Introduction to Biomaterials

Prof. Bikramjit Basu

Prof. Kantesh Balani.

Department of Materials and Metallurgical Engineering

Indian Institute of Technology, Kanpur.

Module No. # 01

Lecture No. # 36

Tissue Engineering and Wound Healing

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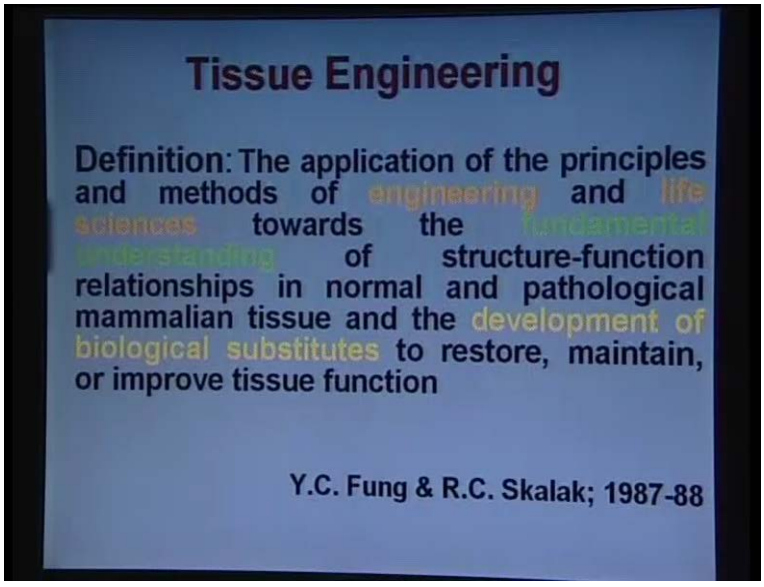

In today's lecture, I will be dealing with the tissue engineering aspect. And the first, this is like a text book type of definition, the tissue engineering the way it is defined: it is the application principles and methods of engineering and the sciences- so, engineering means it is mostly the biological engineering as well as little bit of the material science- biological engineering is already there in life sciences section, so, life sciences means it is a essentially pure biology, biological and engineering means most of that engineering disciplines like mechanical engineering, materials science, etcetera- towards the fundamental understanding of the structure, functions, relationship in normal and pathological mammalian tissue and the development of biological substitutes to be restored, maintained and improve tissue function.

Now, let me explain it little bit more. So, if you remember correctly, in the typical material science the fundamental concept is to understand the structure, property, relationship with different metals, ceramics and polymers, as well as composites. So, similarly, tissue engineering means you apply the fundamental concepts of engineering that can be either material science and engineering or mechanical engineering, or chemical engineering, and life sciences that means mostly biological approach. And why you are trying to, why you are trying to apply the principles of these two disciplines?

Your ultimate aim is to understand the structure, property relationship in normal, and, in normal and pathological mammalian tissues. So, basically you want to understand the structure, property relationship in these tissues and if required, you would with this understanding, you would be able to develop some tissue engineering replacement materials, or in otherwise biological substitutes to restore, maintain or improve tissue functions. So, whatever the tissues would ultimately ((certain purpose)) or certain biological functions, your biological substitute should be able to do those functions- so, that was the ultimate aim of the tissue engineering substitutes.

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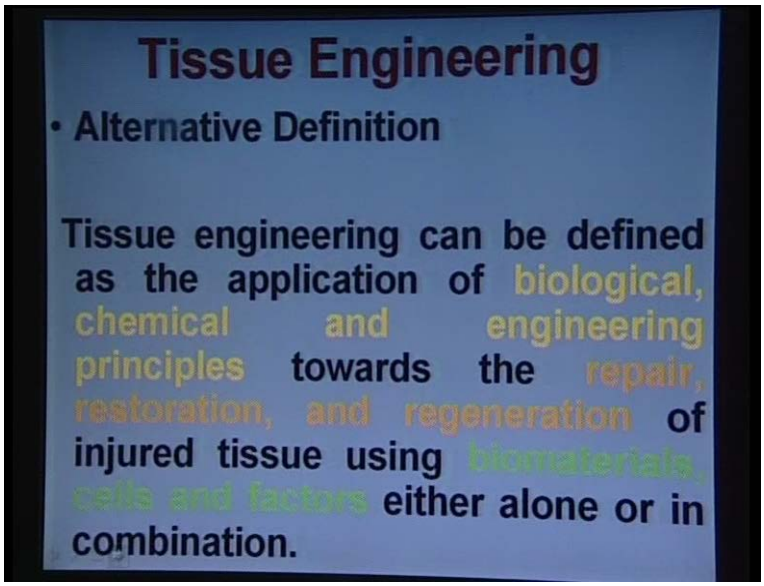

Now, alternative disciplines that they have proposed- the tissue engineering can be defined as the application of biological, chemical and engineering principles. So, as I said that engineering discipline means it also contains that material science, chemical engineering, etcetera, so, essentially here it has been defined as the application of

biological, chemical and engineering principles towards the repair, restoration and regeneration of improved tissue using biomaterials, cells and growth factors either alone or in combination. That means, these tissue engineering, these tissue engineering essentially has been defined as the application of the different engineering discipline principles towards the repair, restoration, repair, restoration and regeneration of the injured tissues. So, basically, we can repair the injured tissues as well as you restore the normal functions of those tissues in that implanted area.

Now, how you can do? You can use the biomaterials, you can combine use the biomaterials, cells and growth factor- so, these three things if you combine together, then you can actually, you would be able to restore and repair the normal tissue functions.

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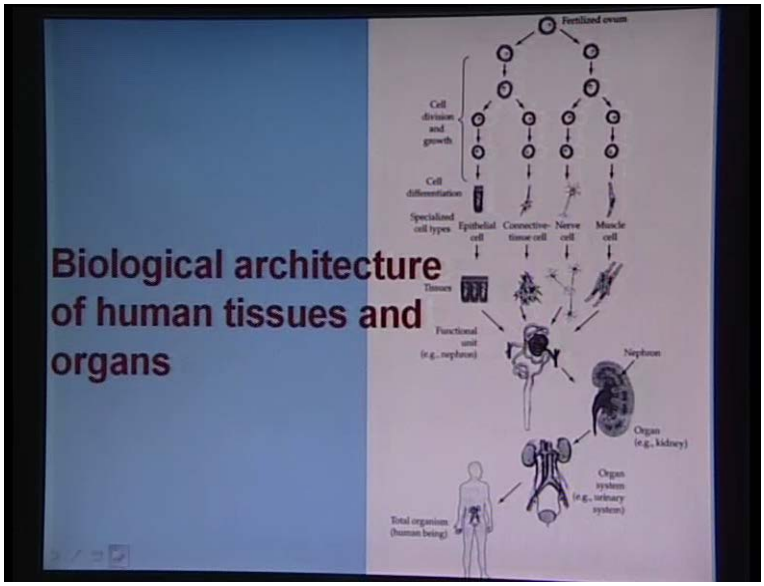

So, this is the biological architecture of human tissues and organs. So, if you see, it starts with the fertilized ovum then, in the cell division process and cell growth, you are actually replicating the cells. So, you start with the mother cell that is, fertilized to ovum then, it goes to two daughter cells, now, these two daughter cells in the cellular lineage then, it will be also differentiate, or it will be also divided into multiple cells.

