant and the least expensive. You can get it from organisms that can be grown for harvesting these cells, but there is a much higher risk of disease transmission and immune rejection. So, it is important to balance these advantages and disadvantages. People do try to use autologous cells as much as possible.

However, it is not readily available. In many cases, what people do is they try to harvest autologous cells and then culture them, expand them to get desired numbers and then use it for treatment of the injured site. In the introductory lectures, we had talked about using autologous chondrocytes for the regeneration of cartilage. Those kinds of things are very commonly done even today.

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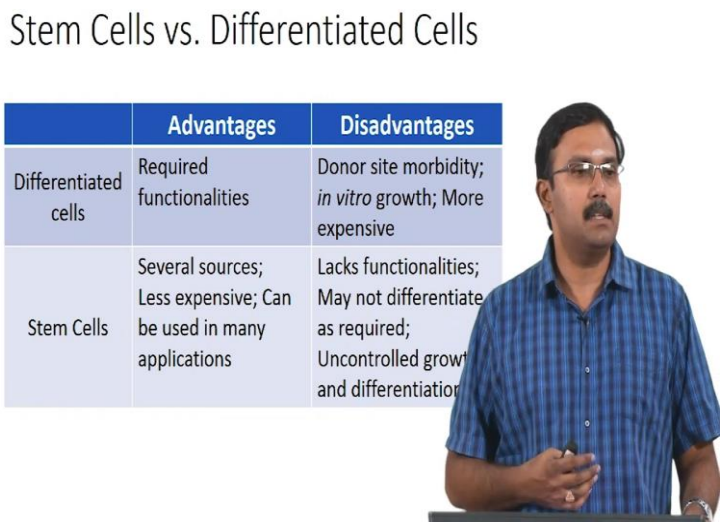

Differentiated cells and stem cells also have their own advantages and disadvantages. Differentiated cells have the required functionalities which are needed for the tissue to function the way we expect them to. Disadvantages are, you would cause donor site morbidity, and in vitro growth is more difficult. You cannot culture them for multiple passages. They die after some time, and they are more expensive.

Stem cells, on the other hand, are less expensive, and there are different sources from which you can get stem cells; they can be used for many applications. If you have one type of stem cell, then you can try to differentiate it into different cells and use it for different applications. So, those are some of the advantages of stem cells; however, the

disadvantage is they do not have the functionality, which means you need to differentiate them properly to get the desired functionality, and they may not do that; they may not differentiate as required. There could always be an issue with differentiation, especially when expecting differentiation to happen in vivo.

In vitro, you have a lot more control over what the cells are exposed to and how you treat the cells to get the required differentiation. Whereas, in vivo, the level of control you have is lesser. There is also a risk of uncontrolled growth and differentiation. This is especially true for embryonic stem cells, which might lead to the formation of teratomas, which could be a problem.

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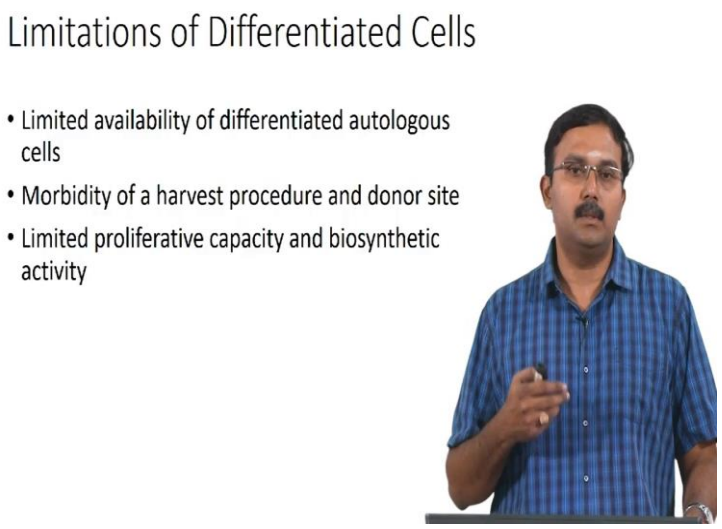

The limited availability of differentiated autologous cells is one of the limitations of why people have looked for stem cells and other sources. Morbidity and limited proliferative capacity are serious limitations when you are talking about expanding the cells. As I was saying, we would not be able to harvest enough cells. If you are trying to harvest a lot of cells, then you will cause significant damage to the donor tissue.

So, you try to harvest only smaller numbers, and then you have to expand them. Expanding them is also a problem because they do not grow as rapidly, and they only multiply for a few passages. They will not multiply for a long time; so, that is a problem. So, because of that, using autologous differentiated cells is not very easy for many applications.

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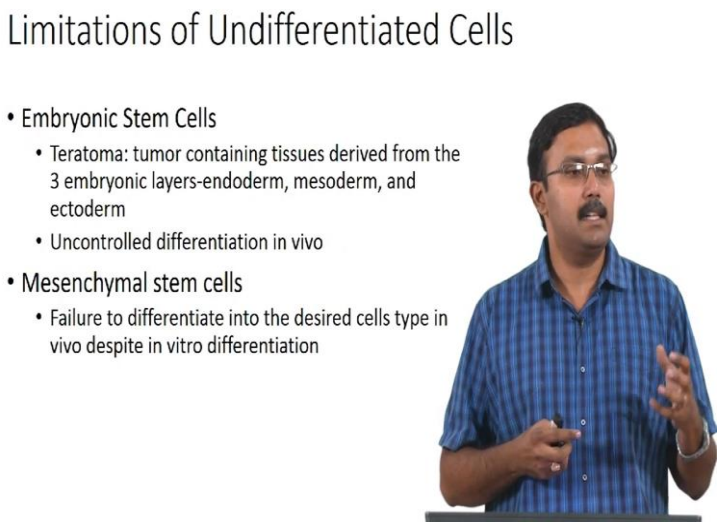

With respect to undifferentiated cells, the limitations are different for the different kinds of stem cells you use. With embryonic stem cells, there is a chance of teratoma formation; teratoma is a tumor containing tissues that are derived from three embryonic layers, which are the endoderm, mesoderm, and ectoderm. There is also a chance of uncontrolled differentiation in vivo. These are technical difficulties, and you also have ethical questions when you are talking about embryonic stem cells.

Mesenchymal stem cells have a problem because they failed to differentiate into desired cell types in vivo even though people have repeatedly shown that in vitro differentiation is very successful. Because of this, it is not very easy to use these stem cells for in vivo applications. Many times what people do is they use stem cells, and they expand them and then differentiate them in vitro and finally, then place them in the site of implantation. So, those are some of the challenges with stem cells.

