Biomaterials for Bone Tissue Engineering Applications
Professor Bikramjit Basu
Materials Research Centre
Indian Institute of Science Bangalore
Module 4
Lecture No 17

Ok in this module, I will discuss the stem cells as a model system to make you understand the cell differentiation better.

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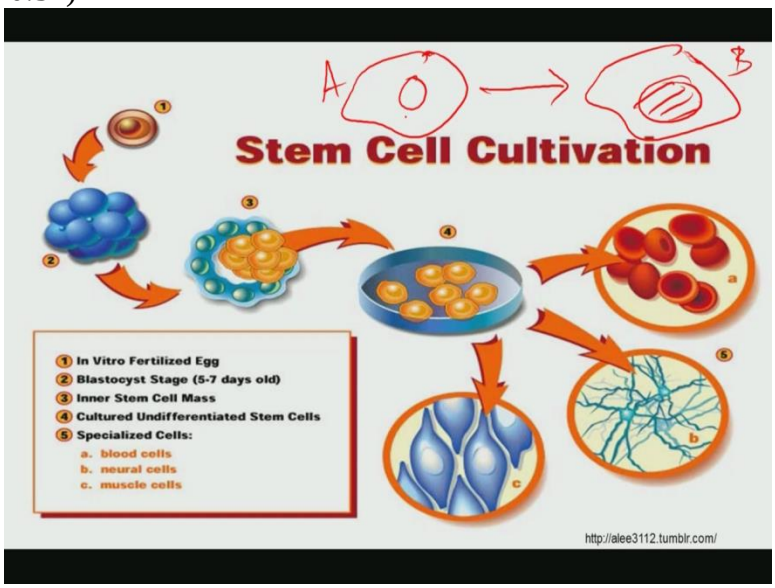

Just to recall the definition of differentiation, so differentiation essentially means differential gene expression and therefore when cell A is differentiated to cell B then one would expect that. When a cell A is differentiated to or a cell type is differentiated to another cell type and every time I so differentiation I always mark or sketch the nucleus in different shade just to show that two cells have different gene expression.

Now so this, some basic theory related to the differentiation was covered in one of the earlier modules. In this particular module, I will discuss the stem cell differentiation process and for this purpose, I will also introduce you:
what is the definition of stem cell? How stem cell is different from other cell types? What are the different types of stem cell types, different type of stem cells, and how they are differentiated to other cell type?

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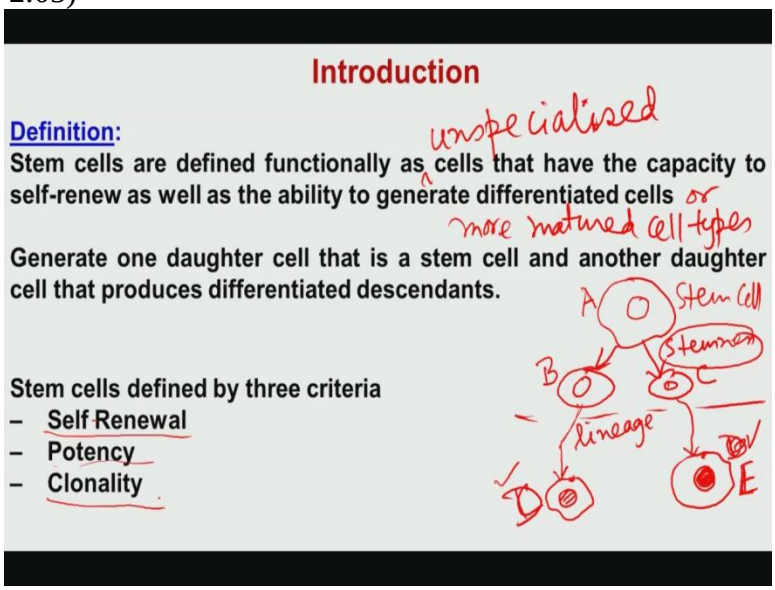

So this is a formal definition. Stem cells are defined functionally as this as some unspecialized cells as unspecialized cells that has the capacity to self renew as well as the ability to generate differentiated or more matured cell types, or more matured cell types. So it can do two things one is that I will show you in some of the sequence lights, so if you have that one stem cell then it can produce two stem cells while maintaining the stemness to stop so it is simply the proliferation of stem cells without being differentiated.

But then each of these daughter cells now can undergo differentiation along certain lineages and they can be, they can finally produce different cell types. and these cell types let us start with marking of cells now A to Band A to C so here up to this part your stemness is maintained, that means stem cell is now growing proliferating from one to two, two to four like that. Now when B goes to one specific lineage to produce matured cell type C or C goes to, sorry this is this will be D and this will be E.

And when C is differentiated to produce different cell type E. Now remember D and E have different functionality. So although they come from the daughter cells, two different daughter cells of one mother stem cell but there functionality of D and E is different and therefore in principle D and E can form different tissue type also. Now as we progress through the subsequent slides we will learn more about it and we will learn more about the, what are the different lineages are possible, what are the different types of stem cells and so on.