Now, then, cell differentiation means, like, it involves the changes in gene expression, or it will be transformed from one type of cell to another type of cell. And if you follow this kind of lineage, then you will see there are four types of cells- one is the epithelial cell, another is connective tissue cells, third one is a nerve cell, forth one is the muscle cell-

and if you combine these four, then you can find that they will form a tissue and finally an organ and this organ, you are talking about that is, an organ system is an urinary system, or organs is the kidney, or nephron for example. So, what I am trying to say here, that cells are at the very base level, on that cells you form tissues, and self organized tissues that will form the organs. So, cells tissues organs- so, this is the hierarchical structure of the biological system.

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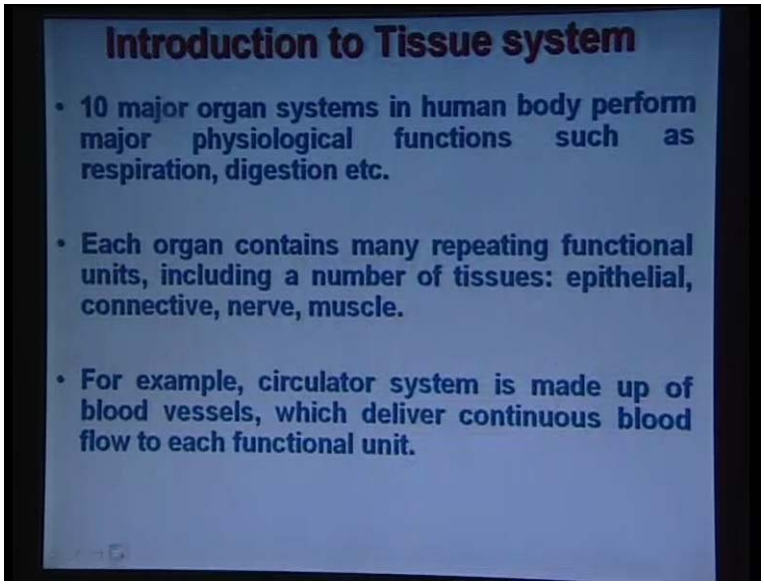

So, there are ten major organs systems in human body, they perform different major physiological functions such as, respiration, digestion, etcetera. And each organ contains many repeating functional units including a number of tissues like, epithelial, connective, nerve tissue, muscle tissue. And for example, circulation system is made up of blood vessels like, blood circulation system, which deliver continuous blood flow to each of the functional unit.

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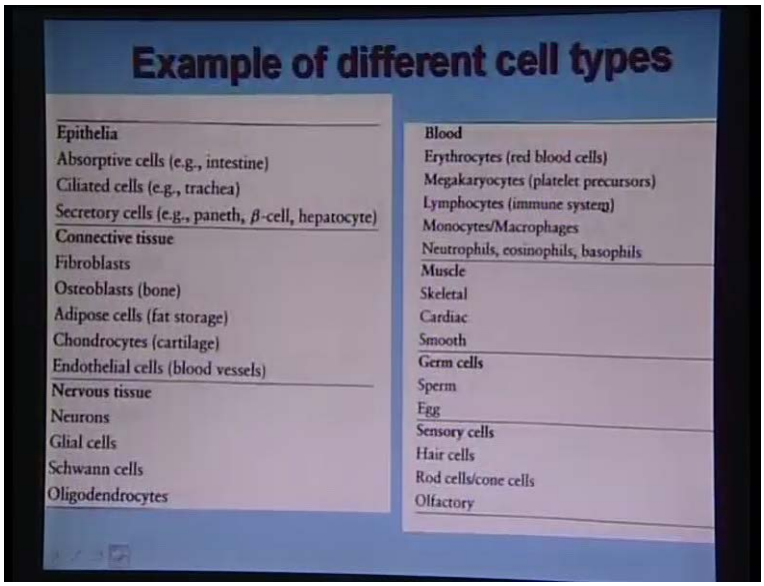

Now, here is that different cell types in different tissues like, epithelia. Epithelia has its absorptive type of tissue like, intestine then, secondary cells like paneth, beta cell, hepatocyte, etcetera. Connected tissue is the fibroblast, osteoblasts that is, bone tissue, adipose cells, chondrocytes that is, a cartilage tissue, endothelial cells that is, the blood vessels cells. Nervous tissue, they have different type of cells again, neurons, glial cells, schwann cells, etcetera. Blood, you have the RBC, you have this lymphocytes, immune system, you have the monocytes, macrophages, etcetera. Muscle cells, you have a skeletal,l cardiac cells, smooth cells. And then, you have different cells like sensory cells like, hair cells, rod cells, etcetera.

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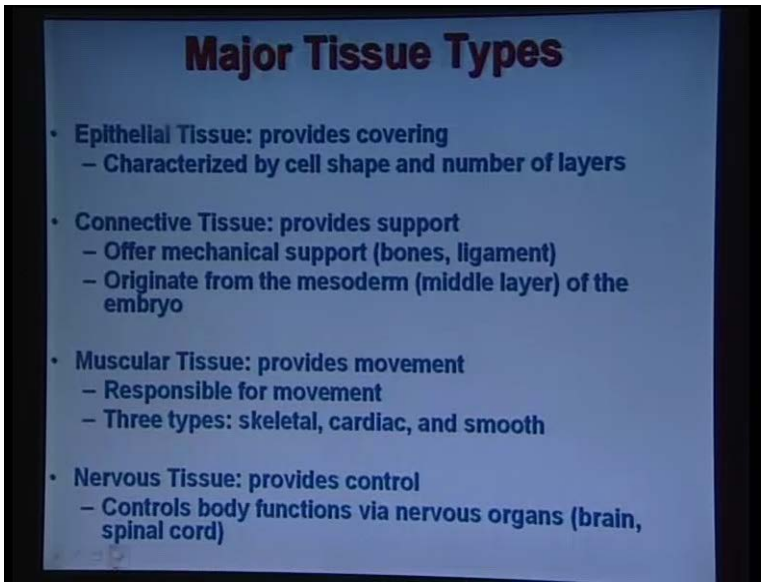

Now, different tissues, they perform different type of functions because as I have explained to you that in a typically tissue engineering means that essentially you are trying to understand the structure properties relationship of different tissues and thereafter, you will apply the concepts of engineering principles to develop biological substitutes so that it will perform its normal function, or restore its normal function, it is, of that damaged tissues.