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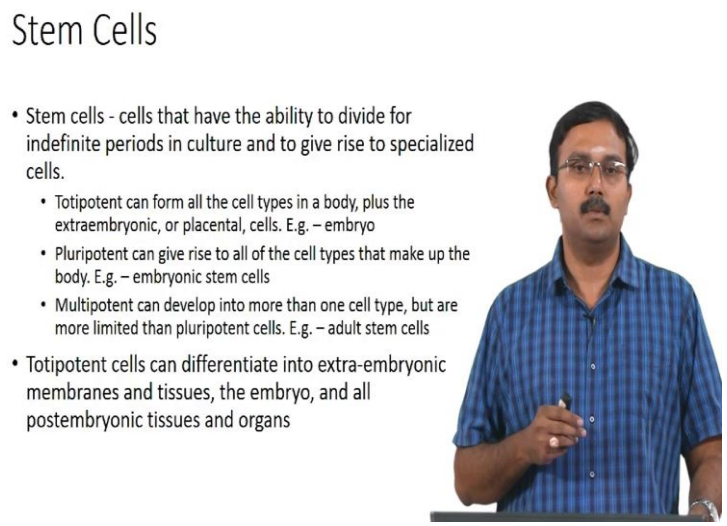

What are stem cells? Stem cells are cells that have the ability to divide for indefinite periods of time in culture and can give rise to specialized cells. So, they themselves do not have any special functions. They are undifferentiated, and they can differentiate into different types of cells.

There are three different types of stem cells; totipotent, pluripotent, and multipotent stem cells. Totipotent stem cells are the cells that can form all types of cells in the body and the extraembryonic cells, which are the placenta. Embryo and the first few divisions of the embryo are the totipotent stem cells, which can form any type of tissue.

The totipotent stem cells have the ability to develop into a whole organism whereas, the pluripotent stem cells can form all the cell types in the body, but they cannot form the extraembryonic or the placental cells. So, those are the pluripotent cells stem cells. Embryonic stem cells and induced pluripotent stem cells are examples of such pluripotent stem cells.

Multipotent stem cells are the ones that can develop to more than one cell type but are more limited than pluripotent stem cells. They cannot form all the cell types, but they can form a significant number of cell types.

Student: Sir at stem cells in umbilical called multipotent?

Yes. The cord blood cells are multipotent stem cells; only embryonic stem cells are pluripotent. Totipotent cells can differentiate into extraembryonic membranes and tissues and the embryo and all the post-embryonic tissue and organs, which means it has all the potential to develop into a whole organism. So, embryo is basically a totipotent cell. The stem cells are broadly classified as adult stem cells and embryonic stem cells, based on where you get it from.

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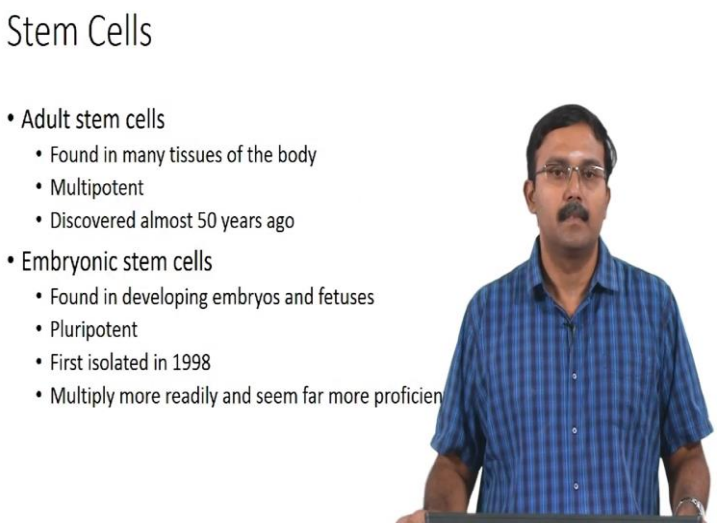

If you are getting it from an adult organism, then it is an adult stem cell, and if you are developing an embryo from which you are harvesting these stem cells, then it is called an embryonic stem cell. Adult stem cells are present in different parts of the body. They are multipotent, and these were discovered more than 50 years ago now. Embryonic stem cells are found in developing embryos and fetuses, these are pluripotent, and these were first isolated in 1998. They can multiply more readily and seem far more proficient compared to adult stem cells.

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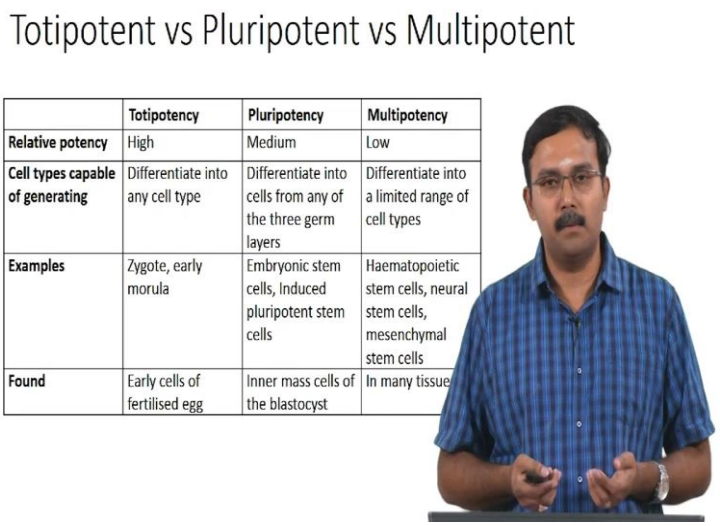

When you are talking about the three major types of stem cells, the totipotent, pluripotent, and multipotent. The potency of the totipotent stem cells is much higher than that of pluripotent and multipotent. Cell types which they are capable of generating totipotent can generate any type of cell, including the extraembryonic cells. Whereas, pluripotent can differentiate to form all the three germ layers. Whereas, it cannot form the extraembryonic tissue. Multipotent is limited to certain cell types.

Examples of totipotency cell types are zygote and early morula, which is the first few cell divisions of the embryo. Embryonic stem cells and induced pluripotent stem cells would be pluripotent stem cells. Hematopoietic cells, mesenchymal stem cells, neural stem cells are all examples of multipotent stem cells. These are basically different types of adult stem cells.

