Tissue Engineering
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Lecture - 34
Bone Tissue Engineering - Part 3

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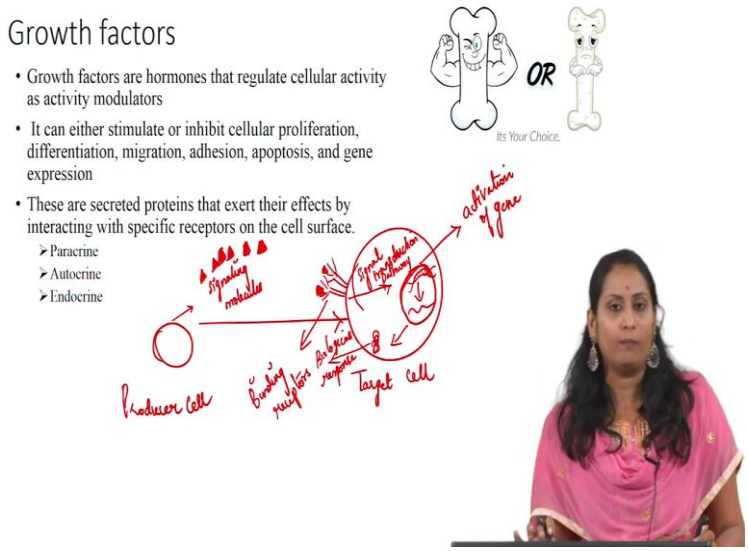

Good afternoon again. I welcome you all for my third final session of Bone Tissue Engineering. I will just explain in a diagrammatic representation, say this is the producer cell; this produces signaling molecules. These are signaling molecules that are nothing but growth factors, and this one is the target cell. So, we need to elucidate the biological response in this target cell. So, this target cell has binding receptors on its surface. So, these are the binding receptors on the surface of the target cell.

These signaling molecules go and bind to these receptors. These are the signaling molecules that are nothing but the growth factors, and they go and bind to these receptors, and then it regulates the signal transduction pathways; it regulates transcription of the gene present in the nucleus. Say this is the nucleus and DNA, it undergoes transcription; this is the activation of the gene. So, this enhances the biological response. This is how the growth factors act. So, signaling molecules go and bind to the receptor present on the target cell, then they regulate the signal transduction pathway, and they regulate gene transcription then they elucidate the biological response.

So, this producer cell has changed this response of the target cell with the help of growth factor; this how it works. In general, they are the secreted proteins that exert their effects by interacting with specific receptors on the cell surface through paracrine. Paracrine is nothing but where they act on the neighboring cells; the signal transfer takes place in the neighboring cells.

In autocrine, as it defines, it acts on itself, the same cell. Endocrine, where it is transferred in the blood and tissues and is transported through the blood and in the targeted site, will enhance its property or elucidate its response. So, these all about the growth factors or the signaling molecules.

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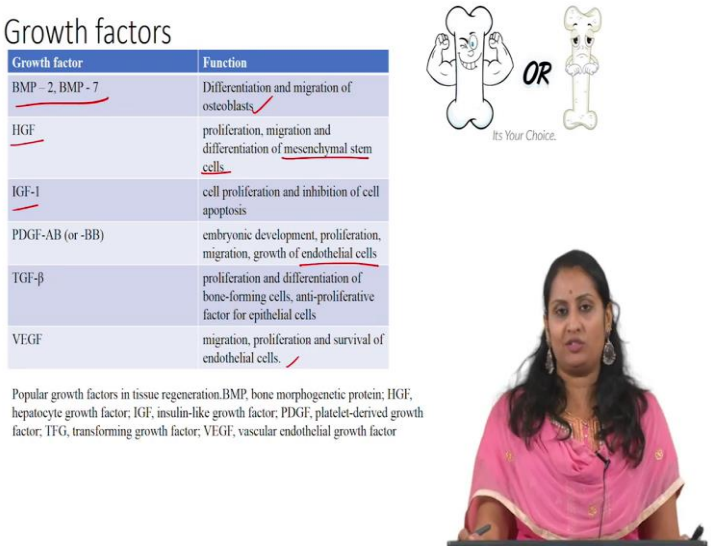

This table shows the certain growth factors which are responsible for bone tissue engineering application. BMP-2, which is Bone Morphogenetic Protein 2, and bone morphogenetic protein 7, are FDA approved growth factors, and they are used in commercially available products, where they help in the differentiation and migration of osteoblast cells. Osteoblast cells help in the formation of bone.