Now, therefore, you have to understand that what are the different tissues, and these different tissues, what are the functions they perform. One is the epithelial tissue that provides covering like, characterized by the cell shape and the number of layers. You have the connective tissue that provides support. Now, this support is actually kind of different supports like, offer mechanical support like, bones, ligament. Now, if you remember correctly, bones and ligaments, they have very good mechanical properties and originate from the mesoderm middle layer of the embryo, so, these offer mechanicals, so, the connect tissues therefore, are important because they provide mechanical support. Muscular tissue, that provides movement like, responsible for movement like, walking. Like, muscular tissue is also important for, there are three types of muscular tissue, one is the skeletal tissue, one is the cardiac tissue, one is the smooth tissue. Then, you have called nervous tissue that controls most of the things like, it controls body functions by nervous organs like, brain, spinal cord, etcetera. So, if nervous tissues, they are injured, then that will lead to the major deterioration of the body

function as you understand like, a brain cannot work, if the spinal cord cannot work, then that will cause severe problem.

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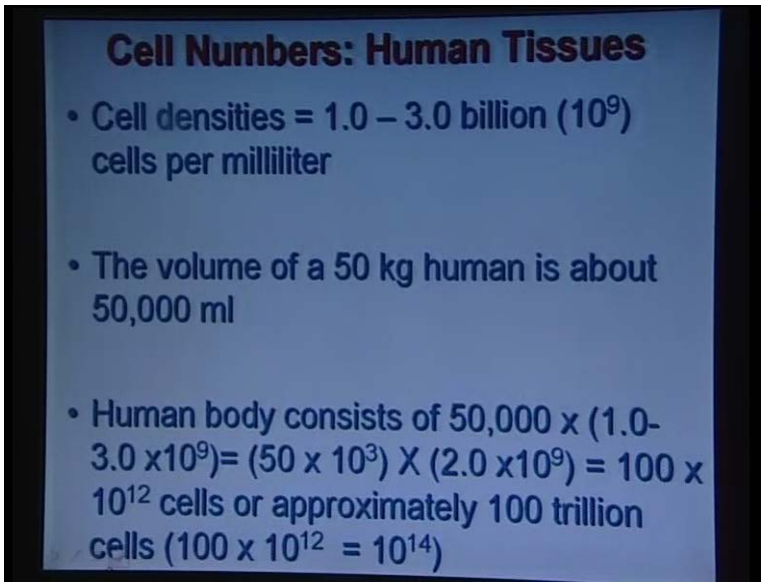

Now, these are some clinically relevant tissues by (( )) numbers. Now, typically, the cell density is per milliliter is type of 1 to 3 billion that is, 10 to the power of 9 order cells per milliliter. And if the volume, if you consider the 50 k g of human, that volume is kind of 50,000 milliliter, so, in the 50,000 milliliter how many cells that will be contained, that is why it has been saying that that human body consist of 50,000 multiplied by 1 to 3 billion cells and that is roughly around 10 to the power of 14 cells.

Now, more the body weight more is the number of cells. For a child when the body weights is let us say, 3 k g or 4 k g then you can see that it will be like let say, one order (( )) less, let us say 10 to the power 13 number of cells. Whether, if a persons whose weight is 100 k g, then you can see that at least two times higher than that of the 2 into 10 to the power of 14 type number of cells. So, 10 to the power 14 is the typical order of magnitude that is, the number of cells that are present in the body, and 10 to the power 14 number of cells, you can understand that that also not a single type, that is also different types of cells those are present inside the human body. So, it is, as I said earlier that it is not the magnitude, but it is the order of magnitude which is important, and what the student should remember that what is the order of magnitude that is, the number of cells that should be present in the human body.

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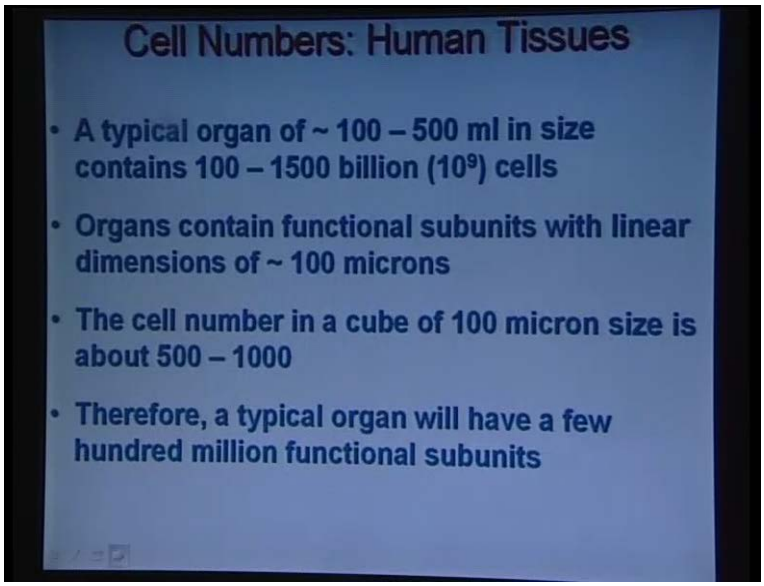

Now, a typical organ of 100 to 500 milliliter in size contains typically 100 to 1500 billion 10 to the power 9 cells. And organs contain functional subunits with linear dimension of typically 100 microns. And the cell number in a cube of 100 micron size would be about 500 to 1000. Therefore, a typical organ will have a few 100 million functional subunits. Now, these functional subunits mostly is related to the polymeric materials, or the monomer unit, that is what we are saying the functional subunits, and typically that 500 to 1000, that is the typical number that this is the cell number that is there in a cube of 100 micron in size. So, if you consider a typical volume element in the human body, which has a size of 100 micron size, so, this should have typical cell number 500 to 1000.

Now, if you consider typically a cell as a square like 10 by 10, so, 10 by 10 is 100 micron square, now, if you consider a cube of 100 micro meter size, now, typically, a cell has an irregular type of structures, so you cannot consider it as a cube, so, it is, mostly it is a spherical like, but it is not spherical, so, it is half spherical and typically the diameter of the cell is somewhere around 25 to 50 microns.