Totipotent stem cells are found in the fertilized egg and the early cells of the fertilized egg. Pluripotent stem cells, which are the embryonic stem cell, are found in the inner mass of a blastocyst. The embryo develops for a few days and forms something called a blastocyst, which is a cell mass. The inner cell mass of this blastocyst is an embryonic stem cell. Multipotent stem cells are obtained from different tissues.

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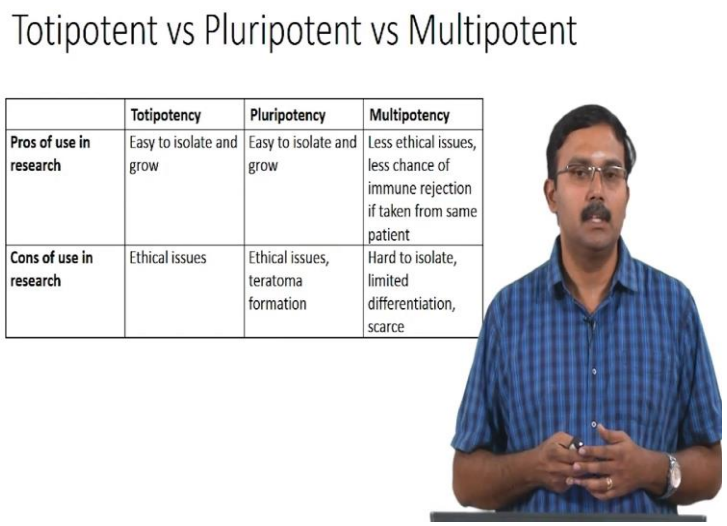

The advantage of each of them is totipotent stem cells are easy to isolate and grow, and pluripotent are also easy to isolate and grow. Cells can be easily separated to be cultured. With multipotent cells, there are less ethical issues, and there is less chance of immune rejection because there is a potential for it to be taken from the same person. So, you can get an autologous stem cell and culture it and use it. That is why people store cord blood, hoping that they would be able to use these multipotent stem cells eventually.

The disadvantages with totipotent and pluripotent are the ethical issue, and with pluripotent stem cells, there is also a challenge of teratoma formation. Multipotent stem cells are very difficult to isolate, and they have limited differentiation, and they are not available in large numbers. When you aspirate blood from bone marrow, It is not very easy to get stem cells; you get only a very few stem cells for the aspiration you get. So, those are some of the problems.

Teratoma is like a cancer tissue that contains tissues from all three derm layers. It is a problem with embryonic stem cells specifically, and induced pluripotent stem cells (IPSCs) may also have the same problem, but people are trying to understand that.

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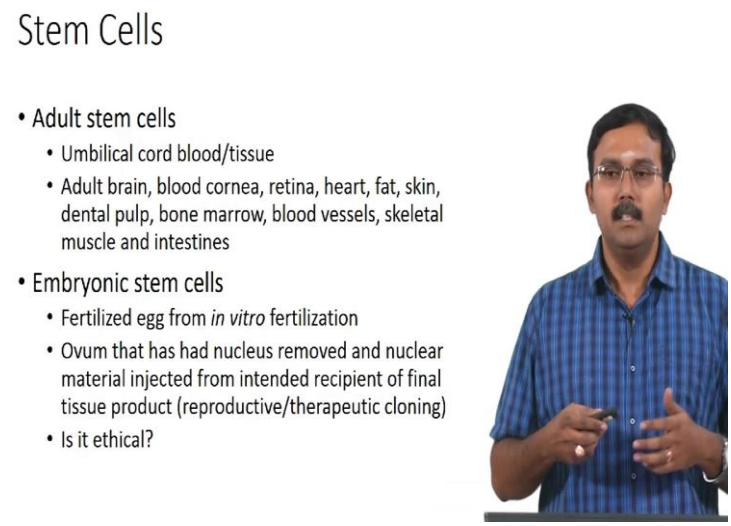

Adult stem cells are primarily obtained from the umbilical cord blood or tissue. They are also cells from the brain, cornea, retina, heart, fat, skin, dental pulp, bone marrow, blood vessels, skeletal muscles, intestines.

So, these are just places where you get different adult stem cells, and the number of cells you harvest will depend on the source itself. The umbilical cord has the most abundant, and bone marrow has significant numbers, whereas, if you were to take it from dental pulp or other places, the numbers are usually smaller.

Student: Sir, I am not sure, I have read somewhere that recently the isolated stems cells from teeth.

That is the dental pulp.

Student: Oh.

The teeth have pulp in the root, and that is where you can harvest it from.

Embryonic stem cells are obtained from the fertilized egg, usually through in vitro fertilization. The ovum that has had nucleus removed, and the nuclear membrane material is injected from the intended recipient; it can be reproductive or therapeutic cloning to get the in vitro fertilized eggs which you culture for a few days and then finally get it. So, the question is always about the ethics of this.

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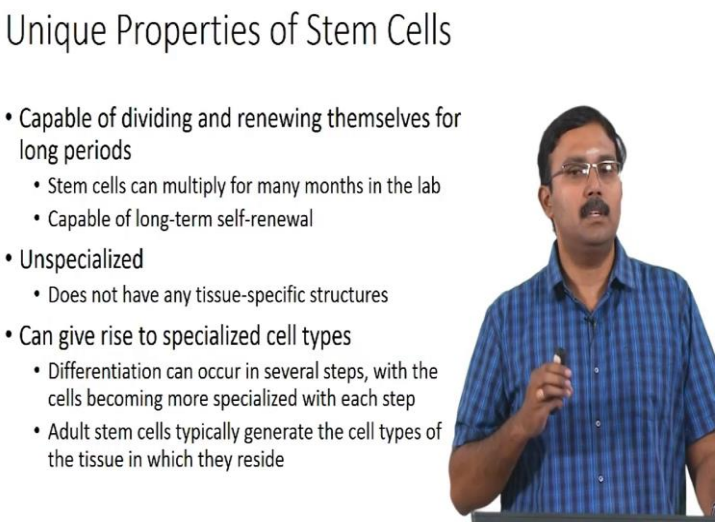

Some of the unique properties of stem cells are they are capable of dividing and renewing themselves for long periods. Stem cells can multiply for many months in the lab, and they are capable of long-term self-renewal, in the sense that even when they are differentiating, they will maintain the numbers. They are unspecialized, which means they do not have any tissue-specific structures.

They can be differentiated into different types of cells. They can give rise to specialized cell types, which will happen through the process of differentiation. Differentiation does not happen as one step; it goes through multiple steps, where the cells become more and more specialized, and finally, they get committed to one lineage and form one particular type of cells.

