Tissue Engineering
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Lecture - 33
Bone Tissue Engineering - Part 2

Good afternoon everyone, I welcome you all for my second session of Bone Tissue Engineering.

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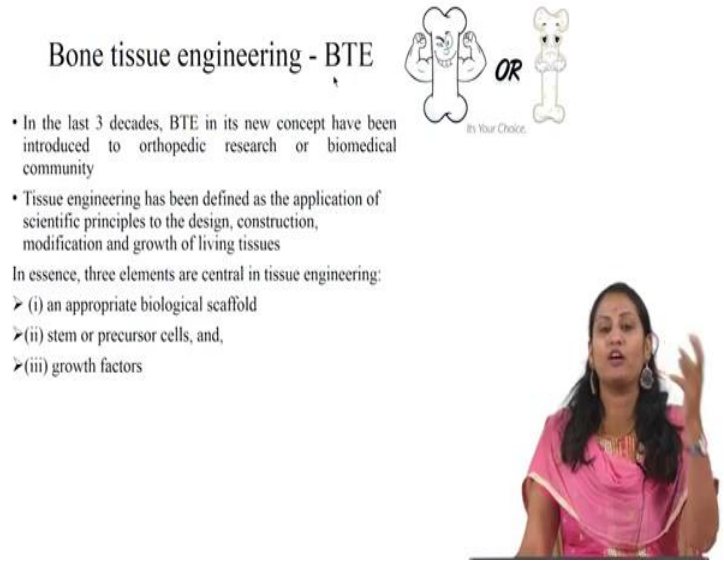

In our first session, we discussed about the function of bone, a brief introduction about what is bone and its function. And, we studied its anatomy and physiology in detail and also the formation of bone as well. Today, in this session, we will be discussing about bone tissue engineering strategies and what are the key components present in tissue engineering application.

In the last 3 decades, bone tissue engineering has been a very new concept and a useful concept for orthopedic surgeons as well as the biomedical community, in order to overcome the limitations for the enhancement and regeneration of bone defects.

To start with, we should know what is tissue engineering. I think by this time, you all would know what is the definition of tissue engineering, which is nothing but where it encompasses the knowledge of life sciences and engineering together for the

development of a biomaterial in order to replace, restore or regenerate a diseased tissue or organ. In general, it has been defined as the application of scientific principles to the design, construction, modification, and growth of living tissues.

There are three major elements present in tissue engineering, which composes of the tissue engineering triad. The first element is biological scaffold, which is biomaterial, and the second one is cells, and the third one is growth factors. So, scaffold, cells, and growth factors altogether make a tissue engineering triad. This is just a brief introduction about tissue engineering and tissue engineering triad.

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What is the need for bone tissue engineering, I think we have discussed that in our first session as well. To overcome the limitations, which we are facing in current clinical operations. Like in grafting procedures, autograft, where we graft tissues from the same individual, but due to limited availability, donor site morbidity. The second option is allograft, where it has the chances of immune rejection and pathogen transfer. So, in order to overcome all these limitations, we need a bone graft substitute to enhance bone regeneration, or to repair the bone defect.

The field of bone tissue engineering focuses on the alternative option that will completely eliminate the above-said limitations, which are facing in the current bone grafting procedures. As I said earlier, over 2 million surgical procedures have been

performed every year; bone grafting procedure, where the bone is considered to be the second most transplanted tissue after blood.

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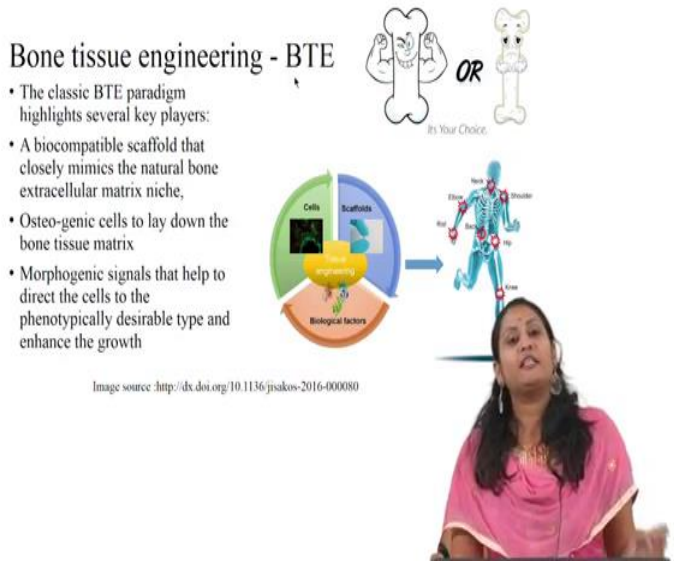

This diagram explains the classic bone tissue engineering paradigm, which highlights several key players. The key players are the three major components of the tissue engineering triad. The first one is the biocompatible scaffold. The scaffolds are the 3D structure, which mimics the extracellular matrix. And, the osteogenic cells to lay down the bone tissue matrix.