Now stem cells as you know that have revolutionary have revolutionized the field of tissue engineering as well as human health care and many researchers claim that almost most of the life threatening diseases can be treated clinically using stem cells and therefore in several countries around the world clinical trials using stem cells are currently underway. So in this perspective it is important to develop some understanding on the stem cells and how it can be useful in research related to biomaterials particularly for bone tissue engineering applications.

Ok now having given this introduction to the stem cells, these stem cells can be defines by the three unique criteria; one is Self Renewal, second one is the Potency and the third one is the Clonality.

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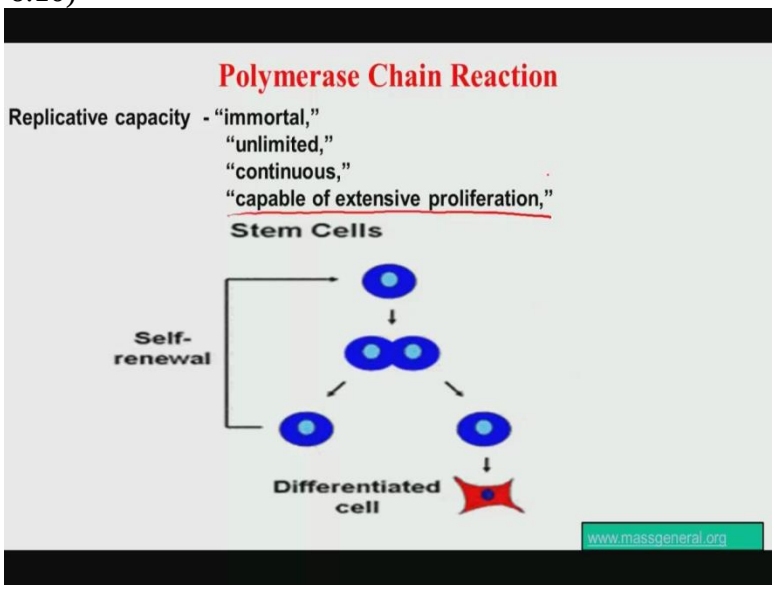

Now each of this term has its own importance. So Self Renewal means that it can undergo normal proliferation to produce a number of stem cells without while maintaining the stemness and this particular case like this essentially that is possible because stem cells have the replicative capacity. Like any other cell types, stem cells also have the replicative capacity otherwise you cannot call it a cell. So essentially it is capable of extensive proliferation while maintaining the stemness and this is immortal unlimited and continuous.

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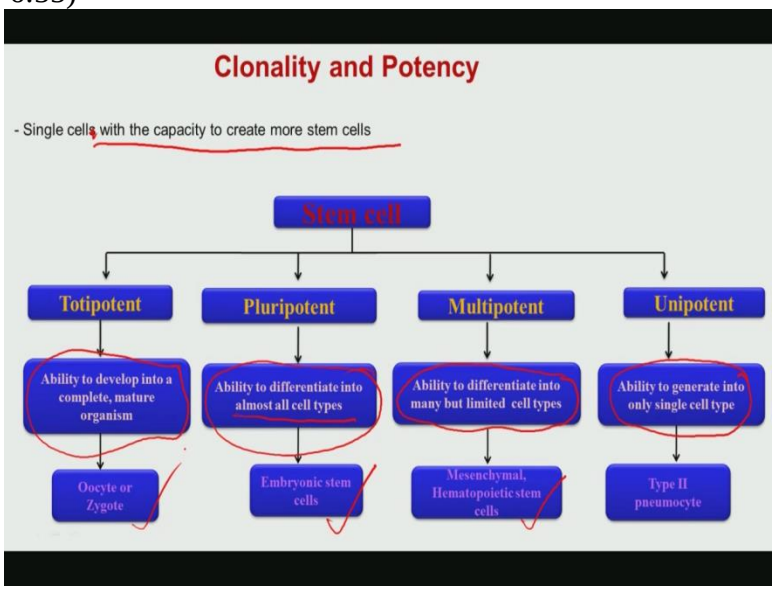

Now Clonality and Potency can be described as follows: Like single cell with the capacity to create more stem cells and in terms of the Potency stem cells can be classified into four different types: One is Totipotent; one is Pluripotent; third one is a Multipotent; and fourth one is a Unipotent. Now, Unipotent essentially means that ability to generate only one single cell type ok? Uni means one so Unipotent means ability to generate only one cell type. Multipotent means, ability to differentiate into many but limited cell types.

It is not all cell types possible but limited cell type. Pluripotent means ability to differentiate to almost all cell types so that is a Pluripotent. And Totipotent means ability to different all kind of cell types so that it can develop, it can mature to a organism. So these are some of the examples given. Pluripotent example is that Embryonic stem cells and you know that most of the clinical trials if it involves

Embryonic stem cells there may be lot of ethical issues as well as religious issues concerned. Multipotent is Mesenchymal and Hematopoietic stem cells. And totipotent is Oocyte or Zygote.