Now, if you consider little bit higher than the 2 cell size that is, a 100 microns cube size, there you can, 100 micron size, then you can consider that they are 500 to 1000 numbers of cells can be squeezed in- so, that is the cell density you can consider at a very finer scale.

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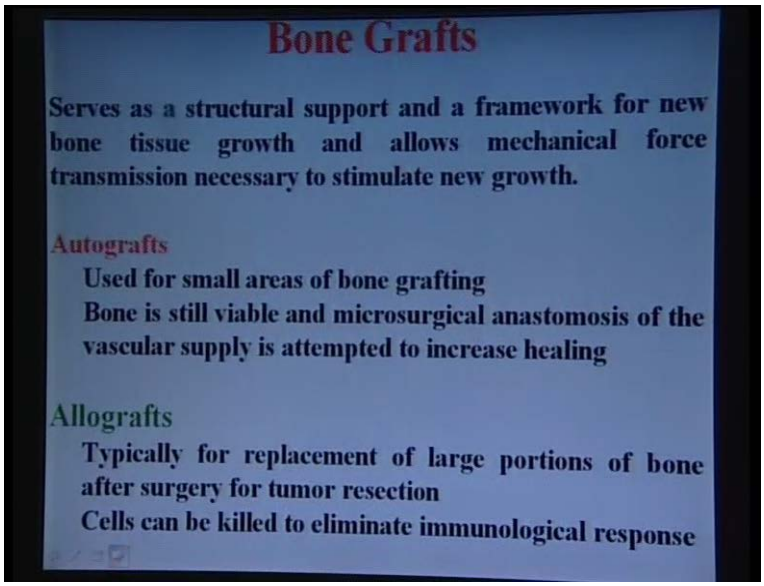

Now, coming to the bone grafts, now, these bone grafts, they serves as a structural support and a frame work for new bone tissue growth and allows mechanical force transmission necessary to stimulate new growth. So, bone graft essentially means because of the some surgery some part of the bone is damaged and it needs to be replaced. Now, bone grafting means you are essentially replacing the damaged part of the bone by some new part of the bone.

Now, autografts and allografts, these are two biological terms. Autografts means this is used for small areas of the bone grafting, and here that is, bone is still viable and microsurgical anastomosis of the vascular supply is attempted to increase healing. And allografts means typically for replacement of larger portion of the bone. So, if it is for the smaller portion, then it is called autografts, and if it is for larger portion, then it is called allografts.

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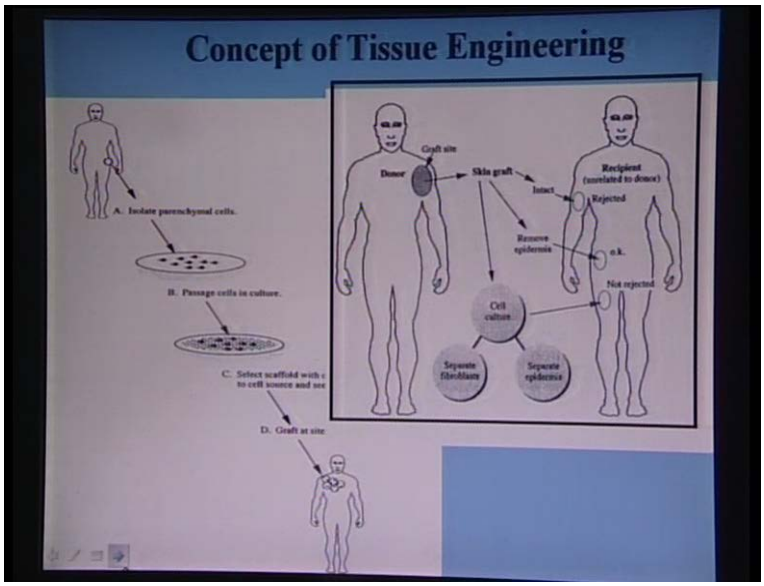

Now this is what is, how this tissue engineering concept is applied. For example, now, if you consider that is, a human being, you take one tissue in, you can isolate the parenchyma cells, then you can passage the cells in the culture. So, passaging, those who know about the, those who know about the biology, the passaging means like, you know, cells, in order to maintain the cells in a normal healthy conditions many times you have to drain out the medium and then you have to use the fresh medium, this fresh medium actually provides more nutrients to the living cells, and that will actually, that are actually used to maintain the living environment in the cells. Now, after the pass aging in the cells then, you select the scaffold of the cells source and seat them. So, essentially, then you have to find out, so, if you remember the definition of tissue engineering, whenever you want to develop some biological substitutes in a damaged tissue then you require a biomaterial, you require cells and you require growth factor, that is the three things.

Now, you have at the stage C, you have the cells then you require the biomaterial and this biomaterial is typically, it is a polymeric scaffold, so, you get this polymeric scaffold, you seat that cells on this polymeric scaffold and then, you can graft them at the sides that where it is required. So, you start with that cells from particular organ then, you go to this biomaterial and you seat them then, after seating you get the tissue engineering subtract where tissue is substituted then, you implant it.

Now, similar things you do it here, as you can see here that this is the donor, donor can be one human being, recipient can be another human being, and this recipient is that unrelated to the donor that means, they do not have any blood relation. Now, what you see here, that is a graft site, so, from that, you know, let us say, you take some skin out of this graft, so, skin graft then, you do some cell culture and then, after the cell culture you can separate the fibroblast and you can separate epidermis and then, you can put the cell culture and then, the biomaterial scaffold then, if you do it, after this scaffold, then you can put it in one place. Now it is not rejected that means, then you can keep this at that place, but then, what has been shown in this slide particularly, that you have this skin graft and then, if you use the skin graft intact that means, without culturing and without seating on the scaffold then, you can put it on the particular side and there is a chance that it can be rejected. So, what is saying that you can take this graft from one donor then, you have to extract the cells like, the extract the cells means, tissue means what, tissue is nothing, tissue is nothing but a self aggregate, or that self organized aggregates of the cells, so, you can always extract the cells from the tissue then, tissue you have to culture them, you have to process them like, this kind of, follow this kind of procedure, after you culture them you can separate fibroblast and you can separate epidermidis.

Now, this fresh cell lines, you can put it on the biomaterials scaffold then, you can put it in the graft materials as a another recipient, but the point is that if you do not take out this cells from this graft material, then there are chances that it can be rejected by the recipient. So, if it is rejected, that will cause lot of (( )) reaction to this substance.