HGF, which is a hepatocyte growth factor, where its function is proliferation, migration, and differentiation of mesenchymal stem cells, which are very important cells. These mesenchymal stem cells can develop into n number of lineages not only the bone, but it can also develop into cartilage, tendon, or marrow; any kind of lineages it can develop.

IGF is an insulin-like growth factor; it helps in cell proliferation and inhibition of cell apoptosis. Apoptosis is nothing but programmed cell death. PDGF is a platelet-derived growth factor, which is responsible for embryonic development, proliferation, migration, and growth of endothelial cells. Endothelial cells are very much important for vascularisation, which helps in the formation of new tissue.

TGF beta is transforming growth factor, which helps in the proliferation and differentiation of bone-forming cells, antiproliferative factors for epithelial cells. VEGF Vascular Endothelial Growth Factor migration, proliferation, and survival of endothelial cells; again, it is for vascularisation techniques. So, there are several growth factors; I just picked up the few important growth factors in terms of bone tissue engineering application.

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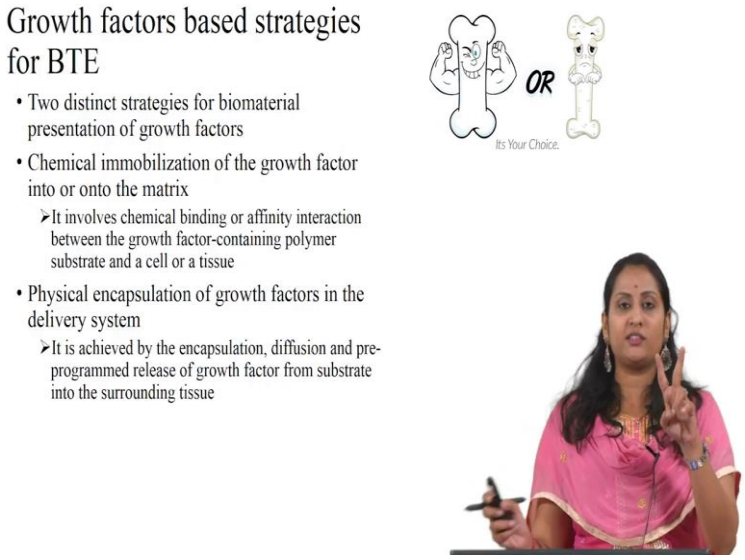

There are two distinct strategies for biomaterial presentation of growth factors. It can be chemically immobilized into the substrate like the polymeric substrate or the matrix, or physical encapsulation can be done. So, there are two mechanisms on which growth factors are encapsulated into the matrix. The first one is chemical immobilization, where it involves chemical binding or affinity or interaction between the growth factor containing polymer substrate and a cell or tissue. And this will produce strong, localized interaction in that area. However, it is mainly based on the physical and chemical properties of the substrate, as well as the growth factor dose.

The physical encapsulation of growth factors in the delivery system. It is achieved by encapsulation, diffusion, and programmed release of growth factor from the substrate into the surrounding tissue. For this, a very good example which we saw in the last session is video. Can you guess where the physical encapsulation or the physical stimulus of the growth factor is done? In which mechanism? Yes, ultrasound. So, through external stimuli, we are delivering growth factors.

So, these are all about the growth factor strategies and what are growth factors and the two main strategies for growth factors.