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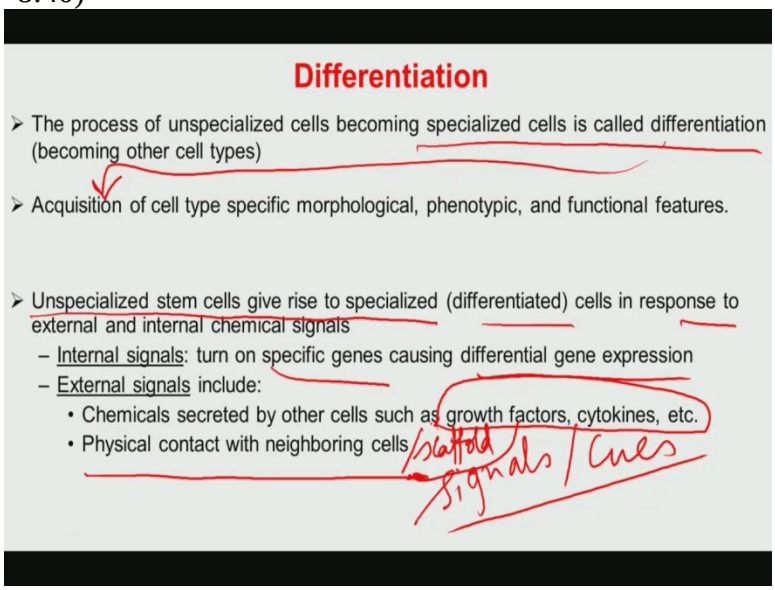

So let me discuss these things in little bit more details. So the process of unspecialized cells becoming specialized cells is called differential, differentiation or becoming other cell types that has been mentioned before. And differentiation also means that the acquisition of cell type or the matured cell type with specific morphological, phenotypic and functional feature. Now unspecialized stem cells can give rise to specialized or differentiated cells in response to different signals.

Now this in biology literature or in barometers literature this signaling signals or cues are kind of used very interchangeably and these internal signals essentially turn on specific genes causing differential gene expression and external signals means either physical contact with neighboring cells or scaffold or biomaterial substrate which has a different elastic stiffness or (())(09:46)stiffness. And also conventional biologist they use different growth factors or cytokines to trigger the stem cell differentiation.

Whereas material scientists use different scaffolds or different materials, biomaterial substrates as physical cues to trigger or to drive stem cell differentiation. So these are two distinct kind of approaches, one is that you can use that biochemical growth factors so cytokines to guide stem cell differentiation. Another approach which is more engineering or more material science-based approach, is to use some elastic scaffolds or biomaterial scaffolds which have a specific elastic

stiffness, which is much higher than that of the cells or tissues, and to use that as a physical cues to guide the stem cell differentiation.

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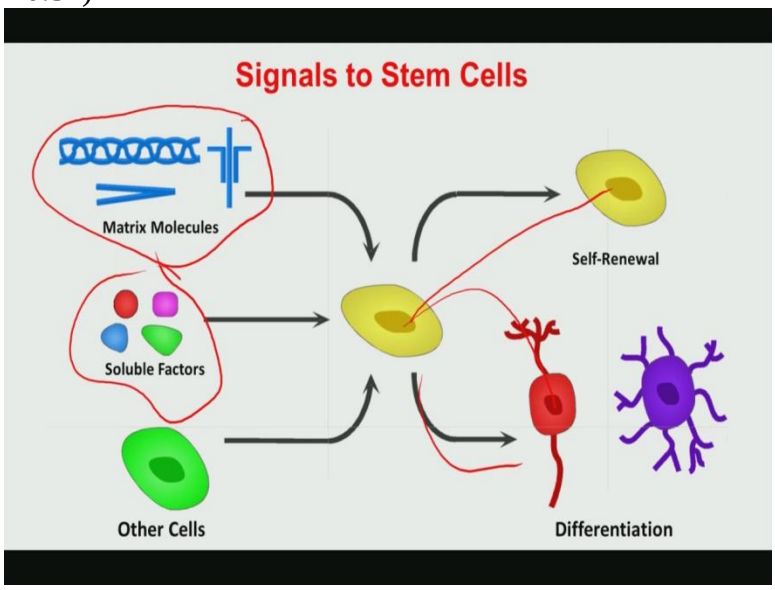

So this is what has been mentioned in the last slide and it is shown here, so we have some soluble factors like growth factors in cytokines which can drive the stem cell differentiation and every time it shows that you know that this you can see that this nucleus is shown in different morphology or different kind of colors. Then you have the matrix molecules or you have that substrates that also can guide stem cell differentiation.

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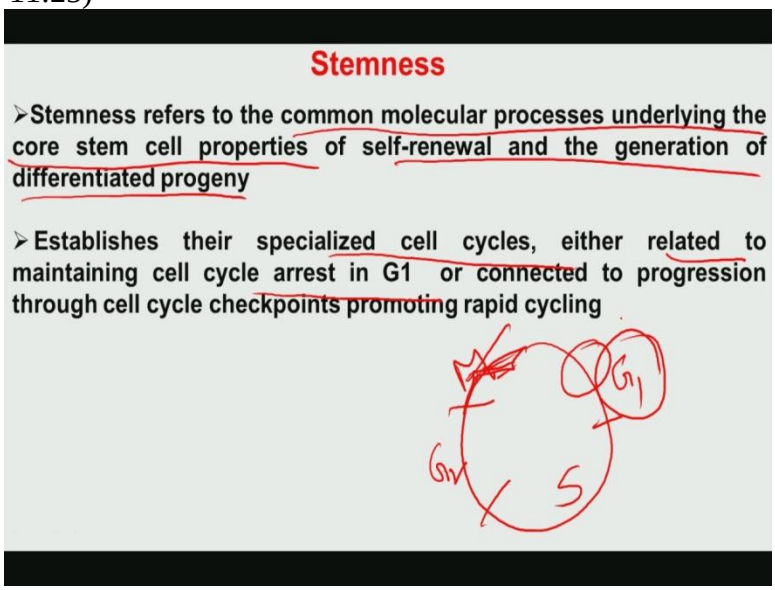