Adult stem cells typically generate the cell type of the tissue where they are obtained from. If you take hematopoietic stem cells, it will form different types of blood cells. It can be differentiated into other cell types by forced differentiation, but in general, it can differentiate only to those types of cells.

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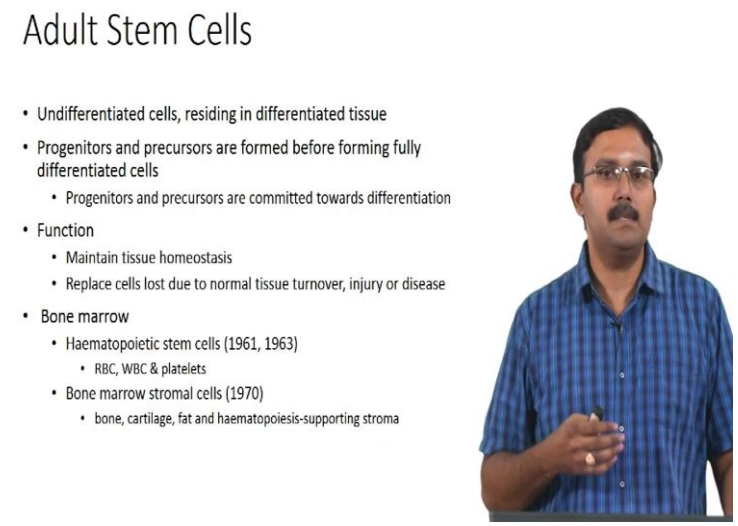

Adult stem cells are undifferentiated cells that reside in the differentiated tissues. Progenitors and precursors are formed before you form the fully differentiated cells; these are the intermediary steps of the differentiation process. Progenitors are, however, committed to a lineage. Whereas, the stem cells are not, so that is the difference. Although in some cases, people do try to use them interchangeably, it is not scientifically correct; progenitor is already committed. One example would be endothelial progenitor cells. So, these can only form endothelial cells right, but they are not fully developed into endothelial cells, but they can grow at a much faster rate compared to endothelial cells.

The function of the adult stem cell is to maintain tissue homeostasis and replace the cells which are lost due to normal tissue turnover, injury, or disease.

Bone marrow is where the first set of stem cells were identified, and hematopoietic stem cells were identified in 1961 and 1963, and these can form the RBC, WBCs, and platelets. Bone marrow stromal cells were identified in 1970, and they have been shown to form bone, cartilage, fat and hematopoiesis-supporting stroma.

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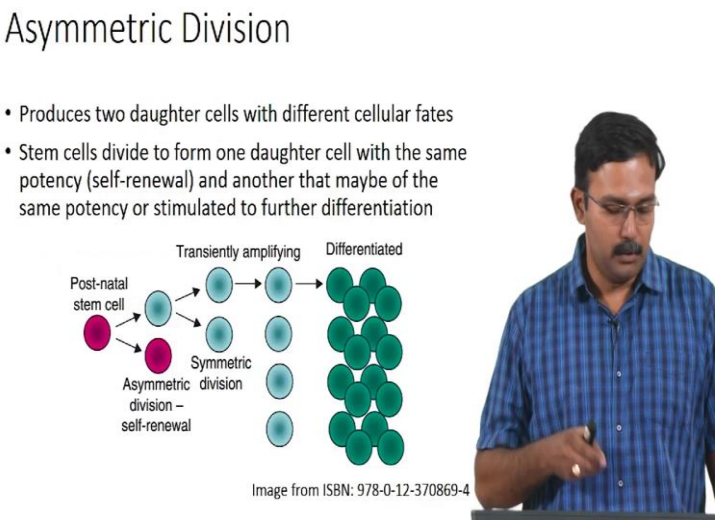

Stem cells have the self-renewal property because of something called asymmetric division. What happens is, whenever you have asymmetric division, there are two daughter cells, which can have two different cellular fate. When you have mitosis, the two daughter cells formed are exactly identical, and they have the same fate. Even with meiosis, that is what happens, right? Meiosis also forms two daughter cells that have the same fate; they can only be gametes, and they cannot be anything else.

Whereas here, that is not what happens; you have an asymmetric division where the stem cells which divide one will be a stem cell, which means the self-renewing property is maintained. One stem cell forms one stem cell and another cell that can get differentiated into any type of cell. So, the number of stem cells in their population remains the same. Basically, one daughter cell has the same potency, which is the self-renewing property, so it is a stem cell, and the other one can either be a stem cell or be stimulated for further differentiation.

Usually, what happens is this daughter cell, which has a different fate, can multiply before it gets differentiated. That is what happens because after complete differentiation, if the multiplication has to happen, then it will be much slower. Multiplication usually happens before complete differentiation. So, that is why these progenitor cells are important.

These progenitor cells will divide at a faster rate, but they are already committed to the lineage. They would not come back to the previous step, they will get differentiated to the final type of cells.

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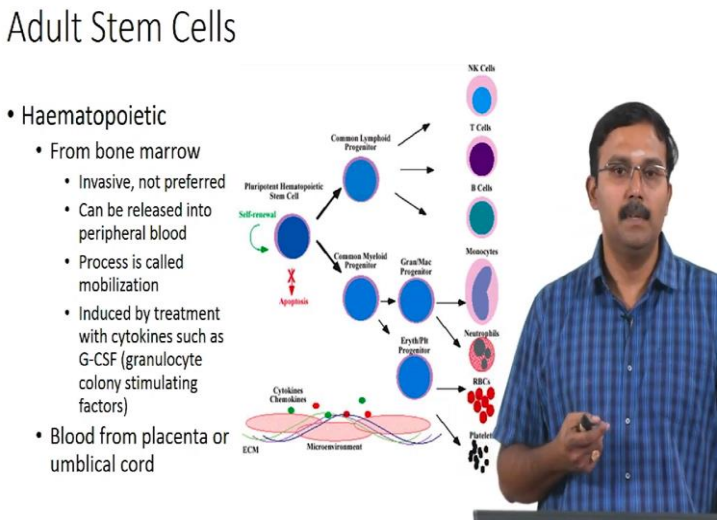

Hematopoietic stem cells are the ones that are obtained from the bone marrow. The way you harvest them is an invasive procedure; you have to drill into the bone marrow to get it. It is not a very preferred mechanism for harvesting, but that is the only way you can harvest hematopoietic stem cells. They can also be released into peripheral blood by a process called mobilization.

This mobilization can actually be induced by treatment with cytokines, and thereby, you can actually get them into the peripheral blood and then try to draw them. Blood from the placenta or umbilical cord would also have hematopoietic stem cells. These are much easier sources to harvest these stem cells.