When I was explaining about the types of cells present in the bone; we have four different types of cells, osteoblast cells, osteocytes, osteogenic cells, and osteoclast cells. The osteoblast is responsible for the formation of bone, osteoclast is responsible for bone resorption, and osteogenic cells are the only bone cells that can divide, differentiate into osteoblast cells. So, we need osteogenic cells to lay down the bone tissue matrix, the second essential component.

The third one is the growth factors, which are the morphogenetic signals, that help to direct the cells to the phenotypically desirable type and also for the enhancement of the growth. The growth factors are the third major component of bone tissue engineering. So, scaffolds, cells, and biological factors, all three together combine to form the bone tissue engineering triad.

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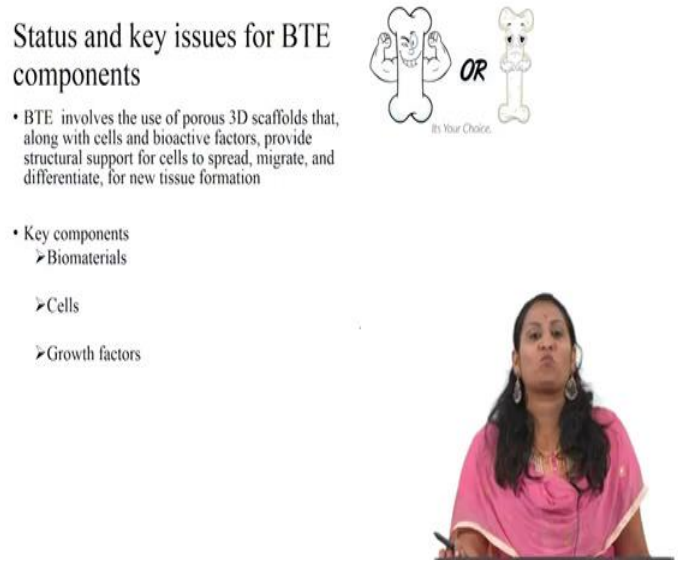

In this session, we will be dealing in detail about each key component of bone tissue engineering. Bone tissue engineering involves the use of porous 3D scaffolds. The 3D scaffolds, as I said, mimics the extracellular matrix. In general, it acts as the structural template for the cell to enter, adhere, proliferate, and differentiate, and thereby it enhances the formation of new tissue, and it has to degrade. So, it should be degradable, and it is the temporary implant; it cannot be assigned to a permanent implant.

It involves the uses of porous 3D scaffolds that along with cells and bioactive factors, can provide support for cells to spread, migrate, differentiate for new tissue formation; So, the three components are biomaterials, cells, and growth factors. We will be seeing the strategies for biomaterials, cells-based approaches, and growth factor-based approaches in the later presentation.

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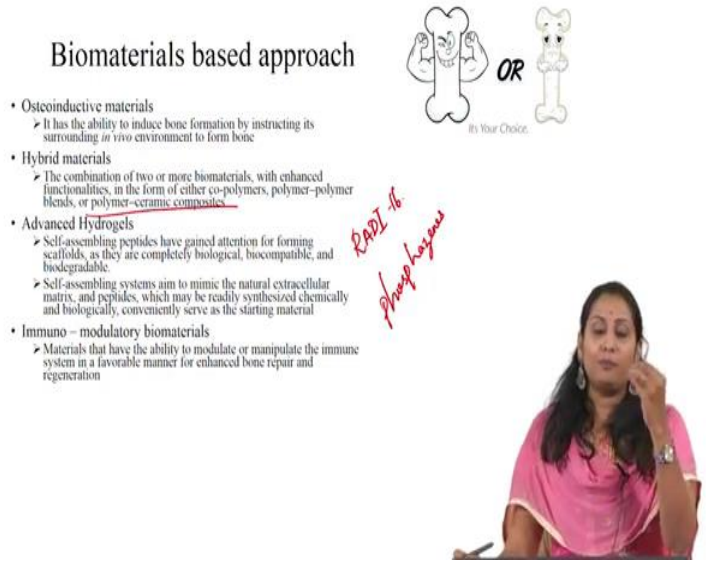

To start with the biomaterial-based approach, we should know what a biomaterial is. What is a biomaterial? A biomaterial is a natural or synthetic substance that has been engineered to construct to perform a biological function with a medical purpose. A medical purpose can be either therapeutic or diagnostic.