So stemness therefore refers to the common molecular processes underlying the core stem cell properties to self renewal and the generation of differentiated progeny. And it is important to mention also that is the stemness establishes stem specialize cell cycles, either related to maintaining cell cycle arrest in G1. So remember that cell goes to a difference.

One cell goes to G1 then there is a checkpoint then S then goes to another check point then go to G2 and then finally M. So if a cell is arrested in G1 cycle itself then it cannot go to unlimited growth conditions but however it can now differentiate. Now those things I will explain to you little later.

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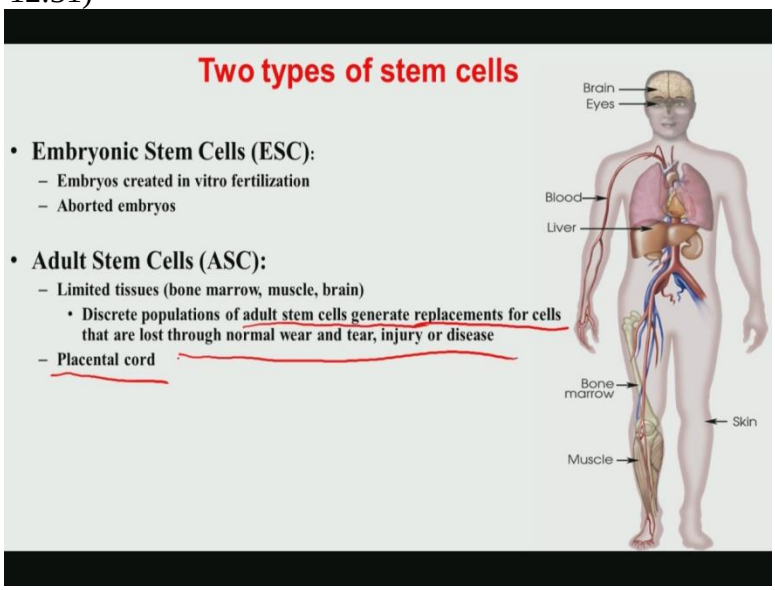

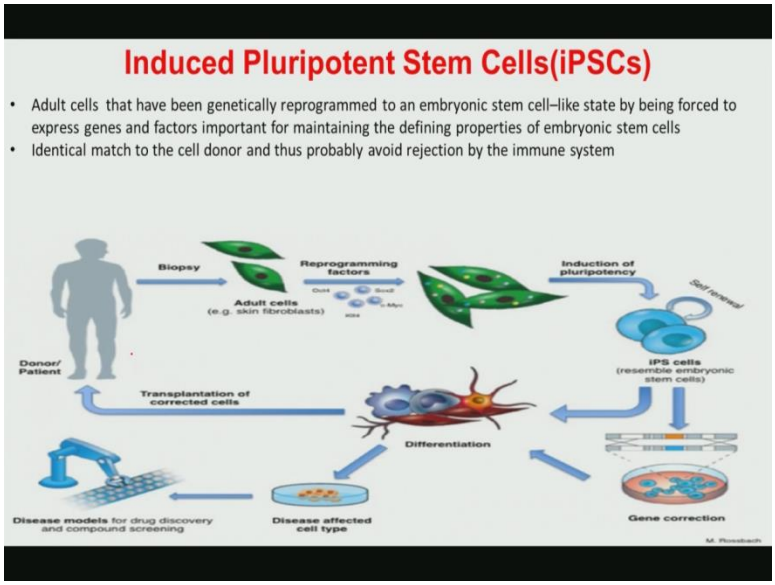

So as I said before that there are two different types of stem cells one is Embryonic Stem Cells like which is like embryos is created in during in vitro fertilization or aborted embryos that can be used to obtain that embryonic stem cells. Another one is a adult Stem Cells and adult Stem Cells are available in bone marrow, muscle and brain. And these are like discrete population of adult stem cells that generate replacements for cells that are lost normal wear and tear, injury or disease. another place where adult stem cells are obtained is Placental Cord.

So right hand side that one person is image is profiled just to show that different sources for stem cells where you can get this different types of stem cells that have mentioned in this slide.