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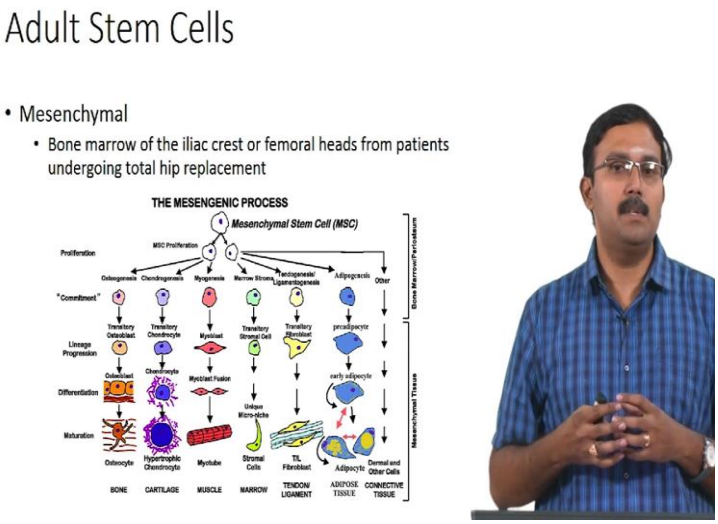

Mesenchymal stem cells are the ones that are extensively studied; they are obtained from the bone marrow of the iliac crest or the femoral head from a patient who is usually undergoing a total hip replacement. You do not harvest mesenchymal stem cells only for that purpose because it will be a quite painful procedure. Iliac crest is from your hip, and you can get it from your sternum, which is the joint between your rib cage. You can also get it from the femoral head; femur is the bone in your thigh, and the head which gets into the ball and socket joint, that is the head from where you can get these crest.

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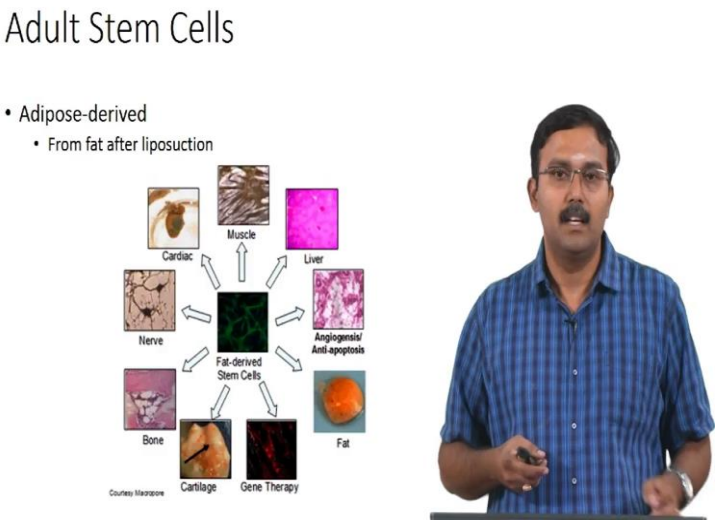

Adipose-derived stem cells can be obtained from fat tissues, usually which is taken after liposuction. Adipose-derived stem cells have also been differentiated to various types of cells, and they have been effective in using for tissue engineering applications.

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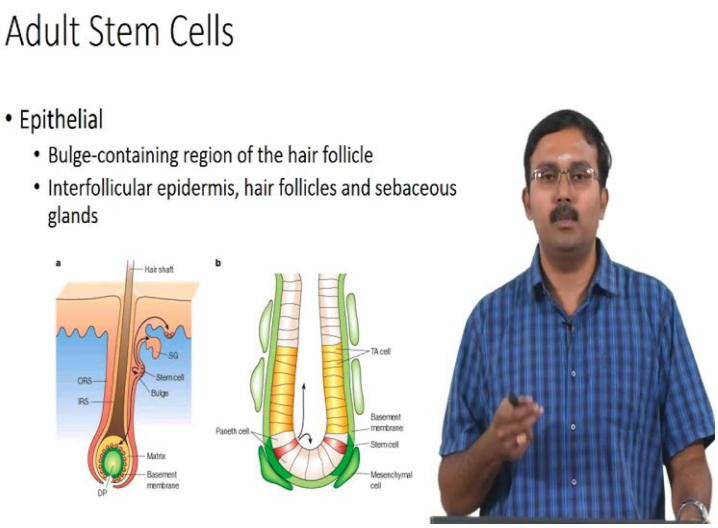

Epithelial cells are usually found in the bulge containing the region of the hair follicle. As I said, the numbers would be small it is difficult to harvest them, but they do have the same multipotency as mesenchymal stem cells.

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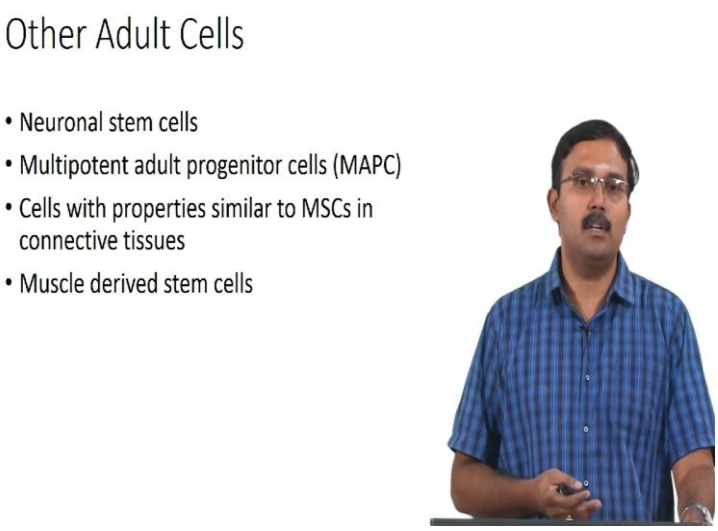

Other stem cells are neuronal stem cells, multipotent adult progenitor cells, and cells which are present in your connective tissues and muscle-derived stem cells. These are all the different adult stem cells which have been used for different tissue engineering applications.