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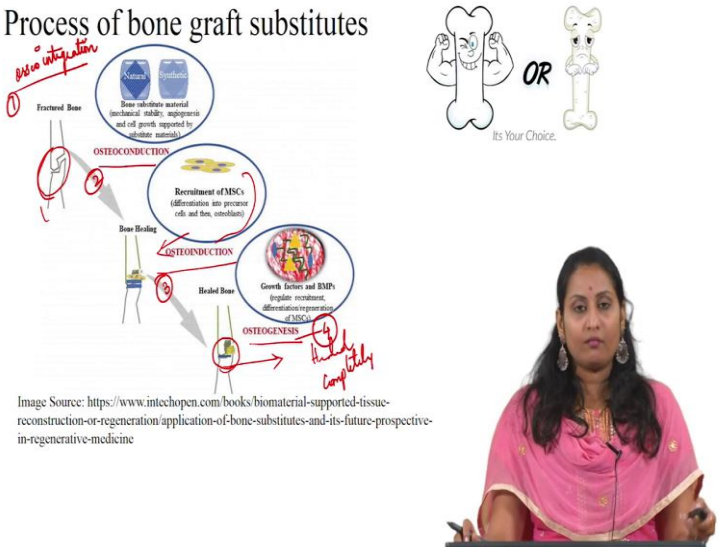

Image Source: https://www.intechopen.com/books/biomaterial-supported-tissue-reconstruction-or-regeneration/application-of-bone-substitutes-and-its-future-prospective-in-regenerative-medicine

To combine everything, all the scaffold, cells, and growth factors development of an ideal bone graft substitute are needed. So, this picture clearly depicts the properties of the ideal bone graft substitute.

The first one, there is the fractured bone; there is a defect in the bone. The first thought we need to think is what kind of base material we need to select either natural material or synthetic polymer material. But it should be mechanically stable, angiogenic, and the cell growth should be supported by the substitute material. So, the initial thinking should be about the matrix, which is nothing but the scaffolds. So, scaffold can be natural or synthetic, but it should be nontoxic, mechanically stable, and highly interconnected porosity.

Then the osteoconduction. So, osteoconduction is nothing but the formation of new bone on the surface. Then the recruitment of MSC cells, mesenchymal stem cells, which then differentiated into precursor cell and then into osteoblast. So, that undergoes with osteoinduction. Recruitment of mesenchymal stem cells, differentiates into precursors cells, and then osteoblast.

Then the osteogenesis takes place. Osteogenesis is nothing but the formation of new tissue. This can be done with the combination of growth factors, which are nothing but bone morphogenetic proteins, where it regulates, recruits, differentiate and regenerate mesenchymal stem cells. So, osteogenesis takes place and this bone is healed completely.

Any ideal bone graft should have three major processes. First, it should be osteointegrative. So, it should have all the four properties osteointegration, osteoconduction, osteoinduction and osteogenesis. These all four important processes is needed to develop a bone graft, to enhance and repair the bone defect without any side-effect and it can heal completely and it also accelerates wound healing.

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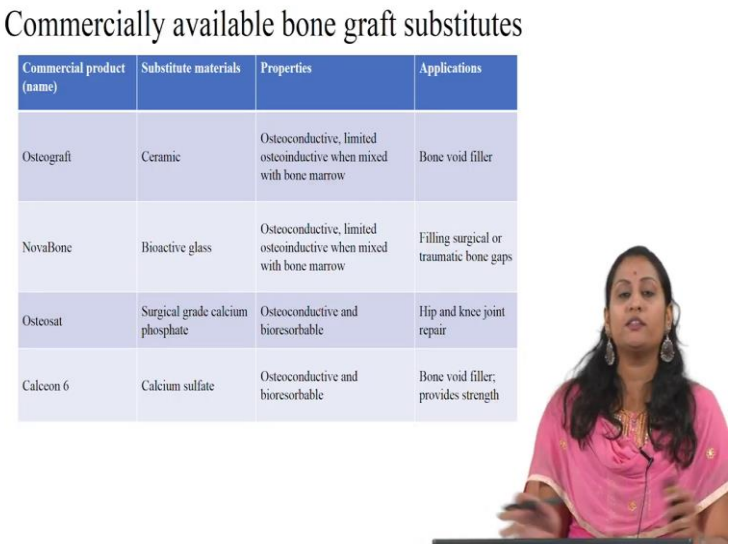