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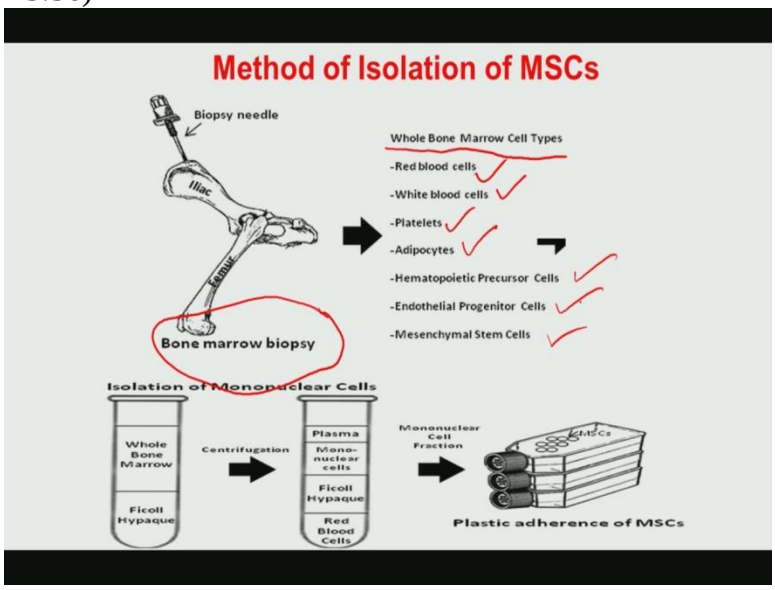

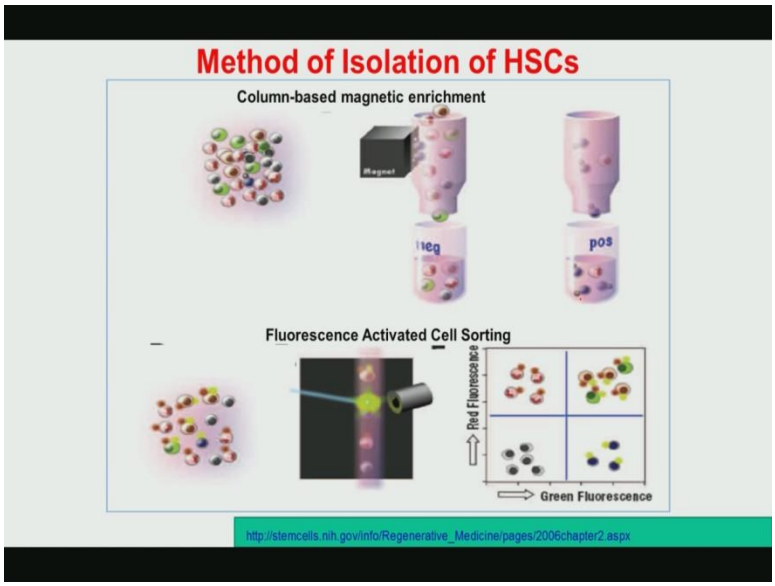

There are specific biological methods to isolate Mesenchymal Stem Cells, so like bone marrow derives the stem cells you can see that, here it is shown like a case of a bone marrow biopsy then you take this biopsy needle then you take it then there is this different whole bone marrow cell types like RBC, WBC, Platelets, adipocytes as well as Hematopoietic precursor cell, Endothelial Progenitor cells and Mesenchymal Stem Cells.

Now the complete procedure of isolation of Mesenchymal Stem Cells, from using this bone marrow biopsy is not under the preview of this course. However it is just to shown that you know it is indeed possible to isolate Mesenchymal Stem Cells in this route.

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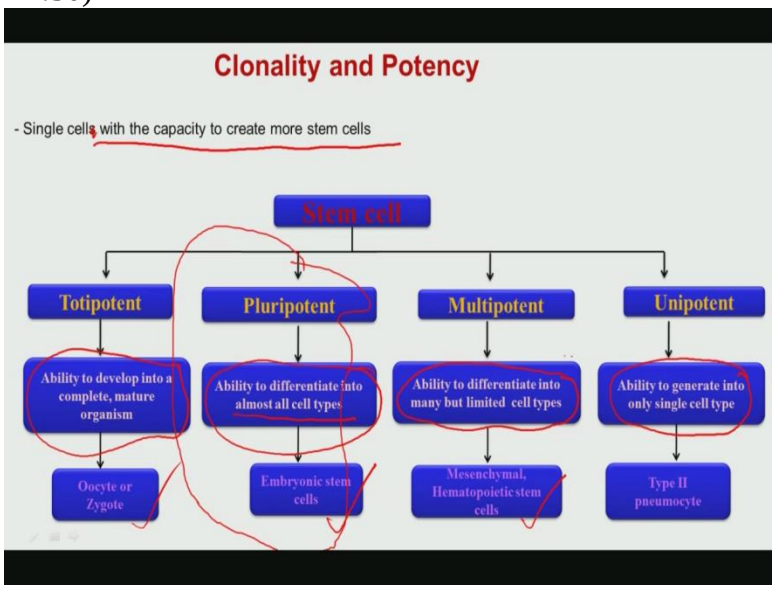