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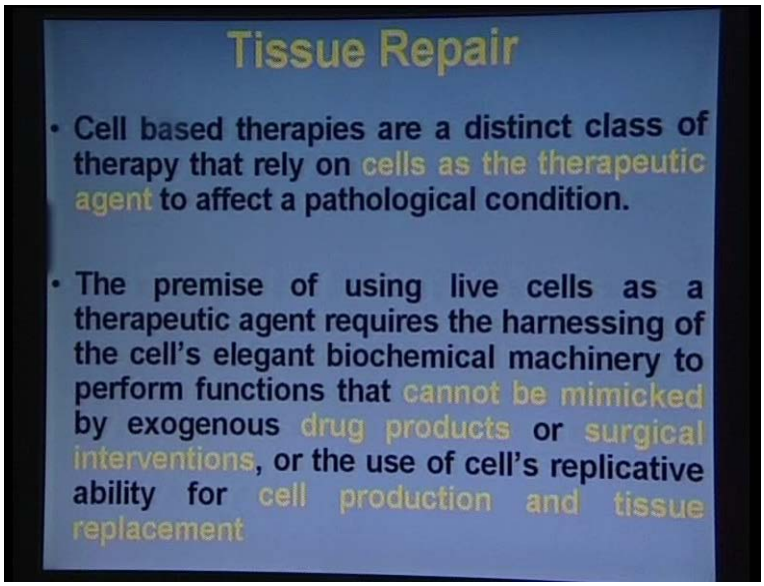

So, tissue repair, that has been defined as, here as the cell based therapies are a distinct class of therapy that rely on cells as the therapeutic agent to affect a pathological condition. What it means it? It means that entire therapy like, whatever you have seen like, you know, the constructing the biological substitutes that is based on living cells. So, essentially you take a tissue from a donor, you extract the different cell lines, whatever you require like, depending on fibroblast cells and other type of cells and then, use this cells as a therapeutic agent to affect a pathological conditions. So, that was the overall idea.

And the premise of using living live cells as a therapeutic agent requires the harnessing of the cell's elegant biochemical machinery to perform functions that cannot be mimicked by exogenous drug products or surgically interventions. So, what has been said here that the premise of using live cells as a therapeutic agent. So, this cells here, it is used as a therapeutic agent in this tissue repair, or the tissue engineering, and this requires the harnessing of the cell's elegant biochemical machinery like, every cell has a particular biochemical machinery for examples, the mitochondria that is called, known as the energy power house and then, you have this ribosome's in the cells, which are used for the protein synthesis then, you have the different, other Golgi apparatus and other things, so, they perform different kind of biochemical function. So, all this, cytoskeleton acting fibers, they provide the mechanical support to the cells. So, those kinds of things,

this biochemical machinery needs to perform its own functions and it cannot be mimicked.

That means, these live cells, biochemical machineries, it cannot be replaced, or it cannot be mimicked by exogenous drug product for example, if a patient requests skin grafts, you cannot, you cannot give the patient some kind of drugs, which will, which will replace the necessity of having that tissue engineering constructs. So, essentially, it means that this requirement of having the skin grafts cannot be made by the, by having some drugs, or by having some medicines for example, or surgical interventions and the use of cells explicative ability for cell production and tissue replacement. That means, what is the next sentence that has been said? Like, each cell, they have a, each living cell, they have a DNA and RNA in the nucleus and they have the unique ability to replicate, if you remember the fundamental definition of cell has been said that it is a self content unit, which is capable of replicating itself provided it is given the sufficient nutrients and the environment.

Nutrients means, cells like a human being has to be fed some nutrients and environment means, like, it has to be, it has to be kept always at certain p H level, certain C O 2 level and certain temperature, if you increase or decrease these things, then healthy state of the cells that cannot be ensured. Similarly, here what has been saying that, why that question that comes that why do we need to use the living cells in the tissue engineering construct, why cannot we, why cannot we replace these tissue engineering constructs with the help of some innovative drugs? The answer is, you cannot replace the need for the tissue engineering constructs with the, with some external agents like, drugs or some surgical intervenes because one has to remember the cells have the unique biochemical machineries like, you know, different cellular organelles they perform different things.

And accordingly, those functions cannot be served by some other drugs, that is number one. Number two is that, cell has a distinct replicative ability, replicative ability means, cells can replicate himself like, one mother cell can give rise to daughter cells and each daughter cells can undergo again cell division, mitosis, cytokinesis process then, they can again undergo replication process, that unique replicative capability cannot be made, or cannot be provided by any other therapeutic agent, that is why that living cells are used as a good therapeutic agent in this particular case.

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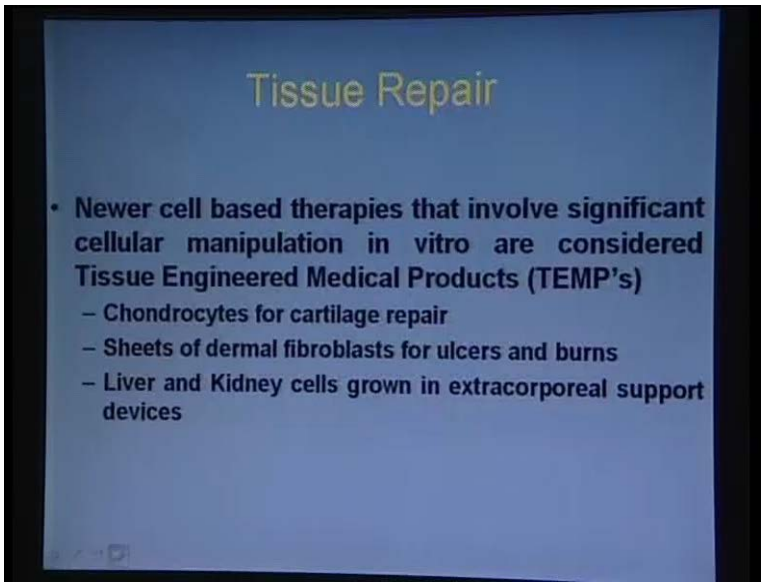

Tissue repair, they have said that newer cell based therapies that involve significant cellular manipulations in vitro are considered tissue engineered medical products. For example, chondrocytes for cartilage repair. So, many times due to etching and so on there is cartilage repair is required and chondrocytes is the cells for the cartilage tissues.

Then, sheets of dermal fibroblasts for the ulcers and burns, and liver and kidney cells grown in extracorporeal support devices. So, these are like three different types of examples that are given.