Evolution of biomaterials. In the 1970s, first-generation biomaterials have been developed. It was developed in an idea, that it has to mimic the physically damage tissue because of fracture or disease or any other traumas. So, they are just bioinert materials, and they do not interact with the biology of the host organism. So, first-generation materials are called as bioinert materials for example, stainless steel and its alloys come under bioinert materials.

Then, they switch the gears from the passive materials to the active materials; the second-generation materials are called bioactive materials. Here, it can interact with the host organism biology, where there will be an interaction between the graft as well as the cellular level interaction between the host organisms.

In the 2000s, the third generation of biomaterials has been developed. These have the bioresorbable properties; where it helps in the formation of new tissue. It combines the properties of bioactive as well as the bioresorbable, where it interacts with the cellular level in the host organism, as well as it gives a specific response. This helps in the

development of new tissue formation. In 2020, they develop biomimetic materials, where they develop a material that mimics the natural material.

In this biomaterials-based approach, we will be focused mainly on third-generation materials for example, polymer and the composites. Again, they are classified into osteoinductive materials, hybrid materials, advanced hydrogels, immunomodulatory materials.

The first category is osteoinductive material. The word osteoinduction means it instructs the surroundings stem cells for the formation of bone. If I say this is an osteoinductive material, that means that material has the ability to induce bone formation by instructing the surrounding cells in vivo environment. For example, hydroxyapatite, calcium phosphate, or ceramic-based materials prone to have osteoinductive property.

The second category of biomaterials is hybrid materials. They are the combination of two or more biomaterials with enhanced functionalities either in the form of copolymers or polymer-polymer blend, or polymer ceramic composites. Copolymers are nothing but it is a substance where it developed from two or more monomeric species. For example, PLGA, where it is developed from the monomeric units of polylactide and polyglycolide.

So, we can tune the properties of both the monomers, where PLA has the glass transition temperature above room temperature, and it has a very long degradation rate. Whereas PGA polyglycolide has a glass transition temperature below room temperature, and it has a shorter degradation rate. So, we can combine and tune the properties of PLA and PGA, which are FDA approved polymers. And, we can develop polymer which is a copolymer, hybrid material, PLGA with tailored property in the end.

Polymer-polymer blends. Again, the same example I will tell you about PLGA, the degradation product of PLGA will be acidic in nature. So, prolong exposure of tissue to the acidic product will lead to tissue necrosis and, eventually, will lead to failure of implants. What researchers will do is, they combine with the polymer; for example, phosphazenes, where the degradation product of phosphazenes are in neutral pH. So, they combine PLGA as well as phosphazenes to develop a biomaterial to give a non-toxic degradation product. The final one is polymer ceramic composites, which are very useful in bone tissue engineering, and this we can call it as biomimetic scaffolds. It has all the desirable properties for bone tissue engineering.

The third class of biomaterial is advanced hydrogels. Hydrogels because of its physical properties and its structure it has been widely used in tissue engineering application. Recent research has shown that self-assembling peptides have gained enormous attention forming scaffolds, as they are completely biological, biocompatible, and biodegradable. For example, RADI-16 is a self-assembling peptide where it aims to mimic the natural extracellular matrix, and it can be readily synthesized. It is injected in the form of nanofibers, once it enters the physiological fluids, and it becomes the gel, and it serves as the template for the starting material.

The final one is immunomodulatory biomaterial. In order to suppress the immune reaction, we can develop a biomaterial that can modulate or manipulate the immune system in a favorable manner for the enhancement of bone regeneration. So, these are about the biomaterials-based approach, where I will focus mainly on scaffolds.