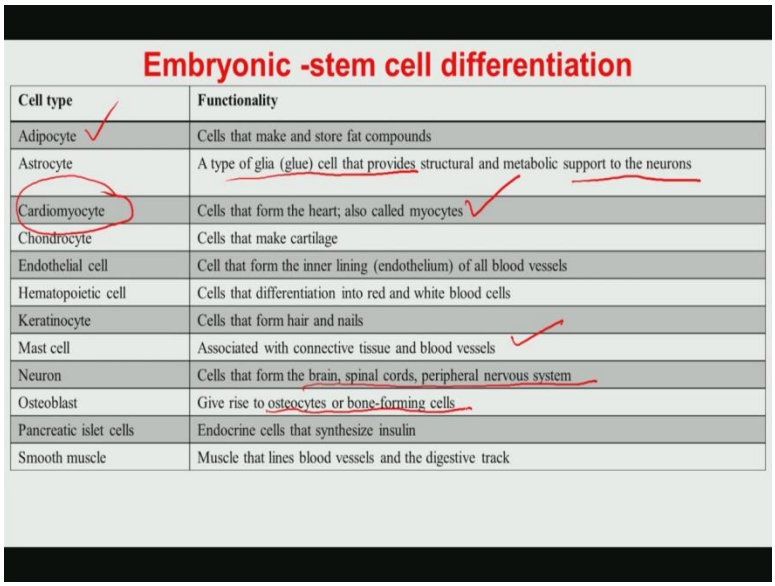

Embryonic -stem cell differentiation

Cell type	Functionality
Adipocyte	Cells that make and store fat compounds
Astrocyte	A type of glia (glue) cell that provides structural and metabolic support to the neurons
Cardiomyocyte	Cells that form the heart; also called myocytes
Chondrocyte	Cells that make cartilage
Endothelial cell	Cell that form the inner lining (endothelium) of all blood vessels
Hematopoietic cell	Cells that differentiation into red and white blood cells
Keratinocyte	Cells that form hair and nails
Mast cell	Associated with connective tissue and blood vessels
Neuron	Cells that form the brain, spinal cords, peripheral nervous system
Osteoblast	Give rise to osteocytes or bone-forming cells
Pancreatic islet cells	Endocrine cells that synthesize insulin
Smooth muscle	Muscle that lines blood vessels and the digestive track

Ok, as I said before that in the context of potency of the stem cells you have Pluripotent Stem Cells and one of the that if you go back to that one of the earlier slides where I have mentioned you have the Pluripotent Stem Cells and this Pluripotent Stem Cells one of the example is that Embryonic Stem Cells. So therefore, Embryonic Stem Cells can differentiate to different cell

type as mentioned in this particular slide. So one of the cell type is adiposite like which make and store fat compounds.

Astrocyte that it is more related to the neurons and neurons tissue applications like a type of glia cell that provides structural and metabolic support to neurons. Third one is Cardiomyocyte that is the cardiac tissue cells, and it actually forms the heart and called Myocytes and remember this Cardiomyocytes is one of the very difficult cells to grow under normal ambient culture conditions.

So therefore you need kind of very specialized tissue engineering scaffold or substrates which can support the differentiation of embryonic stem cells to Cardiomyoctyes, and also I must mention this cardiomyocytes cells they can beat like a normal heart beats in the when electric stimulation is given externally to the cells. Fourth one is the Chondrocytes that is cartilage cells I have mentioned it before also that is Chondrocytes. Fifth one is an endothelial cells that is the inner lining of all blood vessels, for example Endotheliumic mix.

Hematopoietic Cells that differentiate into red cells and white blood cells. So in case of person suffering from blood cancer and in those kind of cases it is useful. Keratinocytes that is cells that form hair and nails, often you see an open advertisement for treating hair loss. People use stem cells and to treat the hair loss essentially they use stem cells so the stem cells can differentiate into keratinocytes and then one can have fresh hair and nails.

Mast Cells that is associated with connective tissues and blood cells. Neuron that is embryonic stem cells can differentiate to neurons that can from brain, spinal cords and peripheral nervous system. Osteoblast as I said multiple times before Osteo means bone, Osteoblast means bone forming cells and from Osteoblast it can differentiate further to a more mature Osteoblast that is called Osteocytes and remember that Osteoblast and Osteocytes have different distinct cellular morphology.

Pancreatic islet cells that produce insulin this can be used to synthesize insulin essentially for diabetic patients and this is kind of useful. Smooth muscle cells, this is like muscle that lines the blood vessels and the digestive track.

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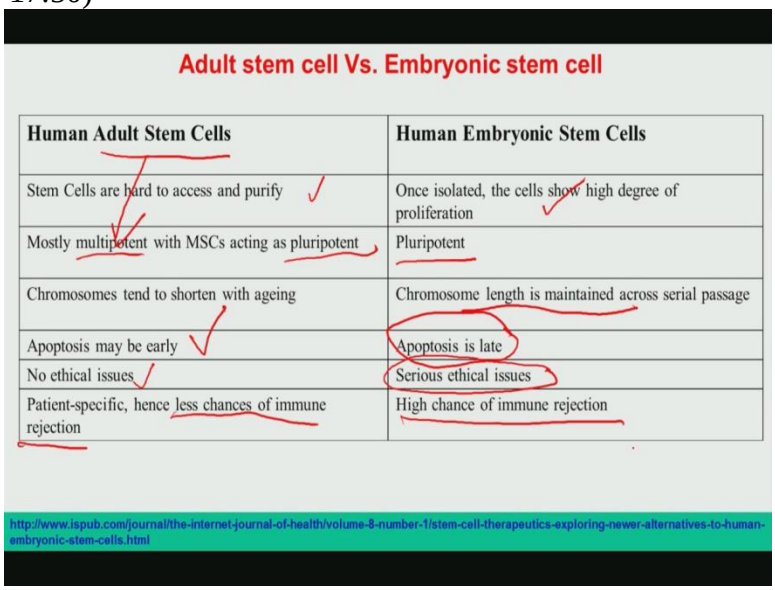