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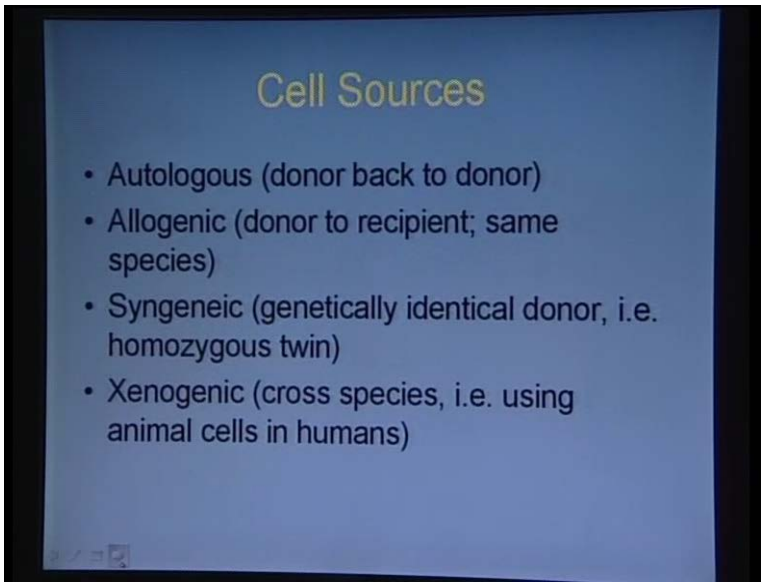

Now, what are the cell sources? Now, the cell sources, the first one is autologous, autologous means donor back to donor means, you are taking from the donor, you are not using that cells for another recipients, but you are taking the donor cells and they are putting it back for donor cells. For example, suppose a person requires some skin graft at the let us say, mouth part, so, what surgeon can do, they can take out some skin from the, some tissues from the thigh of the leg, they can culture it and they can use it back in the same person, so, then it is called autologous cells that means, same person to same person.

Allogenic means donor to recipient, same species. Same species means human to human, human belongs to that, if you know biology, human, the species is Homo sapiens, Homo sapiens is the species.

Then, syngeneic means genetically identical donor like, homozygous twin. So, syngeneic means genetically identical donor. And xenogenic means that cross species like, using animal cells in the human. For example, you take some cells from the mouse or rat and to try to put it in the human being. So, that is like from one animal to another animal, but then it is called xenogenic. So, these are like biological terms like, autologous, allogenic syngeneic and xenogenic.

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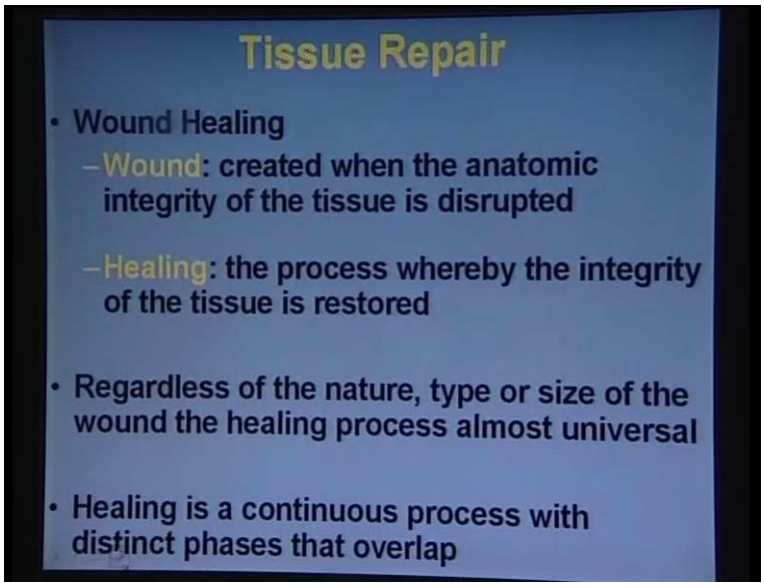

Now, wound healing, that wound, what is the definition? That is created when the anatomic integrity of the tissue is disrupted. So, that is the fundamental definition of

wound and that it said that wound is created at any place in the human body when the anatomic integrity of the tissue is disrupted like, if you suppose you are falling from a cycle, or you are falling from a motorbike, then what will happen? You get wound at some legs, or some knee part, or something, so, locally at that part of the knee, or legs, your anatomic integrity of the local tissues are disrupted. And healing means the process whereby the integrity of the tissue is restored like, then, you put some medicine there, or if it is very severe accident, then you are actually entire tissues need to be replaced by some skin graft materials then, it is, the process is called a healing process.

Now, regardless of the nature, type and size of the wound, the healing process almost universal, and healing is the continuous process with distinct phases that overlap. So, what it says that that it does not matter that where you have this wound, either at hand or leg, it also does not matter, what is the size of the wound, is it very large wound, or is it very small wound, the process of the healing is universal. So, this process of the healing will always be followed, it does not matter the place of wound, it does not matter the size of the wound, so, this is number one point.

Number two point is that this is almost a continuous process. So, healing let us say, has four stages and these four stages can overlap let say, stage A, stage B, stage C, stage D. Now, stage A, can overlap with stage B, stage B can overlap with stage C, stage C can overlap with stage D, but this A, B, C, d they will follow in a continuous manner, it is not like after A suddenly C will start, it is not like after B suddenly D will start, after A B will start, but this A and B can overlap, after B C must start then, B and C can overlap, after C D will start, but C and D can overlap. So, that is why it is said that healing process is universal irrespective of the nature, type and size of the wound and it is a continuous process.

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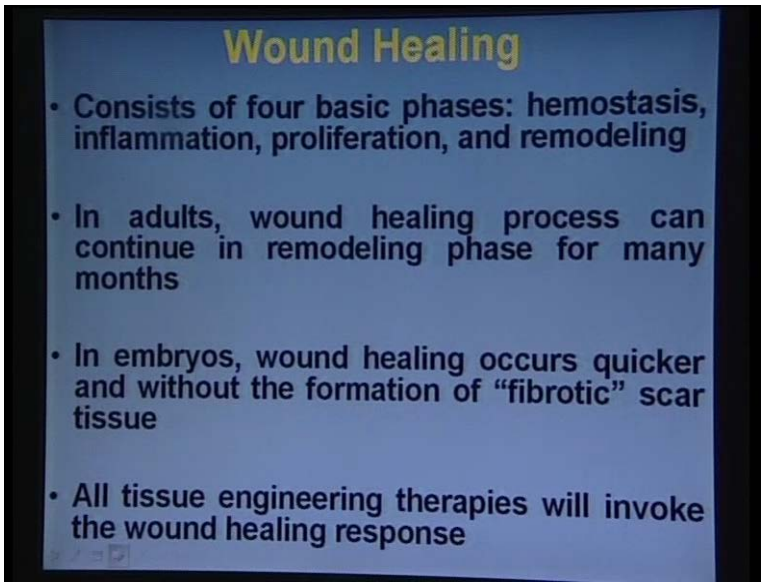

Now, what is the wound healing? Wound healing is that it consist of four basic phases, what I was mentioning in the slide, that is that healing process can contain that A, B, C, D, four phases. Now, these four phases, they are hemostasis, inflammation, proliferation and remodeling. Hemostasis is the first phase, it is followed by inflammation, that is the second stage then, it follows by proliferation, proliferation means you can understand that it is part of the cell-fate process, so, cell proliferation and then, remodeling means tissue remodeling, remodeling means earlier the tissue was in damaged state and now the tissue is coming to a normal functional state, so, that is the process is more or less that can be described as a tissue remodeling.