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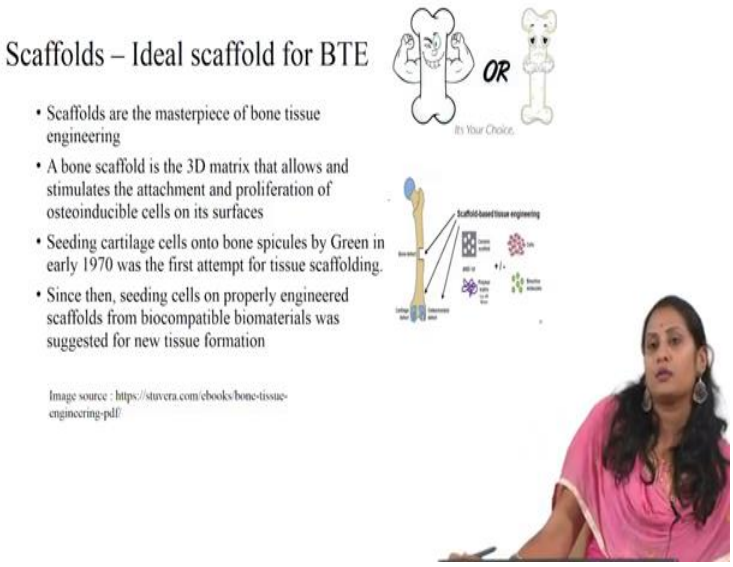

The scaffolds. Scaffolds are the masterpiece of bone tissue engineering. A bone scaffold is a 3D matrix that allows and stimulates the attachment, proliferation of osteoinducible cells on its surfaces. The first one was developed by Green in early 1970, where they seeded cartilage cells into the scaffold; that is how it has developed seeding cells on scaffolds. The image shows there is a long bone where there is a defect, and we have to places the scaffold in the defect and check for the enhancement or regeneration of the long bone.

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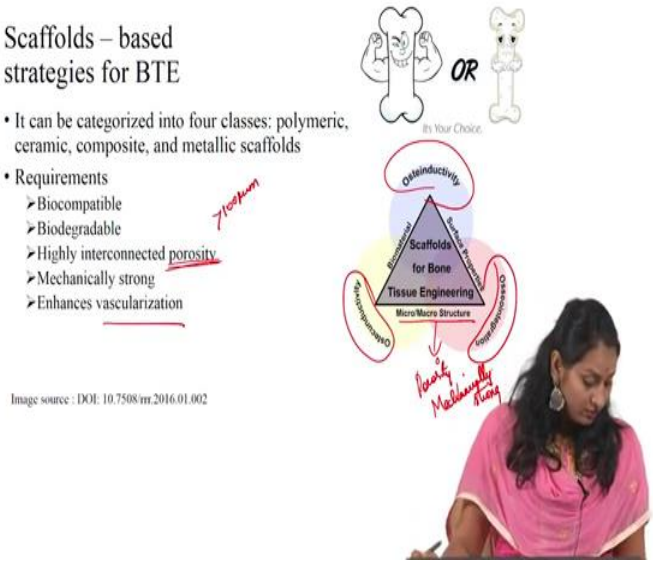

Since the scaffold is a masterpiece of bone tissue engineering, we should be more careful in selecting scaffold and its properties, the materials, and everything for the preparation of scaffold. The scaffolds can be categorized into four classes; polymeric, ceramic, composite, or metallic scaffolds. Polymerics scaffolds can be derived from either natural polymers or synthetic polymers. Composites may be the combination of polymers and ceramics.

The requirement of the ideals scaffold. Any ideals scaffold for bone tissue engineering, it should be biocompatible. It should be biodegradable, and it should not be a permanent implant; it should be a temporary implant. And, It should be non-toxic and have highly interconnected porosity. This porosity plays a very important role in bone tissue engineering. The pore size is very important for the cells to enter and proliferate, differentiate, and also for the diffusion in and out of the nutrients and wastage.

The pore diameter or pore size should be greater than 100 micrometers, and it should be mechanically strong. Since we are aiming for bone tissue engineering, the scaffold should be ideally strong, and it should mimic the natural bone. If, in a case of cortical bone tissue engineering, it should have mechanical properties similar to cortical bone. Whereas, the spongy bone or the cancellous bone, it should mimic the properties of this spongy bone. Also, it should enhance vascularization. Vascularization is the formation of new blood vessels.

So, any ideal scaffold for bone tissue engineering, it should be biocompatible, it should not be toxic, it should be biodegradable, it should have highly interconnected porosity, it should be mechanically strong, and it should enhance vascularization.

The osseointegration which is nothing but the biocompatibility, it should integrate well with the host tissue. And, osteoconductivity, where it helps in the formation of new bone on the surface of the graft. And osteoinductivity, it should direct the surrounding cells for the formation of bone. So, these are the main properties of the ideal scaffold. And, micro and macro structures are nothing but the porosity structures, mechanical properties, it should be mechanically strong. So, these are the main properties of a scaffold to be used in bone tissue engineering.