Ok, now just a simple comparison between human adult stem cells, as I said that adult stem cells have a very limited supply and it can be isolated from specific areas from specific organs of a human patient and Placental Cord is one of the place where adult stem cells can be isolated from. Whereas Embryos or Human Embryonic stem cells are Pluripotent Cells and they can differentiate to so many cell types as I discussed in the last slide.

So some of the points under this comparison that stem cells are hard to access and purify, but how where Embryonic Cell System once isolated the cell once grow with high degree of proliferations. Adult Stem cells or as I abbreviated earlier that is ASC it is mostly multi potent, so it can differentiate to many cell types but not all cell types, that is the difference between Multipotent and Pluripotent.

Now Human Embryonic Stem Cells as I mentioned before they are Pluripotent cells. Then third point is that their chromosomes tend to shorten with ageing but here chromosome length is maintained. Apoptosis may be early, where apoptosis is late. What is apoptosis? Apoptosis is the programmed cell death.

As I mentioned while discussing about the embryonic Stem Cells that use of Embryonic stem cells in any clinical trials or typically in many research projects that can attract serious ethical issues whereas if you use Human adult Stem Cells the ethical issue is much less. The last point is

that adult Stem Cells are functionally more patient-specific, hence less chances of immune rejection. In case of Embryonic Stem Cells, there is indeed large chances or higher chance of immune rejection.

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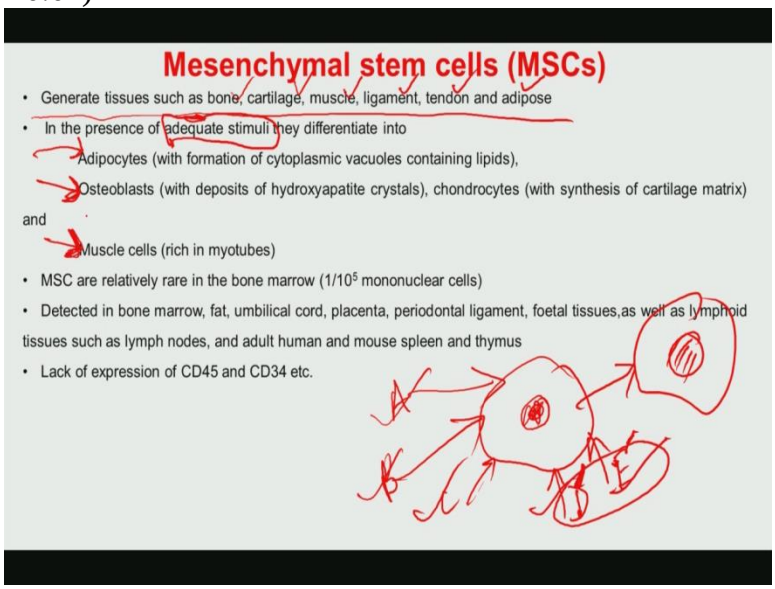

So, having given this difference between this adult Stem Cells and Embryonic Stem cells next I would like to discuss that Mesenchymal Stem Cells and this Mesenchymal Stem Cells are known to generate tissues, first they have the capability to differentiate to cell types like bone cell types, cartilage cell type, muscle cell type, ligament, tendon and adipose. So therefore in a way they have the capability to generate the tissues such as bone cartilage and so on.

And in the presence of adequate stimuli remember the stimulation that is, I have mentioned in the context of cell signaling. So in the context of cell signaling what I mentioned that you have a one cell type or let us say this is one cell so it gets different signal molecules or different signaling cues like A, B and C which is required only for survival. But if it wants to differentiate to another cell types now, if it wants to differentiate to another cell types now then what it needs, it needs some additional signaling, molecules of signaling cues that is D and E.

So that is what has been again reminded here, that in the presence of adequate stimuli, that means you need all these different stimuli so that it can differentiate to adiposite, Osteoblast and muscle cells. Remember that the signaling cues D and E, which helps in differentiation that

would be of different types where the MSC will differentiate to adipocytes whereas different types of signaling cues will be required for Osteoblast and that at the same time different type of using the same logic different signalling cues are needed for MSCs to differentiate to muscle cells.

That is reaching myotubes, so essentially muscle cells come they fuse together then they form a myotubes. And MSCs are indeed rare relatively rare in the bone marrow like one in ten into five mononuclear cells and detected in bone marrow and other places that is mentioned.