In the next few slides, I will be talking about commercially available bone graft substitutes. The first one is Osteograft, which is made up of ceramic material, and these all FDA approved bone graft substitutes. Osteograft is the commercial product name, and it is made up of ceramic, and major properties are osteoconductive, limited osteoinductive when mixed with bone marrow. It has to be mixed with bone marrow in

order to become osteoinductive materials. Bone marrow has a lot of mesenchymal stem cells and they have used for bone void filler.

Then the next product is Novabone, where it is bioactive glass. Bioactive glass is nothing but the silicates. So, it is osteoconductive, limited osteoinductive, and again mixed with bone marrow and used in filling surgical and traumatic bone gaps. And the third one is Osteosat, where it is surgical grade calcium phosphate. Again it is osteoconductive, and bioresorbable thus helps in hip and knee joint repair.

The next one is Calceon 6, where it is made up of calcium sulfate. It has osteoconductive and bioresorbable properties. Again the applications are bone void filler and it provides strength. These all the ceramic and ceramic derived composites which are used for bone graft substitutes. As I said in the second session, in the osteoinductive biomaterials, ceramic plays a role. Ceramic-based materials are very widely used in bone graft substitutes. Because, we already know that bone is a composite material, which is made up of inorganic hydroxyapatite as well as the organic collagen matrix. Again hydroxyapatite comes under ceramic family.

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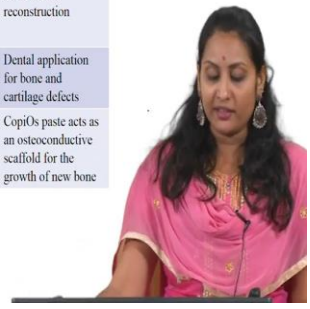
Commercially available bone graft substitutes

Commercial product (name)	Substitute materials	Properties	Applications
Norian	Monocalcium phosphate, tricalcium phosphate, and calcium carbonate	Good compressive strength	Skull bone defect; injectable paste, craniofacial reconstructions
Hard tissue-replacement (HTR)	Poly methyl methacrylate (PMMA)	Good strength, durable, and surface osteoconductive	Craniofacial reconstruction
Alpha BSM	Calcium phosphate cement	Good compressive strength	Dental application for bone and cartilage defects
CopiOs (Zimmer Biomet) Bone Void Filler	Calcium phosphate, dibasic (DICAL), and highly purified Type I bovine collagen	DICAL provides significantly more calcium and phosphate ions at equilibrium than either β-TCP or HA	CopiOs paste acts as an osteoconductive scaffold for the growth of new bone



There are many works based on ceramic-based materials. The next one is Norian, where it has been prepared by monocalcium phosphate and tricalcium phosphate and calcium carbonate. It gives very good compressive strength and used in skull bone defect and craniofacial reconstruction, and also it is an injectable paste format.

And the next one is Hard tissue replacement, where they have used polymethyl methacrylate, which is a synthetic polymer. Now it is a synthetic polymer-based bone graft material. It has good strength, durable, and surface osteoconductive; it is used for craniofacial reconstruction.

The next one again ceramic-based material Alpha BSM which is made up of calcium phosphate cement. It shows good compressive strength; it is used in the dental application for bone and cartilage defects. The next is CopiOS paste, which is made up of calcium phosphate and type I bovine collagen; natural and synthetic composites polymer. It provides significantly more calcium and phosphate ions at equilibrium then either of beta TCP or HA, hydroxyapatite. It acts as the osteoconductive scaffold for the growth of new bone.