In adults wound healing process can continue in the remodeling phase for many months. So, basically what is saying that many times depending on the age of the patient the tissue remodeling phase can takes place for more than one months, in embryos wound healing occurs quicker and without the formation of the fibrotic scar tissues, in embryos the wound healing process can take place very fast and in all tissue engineering therapies will invoke the wound healing response. Basically, all tissue engineering therapies what it means like, therapies means you are again producing the biological substitute to restore the normal healthy function of tissues. So, this, all tissue engineering therapies will essentially invoke the wound healing response. So, they will always involve some wound healing response like, hemostasis, inflammation, proliferation and remodeling, these four

stages, they must undergo all the tissue engineering constructs. Now, let us go through these stages each one by one.

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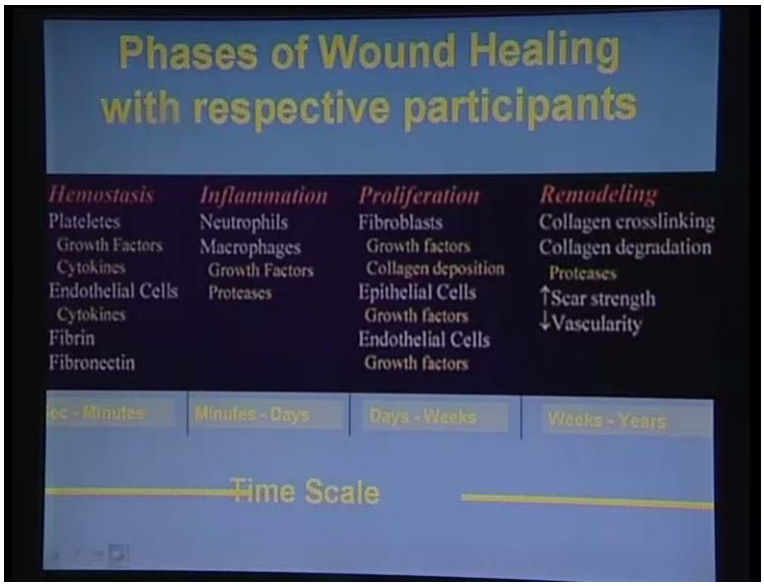

So, the first one is the hemostasis. So, here the platelets, platelets means, blood platelets, they take part in fibrin and fibronectin, these are like proteins, so, fibronectin is a protein; endothelial cells, they also take part and cytokines, and then platelets cells growth factors and cytokines then, that takes place from seconds to minutes.

So, initially, you have a wound, within few minutes this hemostasis part takes place, phase takes place then, comes the inflammation, inflammation means here the neutrophils, macrophages and then growth factors, proteases, they also take part, and that takes part in the case of months to days, so, for few days for example. Then, proliferation, now, proliferation is the place where the fibroblast cells, that is connective tissue cells and then, collagen deposition also take place- so, fibroblast is the very early type of cells which comes in contact during this wound healing process. Epithelial cells, and endothelial cells along with growth factors, they take part then, these will be few weeks, so, from days to weeks.

Now, if you see the process what it says that seconds to minutes means it is quicker, kinetically faster, so hemostasis process is the very fast process, immediately within the wound, that is the fast stage of healing that is, hemostasis. Then, inflammation that little bit slower than the hemostasis process, so, slower in a sense that it takes place still some

days. Proliferation it takes place from days to weeks, here the fibroblast cells that is, connective tissue cells, epithelial cells and endothelial cells, they take part. And remodeling, you can see the last stage that is, the collagen cross linking, collagen cross linking means collagen is also, if you remember the structure, the triple helix type of structure, collagen, and cross linking means you have the one collagen molecule, or collagen fibers, and there is another collagen fibers, if some sulphur molecules, sulphur is atom is there, then that will form a kind of cross linking between one collagen to another collagen, but there may be some other cross linking agents, that is possibly used. Then, collagen degradation and shear strength and vascularity, vascularity means there are lot of network formation through with the blood channels and other things they forms, and that takes place weeks to years.

So, kinetically the slowest step is the tissue remodeling, kinetically fastest step is the hemostasis process- is it clear? And then, this is the time scale, what is the other thing that you notice that I have mentioned earlier that hemostasis and inflammation that can overlap similarly, inflammation and proliferation, that can overlap, and proliferation and remodeling, that can overlap, but this is the sequence of event that take place- so, this is number one, this is the number two stage, this is the number three stage and this is the number four stage. So, this is the different stages that are present in the wound healing process. And you must remember then all these stages of this wound healing process they take place in a continuous manner, that is number one, number two is that all these stages take must take place irrespective of the size or type or place of the wound.

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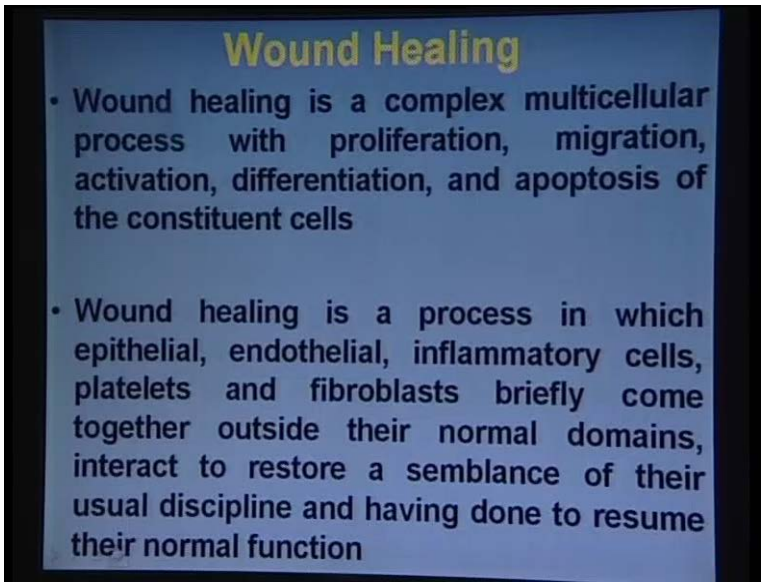

So, this is a definition of the wound healing, what it mentions here that the wound healing is a complex multicellular process with proliferation, migration, activation differentiation and apoptosis- with proliferation, migration, activation, differentiation apoptosis of the constituent cells. So, what has been said here that wound healing is essentially a complex multicellular process, multicellular means as we have seen here blood platelets that are also one type of cells, fibroblast cells, endothelial cells, epithelial cells, all these different type of cells they participate.