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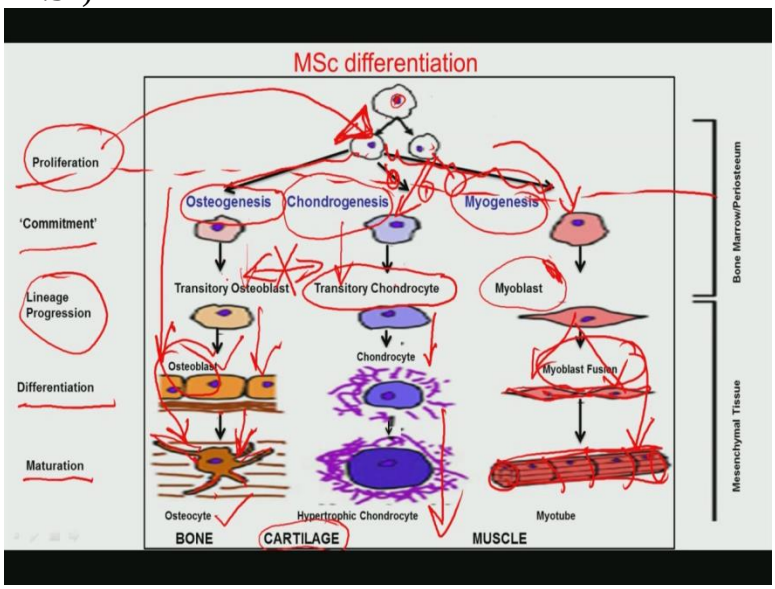

So let me take little bit more time to explain this slide so that you understand and perhaps you can remember this important slide. This slide is little crowded so therefore I need to spend little bit more time. What I m showing here that is class that is, I am trying to describe that is I am trying to describe that differentiation of Mesenchymal Stem Cells. So you have, this is your mother stem cells or parent stem cells.

So here I can draw another cells and let us consider this nucleus with blue like this mother stem cells. Now this is the three stem cells here and three daughter stem cells . So so this is the one stem cell this is the one daughter stem cells this is the second and then from two other then you can produce two more, so then you can produce two more . Now each of this stem cells type now can undergo differentiation along specific lineages.

So first one is that Osteogenesis, osteo means bone so essentially it will undergo differentiation along this lineages it will undergo differentiation to bone cells. Now this lineages in more simplistic terms I can define like each one of us come from a certain lineage, for example our father, grandfather, great grandfather so it comes from that lineage and then you carry a specific family name and so on because you come from that family tree.

So similarly once you try to understand how stem cells will differentiate, now each type in a specific lineage you can consider it as a grandfather or great grandfather or father and yourself, so essentially it comes through that particular lineage. So when it consider that Osteogenesis the first one it comes Transitory Osteoblast like it not yet matured Osteoblast but is it in a transition stage, then it forms that Osteoblast that is bone forming cells and finally that end member of this Osteogenesis is Ostocytes.

Now if you see morphologically Osteocytes is much more star shaped cells, whereas Osteoblast has its specific cell it is more flattened kind of shape. So morphologically Osteoblast and Osteocytes are different and you should be able to distinguish Osteoblast and Osteocyte morphology on a fluorescence microscope. Now along the same line if you want to understand the Chondrogenesis process that is again to make that cartilage cells, so essentially the first one is Transitory Chondrocytes then comes Chondrocytes then you go to the Hypertrophic Chondrocytes like you know it is growing now.

And so this Chondrocytes cells essentially makes the cartilage which is also shown here. Along the third one that is Myogenesis that is the muscle cells, it can form Myotube, it can form initially Myoblast and then subsequently Myotube and subsequently it form muscles. So in that process of the Myogenesis the first member you can imagine that is Myoblast, so that is a muscle cell now . When this muscle cells grow like one to two and two to four then two muscle cells they come together and they have a very characteristic spindle shape.

And you see that spindle shape so it has a very characteristic spindle shape and this spindle shape they can come they can fuse and they form a Myotube. So this Myotube has a larger link and also has a specific aspect ratio and that forms a smooth muscle cells. So this three along this three lineages I have mentioned I repeat like it is Osteogenesis, Chondrogenesis and Myogenesis.

Now let me discuss little bit more on this underline biological aspect, now first stage is, I I dot it I separate it out as a Proliferation. Proliferation is where your stemness is maintained. Like stem cells are now proliferating to large number of stem cell population with each of them having the capability to undergo differentiation along a specific lineage. Now what is commitment? Commitment means once a stem cell is committed to undergo differentiation along Osteogenesis pathway then it will follow that specific pathway.

It cannot suddenly go from transitory Osteoblast to transitory chrondocyte. So these kind of thing is it is not a reversible process so I have just marked a cross there. So once it is committed then it will ultimately form Osteoblast and Osteocytes if it is commited along this lineage so that is called commitment. Now Lineage Progression means that like along this particular lineage, now transitory Osteoblast will now differentiate to Osteoblast and to Osteocytes.

Now fourth one is the differentiation that means it has a differential gene expression, it has a different functionality, it is more matured or more specialized cell type than your initial stem cells. And last one is the Maturation. Maturation means now Osteoblast also is now matured to a different cell type is Ostoecytes, whereas Myoblast fusion which leads to the formation of the Myotube. So this this I think the entire thing I have kind of covered in this module which discuses, Differentiation of the stem cells.