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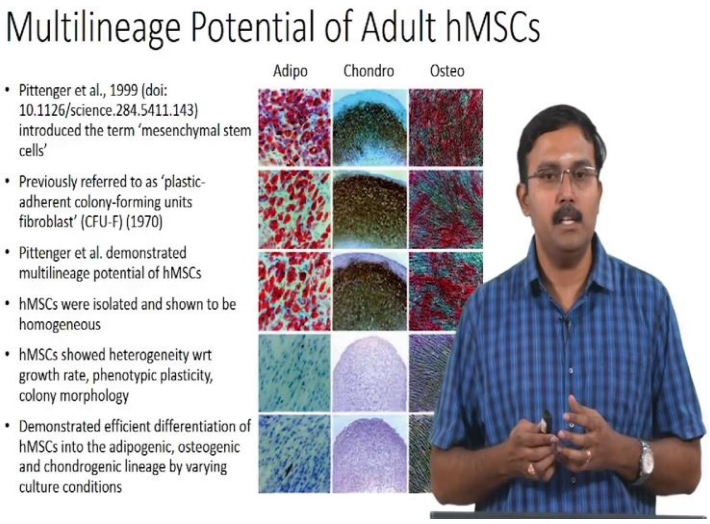

Multilineage potential of the adult human mesenchymal stem cells was demonstrated in 1999 by Pittenger et al., and this was published in science. This is one of the extensively cited papers which has almost 23,000 citations currently. These are cells that were previously called plastic adherent colony-forming units fibroblasts. The term mesenchymal was coined in this 1999 paper.

What happened was Pittenger et al. demonstrated that these human mesenchymal stem cells have a multilineage potential, and they form adipocytes, chondrocytes, and osteoblast. They also showed that these mesenchymal stem cells had heterogeneity with growth rate, phenotypic plasticity, and colony morphology when cultured in different conditions.

Initially, they were identical, right? When you harvest these mesenchymal stem cells, they are all identical; all the morphology, all the properties are the same, but when you culture them in different conditions, they became different cells completely, and that was shown for the first time in this paper.

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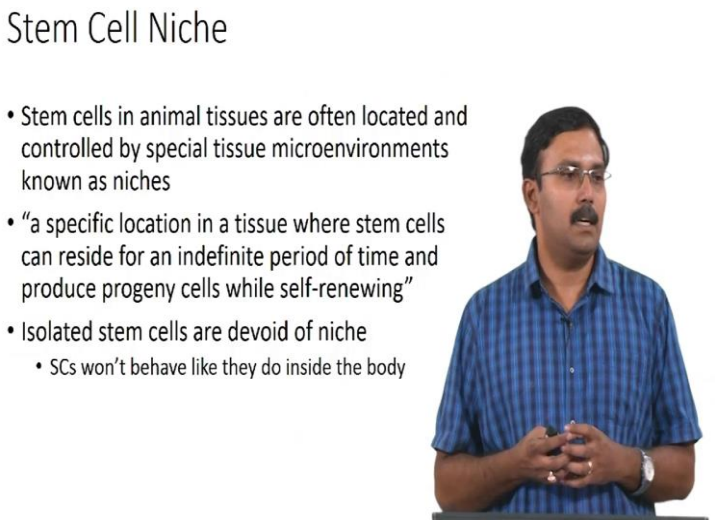

Stem cells are present in your body in a place called stem cell niche. This is a microenvironment that is well controlled to maintain the stemness of the cells. This is a specific location in which the cells can reside for an indefinite period of time without actually differentiating. They can produce progeny while self-renewing, in the sense that they can go through asymmetric division. The cell which is fated towards differentiation will leave the stem cell niche, and the cell, which is going to remain with the same multipotency, will reside in the niche itself.

This is one of the issues when we handle stem cells. We do not have the stem cell niche outside the body. So, you can have the stem cells, but they do not behave the same way. When you have it in a stem cell niche, they have certain properties which are, which will be lost when you take it out of them.

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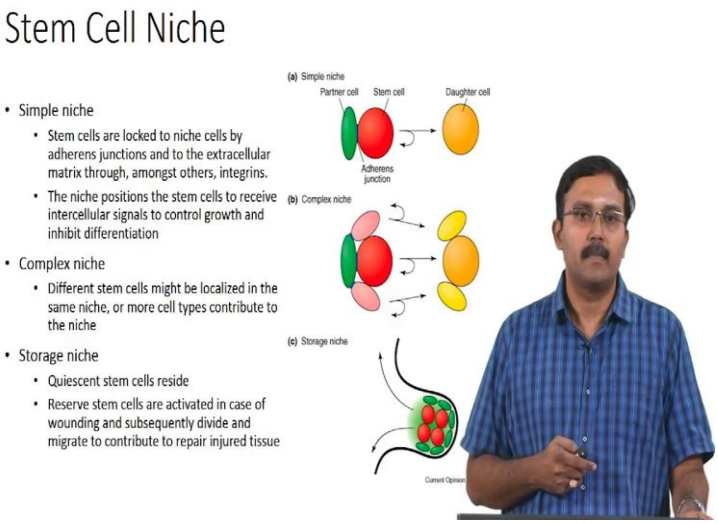

There are different types of stem cell niches, so you have a simple stem cell niche. These are niches where the stem cells are locked to the niche by adherent junctions and to the extracellular matrix through different junctions like integrations. The niche position of the stem cells is to receive intracellular signals that control the growth and inhibit differentiation.

In a complex niche, different stem cells might be localized in the same niche, and more cell types can contribute to the niche, instead of just one single cell type contributing to the niche.

The storage niche is the stem cell niche where the cells are just stored. The cells are quiescent inside this niche. These act as the reserve of stem cells, and they are activated only during injury or some kind of damage to the tissues. So, these cells can go and repair the injured tissue.

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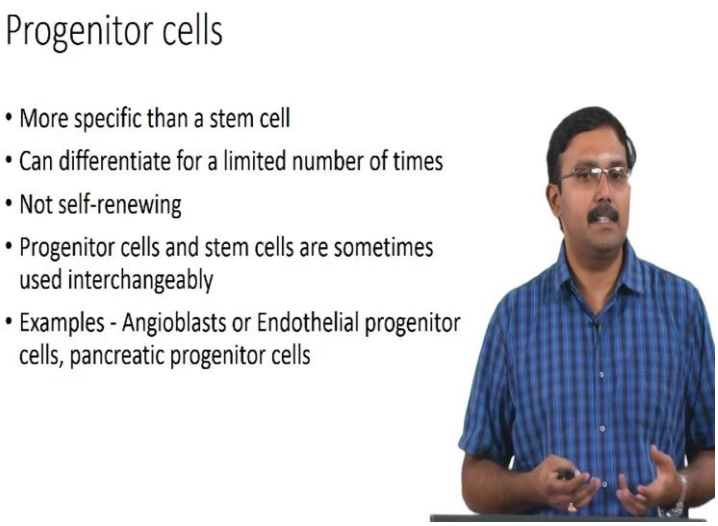

Progenitor cells are cells that are more specific than the stem cells, and they can differentiate for a limited number of times. These are not self-renewing, but in some literature, you would see that they are using it interchangeably, which is not correct. Endothelial projector cells or pancreatic progenitor cells are cells, which are committed towards the particular lineage.