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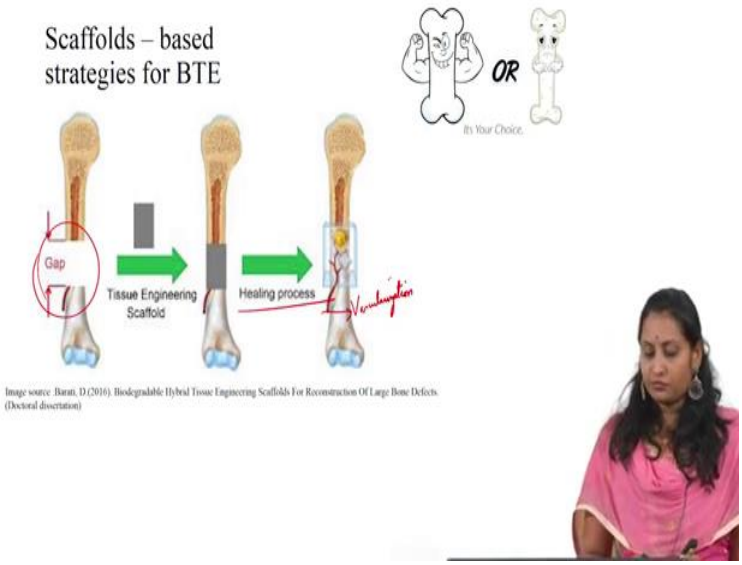

This picture shows the treatment of bone defects with the presence of scaffold. First, there is a bone defect in the bone defect gap in the long bone, where we place scaffold in that defective gap. And, this scaffold first it should be osteointegrated, then it should be osteoconductive, osteoinductive, and then the healing process. This is nothing but the vascularization, the formation of new vessels.

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From here, there will be a few graphical images just for teaching purposes. You should be aware of graphical images that are taken from the literature; for understanding about the information that they have done for scaffold-based strategies. These are first-generation scaffolds, made by the group at the National University of Singapore collaborated with Temasek polytechnic, where they optimize the parameters for PCL, polycaprolactone and its composites, for the development of scaffold by FDM method. FDM method is Fusion Deposition Modelling, which is the 3D printing method for the development of biomimetic scaffold. They considered this as a first-generation scaffolds, where they perform clinical studies for 5 years. After 5 years, they said that the outcome was positive.

Again another research group uses this kind of burr hole plugs for the treatment of cranioplasty. This group tested with another set of experiments, where they used these PCL burr hole plugs, and they studied in rats. After 12 months, they got a positive outcome, and these CT images show the perfect alignment of PCL sheets to the 3D orbital cavity. The first image where the use this PCL sheet in the autologous iliac crest, where they removed the skin from there, and they place this sheet and tested for the regeneration of bone, and they found it successful. The second image where they did for skull defects, where they place this PCL burr hole sheets on the skull defects. Then after 12 months of the study, they found that they have perfectly aligned, and they heal the bone defect.

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Then they have moved to the second generation of scaffolds, where they wanted to study with PCL and composites. They prepared scaffold using PCL tricalcium phosphate and collagen. And, they created defects around 5 mm diameter in rat parietal bone, and then they tested for the healing of the bone defects. In the C and D, you can see the micro CT scanning image where the skull defect, after 14 months, there is the complete closure of the bone. There is the closure of the bone with the presence of the scaffold when it is compared with the blank, where there is no scaffold at all. This was done in rat parietal bone using PCL sheets and its composites; these are the biomimetic scaffolds.

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This one is the pig's spine fusion model, and the below pictures are the New Zealand white rabbit skull defects. They have tested for cranioplasty, again analysis of tissue-engineered constructs shows that the formation throughout the entire scaffold and no fibrous tissue formation is seen. And, micro CT analysis revealed that 30 percent of PCL-TCP is resorbed and replaced by bone.

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This is for the treatment of load-bearing osteochondral defects. Again, with the PCL and calcium phosphate where they have drilled holes in a load-bearing osteochondral defect. They created load-bearing osteochondral defects, where they have seeded mesenchymal stem cells along with the PCL CaP scaffold. And, then they inserted on the defect, and after a certain time, there is a closure of these bone defects in the presence of scaffold.

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So, this all about the scaffold. We have seen about the properties of the scaffold required for the ideal bone graft, and a few examples about the biomimetic scaffold, where they developed first-generation scaffold and second-generation scaffold. Now we move on to the strategies for bone regeneration cellular-based approach. First, we saw about the scaffold-based approach and now it is the cellular-based approach.