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Commercially available bone graft substitutes

Commercial product (name)	Substitute materials	Properties	Applications
Collagraft	Mixture of tricalcium phosphate, bovine collagen, and hydroxyapatite	Bioresorbable and osteoconductive	Use for the treatment of long bone fracture and void filling
BESTT	BMP-2, collagen sponge	High vascularization, Osteoconductive	Bone fractures
OP – 1 Putty	BMP-7, collagen sponge	Osteoconductive and bioresorbable	Bone defects

The Collagraft, which is the mixture of tricalcium phosphate, bovine collagen and hydroxyapatite. Again, it is bioresorbable and osteoconductive; it can be used for the treatment of long bone fracture and void filling. And the last one is mainly based on growth factors, which I already told like BMP -2 and BMP- 7 are FDA approved ones; BESTT and OP-1 Putty. In BESTT, BMP -2 along with collagen matrix is used, and it highly enhances vascularization, osteoconductive and helps in healing of the bone fracture.

The next one OP-1 Putty, which is again BMP- 7 with a collagen matrix, osteoconductive, and bioresorbable it helps in the healing of bone defects. So, there are so many bone graft material approved and they are still in progress. I have just picked up a few bone graft materials mainly based on calcium and few on polymer and ceramic and few on natural and synthetic polymer and some FDA approved bone graft substitute based on growth factor strategies. Since the first one is fresh bone marrow cells, and it is autologous, no need for FDA approval.

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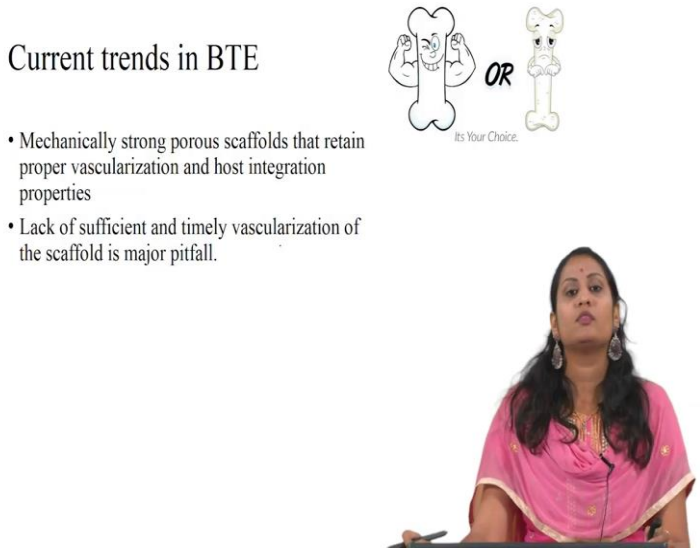

So, that is all about the commercially available bone grafts and a few examples of commercially available products. Now the current trends in bone tissue engineering. Currently, people have shown more interest in developing mechanically strong porous scaffolds that can retain proper vascularization and host integration. The major pitfall of bone tissue engineering is the lack of sufficient and timely vascularizations of the scaffold.

For example, if you place a scaffold in the construct, there should be an immediate acceptance of the construct with the host, or the vascularization should happen deep into the construct. It should not stop at the upper layer of the construct. So, it happens at many stages, and there are many failures because of this kind of issue. So, vascularization is a very important phenomenon or strategy for a successful ideal bone graft material.