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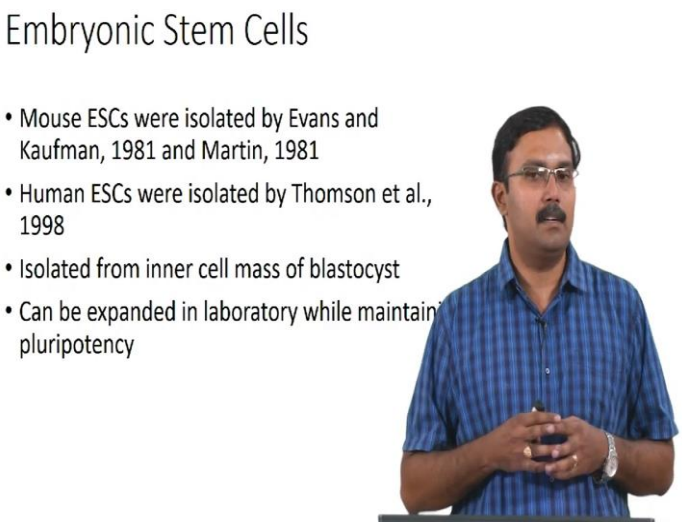

Embryonic stem cells were first isolated from mice in 1981. It was done by two different groups independently, and in 1998, human embryonic stem cells were isolated by

Thomson et al. These are isolated from the inner cell mass of the blastocyst and can be expanded in the lab while maintaining the pluripotency.

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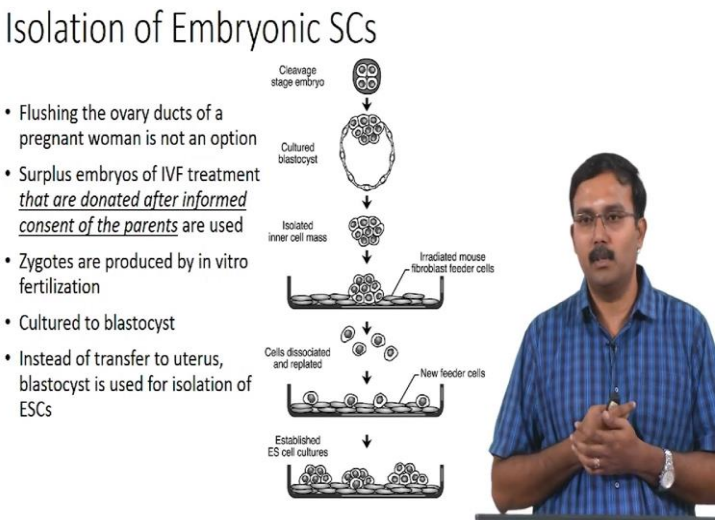

This is what is done for the isolation of embryonic stem cells. If it is natural fertilization, you would have to get the embryo out of the ovarian duct, right? So, then that would mean flushing of the duct, which will destroy the embryo as well, so that is not really an option. Because of this, you need to do in vitro fertilization. That is how you create these embryos for getting these embryonic stem cells. Surplus embryos during IVF treatment can be used for this. This needs to be donated by the parents after informed consent.

In India, you cannot pay for these things, as well. Whereas abroad, people do pay for some of these donations which people make. For blood collection in the US, we used to go and give blood very commonly for research because many of my friends are working with blood. They would need 30 ml of blood for research, and they will call one of us, and we will go and give blood; we will get like 20 dollars or 30 dollars. We used to do that quite regularly, but in India, you are not allowed to do that because you can exploit the poor in the name of doing that. So, in the US, you only exploit the grad students.

Zygotes are produced through in vitro fertilization, and they are cultured to form the blastocyst phase. At this stage, what happens is, this is placed inside the womb for it to develop into a fetus. Here, instead of that, the blastocyst is used for the isolation of embryonic stem cells.

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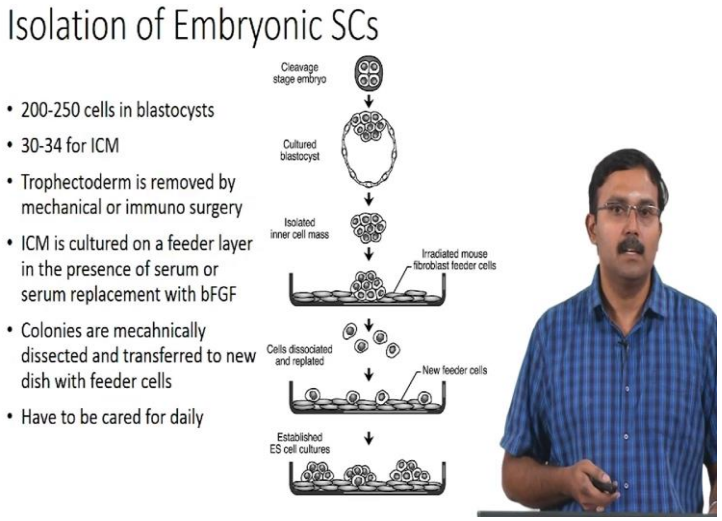

There are only about 200 to 250 cells in the blastocyst and 30 to 34 of them as the inner cell mass, and the outer layer is removed either through mechanical surgery or through Immuno surgeries. After that, you take the inner cell mass and culture it on a feeder layer, and the colonies are mechanically dissected and transferred to new dishes. These need to be cared for daily. So, that you make sure that the differentiation does not happen because when you are culturing it in vitro, there could be stresses, which are caused that could cause differentiation. To maintain its stemness, you need to make sure it is cared for, and it is maintained in the way it can be.