As I said earlier, there are four types of cells: osteoblast cells which are responsible for the formation of bone. Osteocytes are the primary cell for a matured bone and are responsible for maintaining the mineral concentration of the matrix. And, osteogenic cells which are responsible for the differentiation into osteoblast and osteoclast cells, which are responsible for bone resorption. Though the volume of cells present in the bone is less, but its function is crucial. So, we need to have bone cells in order to repair bone defects; these are the proposed approach.

The first proposed cellular-based approach is the implantation of unfractionated fresh bone marrow. And, the second one is the purified culture expansion of mesenchymal stem cells and the third one differentiated osteoblasts and chondrocytes. And, the fourth one is the cells that have been modified genetically to have a recombinant bone morphogenetic protein.

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We will look into the first approach. All the cellular based approach in bone tissue engineering primarily targets the early stages of bone repair when the skeletal progenitors become impaired. So, this can be mainly due to either trauma or any disease conditions, or due to aging as well.

This proposed mechanism by which implanted cells enhance bone regeneration in bone tissue engineering involves; first is the early release of key osteogenic and vasculogenic molecules and growth factors. And, the second one is it forms the template to recruit the osteogenic cells and vasculogenic cells. And, the third one is actively laying down the bone matrix and vascularizing the bone construct. These are the steps that follow in cellular based approaches.

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The first approach is the unfractionated fresh bone marrow approach. In the first picture, the patient is first anesthetized. And, the second one is the defect and where they directly remove the fresh bone marrow and inject the cells into the defective site. We all know cells present in the bone marrow region. So, they remove the cells from the bone marrow and they inject into the defect site directly as well as along with some matrix.

After a few days or a few months, we can see from the radiograph images where there is complete healing of the bone defect. We can see the difference in picture B and C, where there is complete healing of bone defect, due to the injection of bone marrow cells. Osteogenic cells directly from the posterior wing of the iliac crest, and we inject into the defect side. But, the disadvantages of this are availability, again it is autologous, as well as it increases pain, and it requires two surgeries where we need to remove and we need to inject into the defect sites. So, it results in a lot of pain and time consuming and everything.

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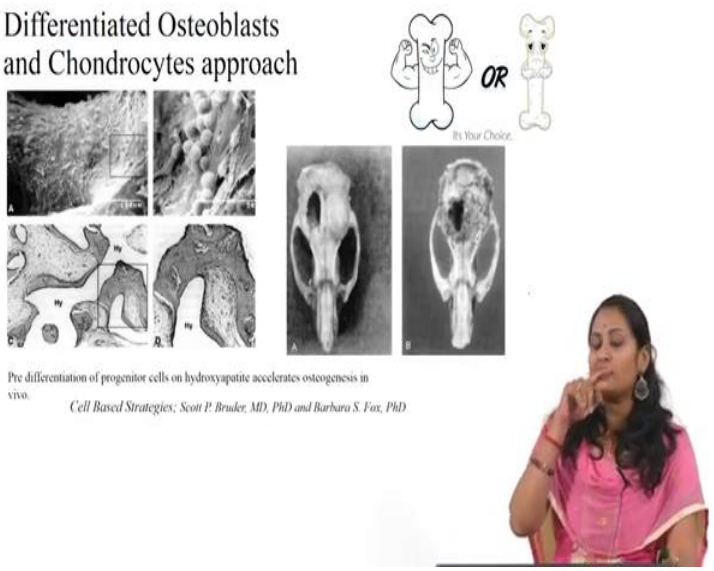

The next approach is differentiated osteoblasts and chondrocytes approach. We all know the formation of bone mechanism. As I explain in my first session, the formation of bone occurs in two mechanisms via intramembranous and endochondral ossification. So, what researchers have thought, why do not we inject differentiated osteoblast cells or chondrocytes directly into the defect or along with the mesenchymal stem cells.

So, first, they have tried and inject differentiated osteoblast cells; they achieved a positive outcome in that. They achieved an increase in the enhancement of bone regeneration, whereas, in the second picture, what they have tried is they tried to inject chondrocytes. Because they thought that endochondral ossification occurs first by the formation of a cartilaginous template, thereby laying down the bone matrix. So, what they thought, why do not we inject chondrocytes directly into the defect sites. Vacanti and his group did this research; what they did it is, they injected chondrocytes into the bone defect and they compared with the periosteal cells. What they found is, there was a formation of the cartilage layer in both the defects; they compared with periosteal cells and chondrocytes cells. But, after cartilage formation, the defect where they inject only chondrocytes, they did not find any formation of vascularization or angiogenesis. In turn, they found that the chondrocytes produce the precursor cells which has the cues, which inhibit vascularization.