Then, it also involves the proliferation, cell proliferation; migration means cells can walk, cells can crawl; this one cell differentiation process also takes; apoptosis means programmed cell death, so, that is also takes place. And then, wound healing is the processes in which epithelial, endothelial, inflammatory cells, platelets and fibroblasts briefly come together outside the normal domain, interact to restore a semblance of their usual discipline and having done to resume their normal function. So, this is also another unique definition. What has been mentioned here that it has been mentioned the different type of cells, they come briefly, briefly means for a shorter time period, they interact with each other and then, they will start, all these groups of different type of cells, they will start performing their own functions for example, connective tissue, their function is to give support, connective tissue cell lines like, fibroblast cells like, epithelial cells, their function is to cover, cover means to cover the entire wound site from the outside environment. So, similarly, muscle cells, that is smooth cells, that also take part. So, all

these cells that come together outside the normal domains, they interact to restore a semblance of their usual discipline, discipline means they perform their own duty, and having done to resume the normal function. So, this brief interaction essentially leads to the formation of a biological tissue there locally which can perform its normal function.

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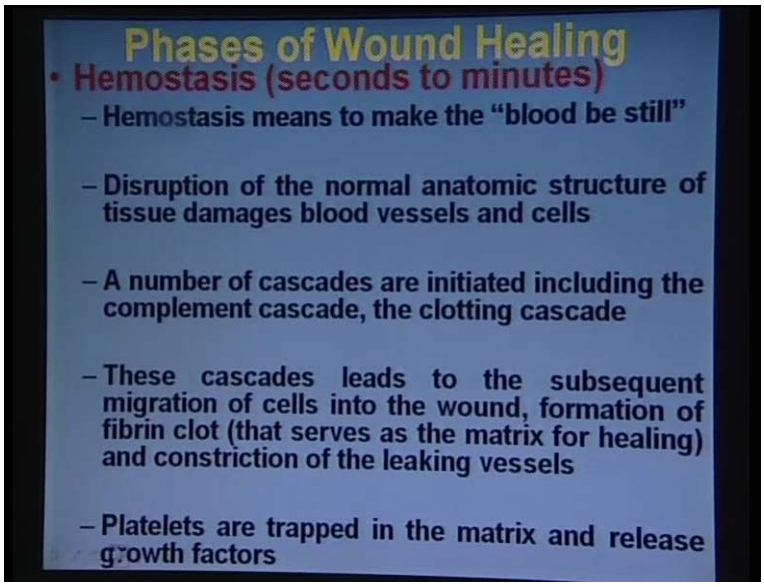

Hemostasis that is, seconds to minutes, so, that is the first stage here. Hemostasis means to make the blood be still, and disruption of the normal anatomic structure of a tissue damages blood vessels and cells. So, if you see that whenever you have the wound, immediately it starts wound bleeding, bleeding means like, it starts bleeds, it bleeds like, lot of bloods that will come out. Why? Because blood is flowing through a- what?- arteries, now, whenever you have a wound all the arteries, they are disrupted, they are are fractured, so, bloods will automatically come out, it will come it will automatically come out and spill.

And because anatomical structure of the tissue damages in number of cascades are initiated including the complement cascade, the clotting cascade, and these cascades leads to the subsequent migration of the cells into the wound formation of fibrin clot and constriction of the leakage vessels. And platelets are trapped in the matrix and release growth factors. This means that there are different cascades which are formed and this, through this cascades that migration of the different type of cells for example, in the wound place is now exposed to the normal environment, now, in the wound area you do

not have initially fibroblast cells, endothelial cells, epithelial cells, so, those cells have to be migrated to the wound site, so, it has, if it has to be migrated, then it has to migrate to some channels, so, these channels are formed. So, biologically this channels are formed and then, through that will enable the transport of the different type of cells to the wound site. And the second thing, that whenever the arteries, blood arteries, they are fractured some constriction takes place, constriction means at the damage side or the fracture side some constriction so that this bleeding is stopped and the blood flow, blood, coming out of the blood that incident is avoided.

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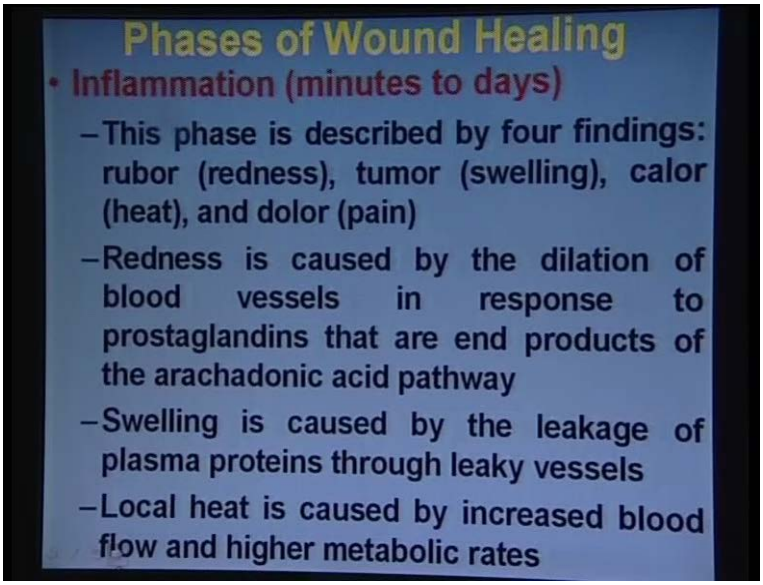

Now, inflammation, this phase is described by four findings that is, redness, tumor is swelling, heat and dolor is pain. So, patient here actually feels the pain because that is inflammation. And redness is caused for the dilation of blood vessels normally, you see that at the point of the wound that there is a dilation of blood vessels in response to fact that are end products of the some particular acid pathway is blocked. And swelling is caused by the leakage of plasma proteins through leaky vessels. And local heat is caused by increased blood flow and higher metabolic rates. So, increase blood flow actually can cause to local heat. So, locally at the wound area, there it will, you will feel little bit hot compared to other parts of the body.