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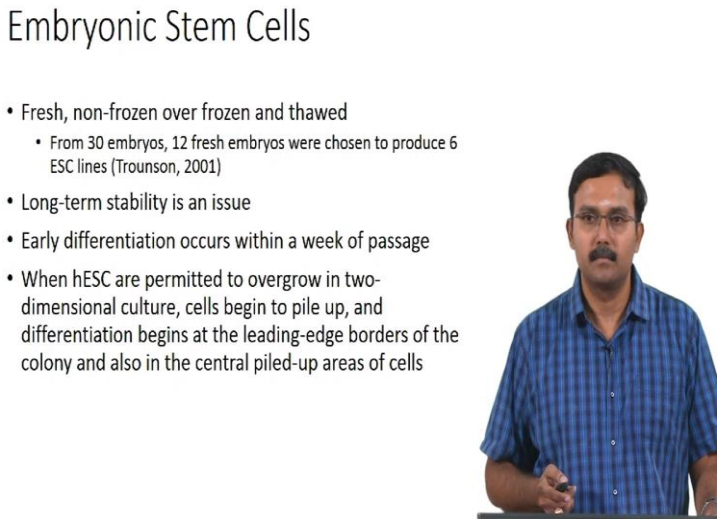

Student: Sir, what was that feeder layer?

The feeder layer is basically another monolayer of cells that is used, on which the cells are cultured so.

Student: What is that?

Usually, they are fibroblasts; fibroblasts are cultured as feeder layers. This goes for any cell type. Fresh, non-frozen cells are always preferred when you are talking about primary cells compared to frozen and thawed cells. The cell lines which you use are different; those are immortalized. Those, you can put it in liquid nitrogen and then take it out and use it. But for primary cells, you would not want to do that, so that is why you need to harvest them regularly. This means it is a problem; you are not going to get enough cells with respect to embryonic stem cells. There are also issues with respect to long term stability, and there are cases where there is early differentiation.

When you culture these cells in the 2D environment, the cells can pile up. Instead of forming a monolayer, once there are more cells, it can start piling up, and this will create stresses causing differentiation around the edges or in the piled-up areas. So, these places would experience more stresses, and it results in the differentiation of cells.

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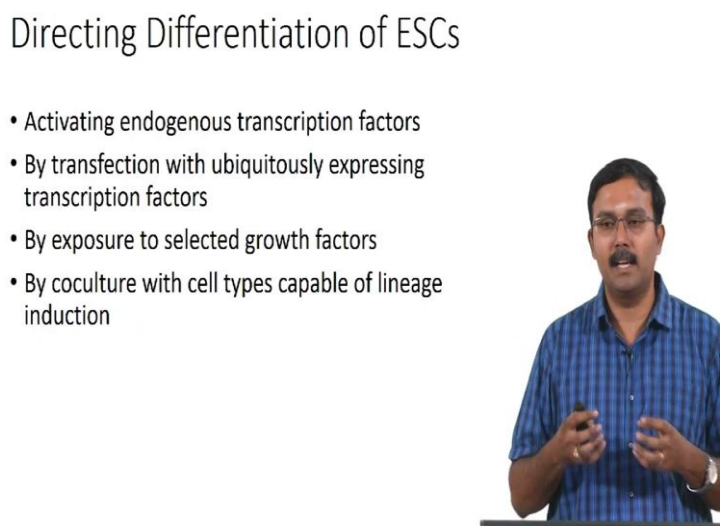

You can use endogenous transcription factors to activate cell differentiation. Using different growth factors, using different cytokines and other markers, you can control

how the cells are differentiated, and you can also co-culture with cell types that are capable of lineage induction. There are studies where people have shown that culturing stem cells, along with pancreatic cells or islet cells, would cost them to differentiate to form islet-like cells. So, those kinds of things are possible.

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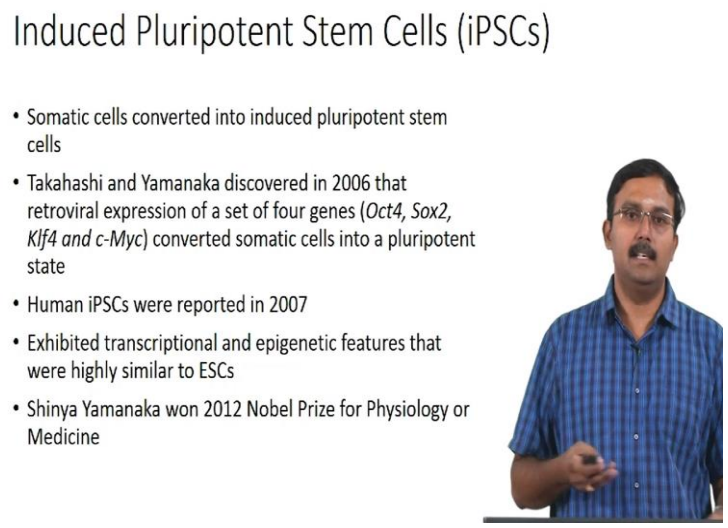

IPSCs are the last type of stem cells which we will quickly go into. These are somatic cells that are differentiated into induced pluripotent stem cells. This was first shown in 2006 by Takahashi and Yamanaka, and what they showed was, there was a set of four genes that can be transmitted into the cells to form them into pluripotent stem cells. Human IPSCs were then reported a year later. These cells have very similar properties as embryonic stem cells. Yamanaka won the Nobel Prize in 2012 for physiology and medicine for this discovery.

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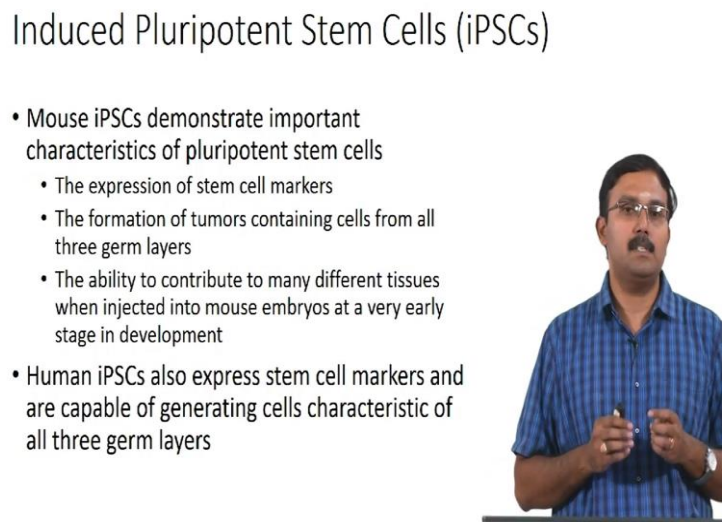

Mouse IPSCs demonstrate certain important characteristics that are seen in pluripotent stem cells. Basically, the expression of stem cell markers ability for the formation of teratomas, which are tumors containing all three germ layers. The ability to contribute to many different tissues when injected into the embryo at the early stages of development.

Human IPSCs also have similar markers and the ability to form cells from all three germ layers. So, there is a lot of studies going on in this direction, and it is a very recent development. So, less than 12 years old, it is a very interesting domain.