So, the other defect, they found the cartilaginous template, as well as the formation of bone and everything. And whereas, in the defect, where they injected only chondrocytes, they could not find the vascularization network or neoangiogenesis which is the primary formation of blood vessels. So, they found that injecting chondrocytes alone will not support in cell-based approach.

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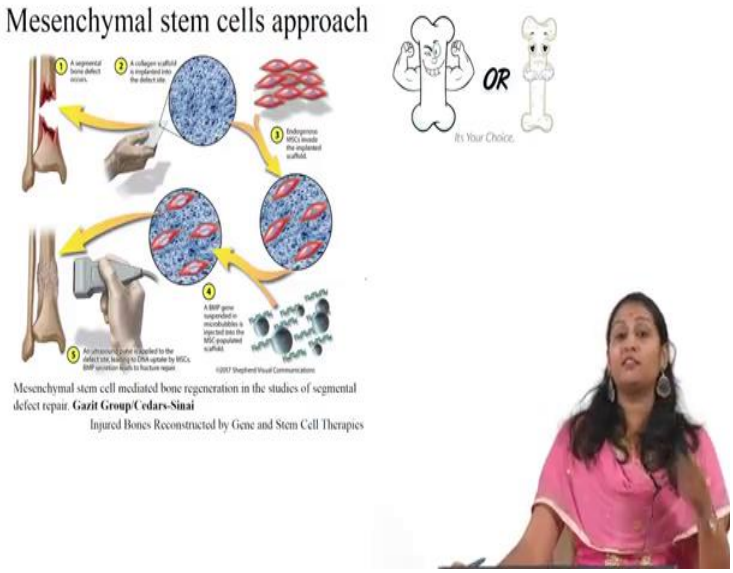

Then this approach, the mesenchymal stem cells approach, is an effective approach where these mesenchymal stem cells can undergo replication without differentiation. We can passage 30 times of these mesenchymal stem cells on which increases up to 1 billion-fold of cells without differentiation; it has to differentiate only in the presence of implantation.

So, this is one of the research where in the segmental defect, they created a bone defect with the inserted collagen matrix. What happened was the endogenous mesenchymal stem cells slightly invaded into the matrix. Then this research group, with the help of ultrasound injected BMP, Bone Morphogenetic Protein in order to start the bone repair process with microbubbles. So, they suspended in microbubbles and injected them into the mesenchymal stem cell populated scaffold. And, with ultrasound, these genes enter into the cells with the external stimuli. After a few months, this segment got healed completely.

All the rat which are treated with this kind of approach has been completely healed with this mesenchymal stem cell approach.

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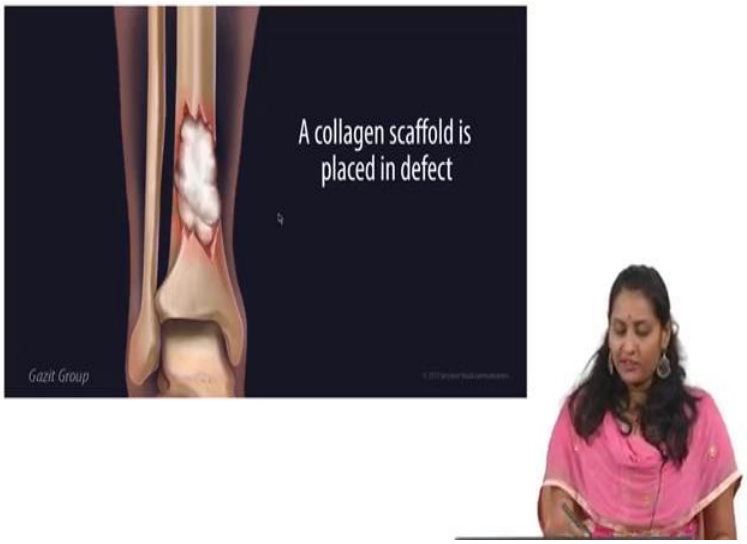

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First, there is the creation of segmental defects, they implanted the collagens scaffold, placed this collagens scaffold; this is the scaffold and bone marrow.

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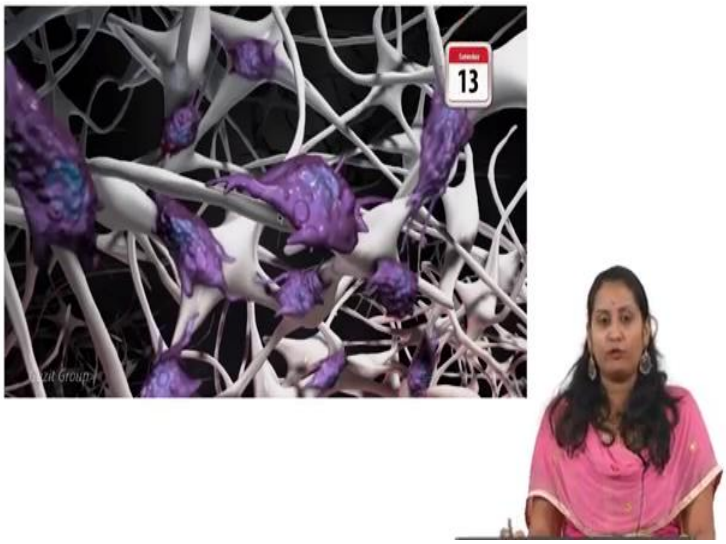

After 2 weeks, these mesenchymal stem cells slowly are invading these scaffolds.

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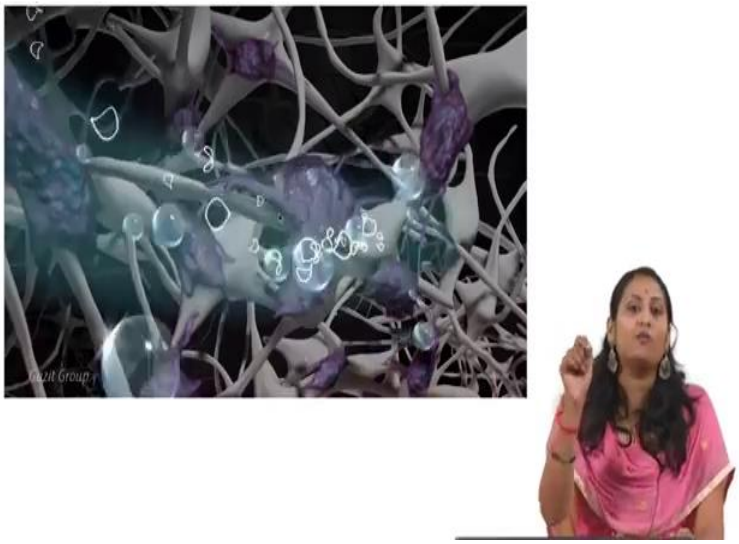

And, then they inject DNA and microbubbles, which has bone morphogenetic protein.

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And, they apply ultrasound, the external stimuli. Now, all the DNA will get into the cells; now, it will start the bone repairing process. See how beautiful is this approach. So, where they have scaffold, cells and growth factors as well.

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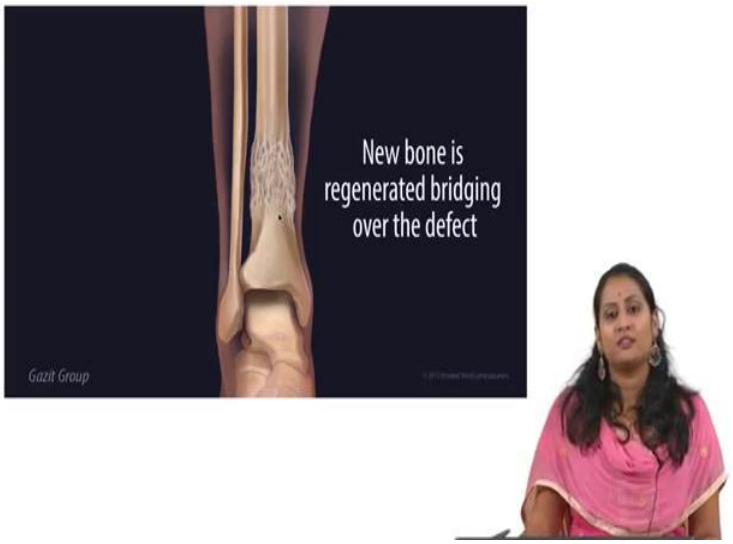

All three combined together and used for the repair and regeneration of that, that big bone defect.

The next session, we will be dealing about the growth factor-based approach and the commercially available bone grafts and what are the current trends and future direction of bone tissue engineering.