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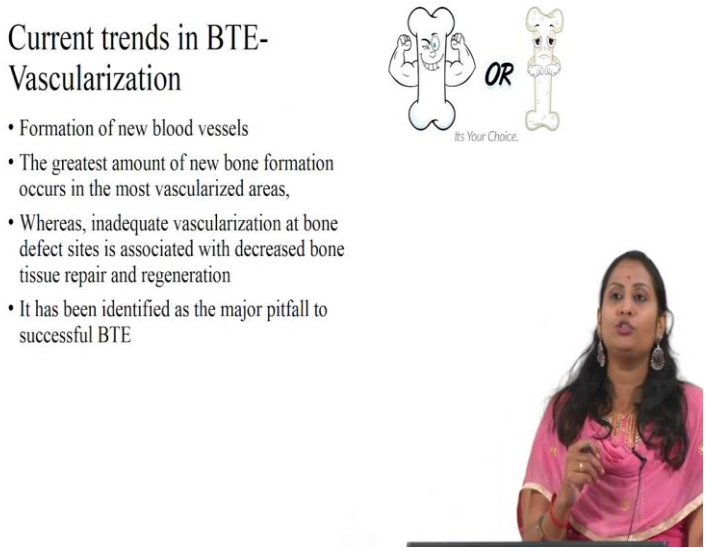

So, we should know about vascularization. Vascularizations is nothing but the formation of new blood vessels. The greatest amount of new bone formation occurs in the most vascularized area. Whereas, inadequate vascularization at bone defect sites is associated with decreased bone tissue repair and regeneration. So, vascularization has been identified as the major pitfall for developing successful bone tissue engineering grafts.

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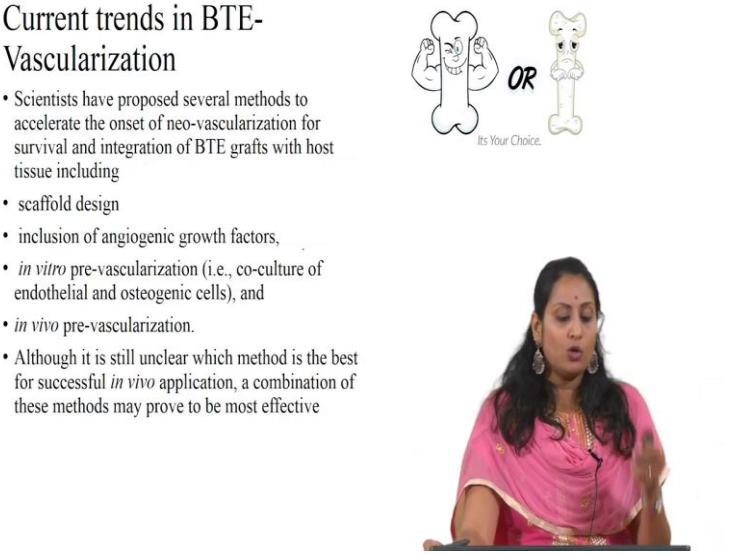

So, people have done strategies proposed to enhance vascularization, thereby to increase the success of the bone graft material. Scientists have proposed several methods to

accelerate the onset of neovascularization, which is the formation of new tissue, for the survival and integration of bone grafts with host tissue.

First, is scaffold design. They have changed many fabrication techniques, where they have started doing 3D printing, where did we have seen few examples of the first generation and second generation scaffold, biomimetics scaffolds, which are nothing but 3D printed scaffolds done by fusion deposition modeling and inclusion of angiogenic growth factors for the enhancement of endothelial cell.

And there are two techniques in vitro prevascularization and in vivo prevascularization. In vitro prevascularization is a coculture of endothelial cells and osteogenic cells in in vitro, and then we transfer into in vivo and check for vascularization. In vivo prevascularization is nothing but two there are two more, where we place the scaffold in vivo vascularized area subcutaneously or intramuscular region. After two weeks or after certain time, we harvest that scaffold out and we place that scaffold in the bone defect, but this method requires two surgeries. First, we need to place the scaffold to get it vascularized; then we have to remove that scaffold and place it on the defect sites. So, it requires a lot of pain, surgeries, time and everything.

On the second method is like where we have vessels, for example, jugular veins; we place that inside this construct and we place in the in vivo. The second method happens to be kind of successful, but still, it is very unclear which method is best for the successful in vivo application. Maybe the combination of all the methods may prove to be more effective for the enhancement of vascularization.

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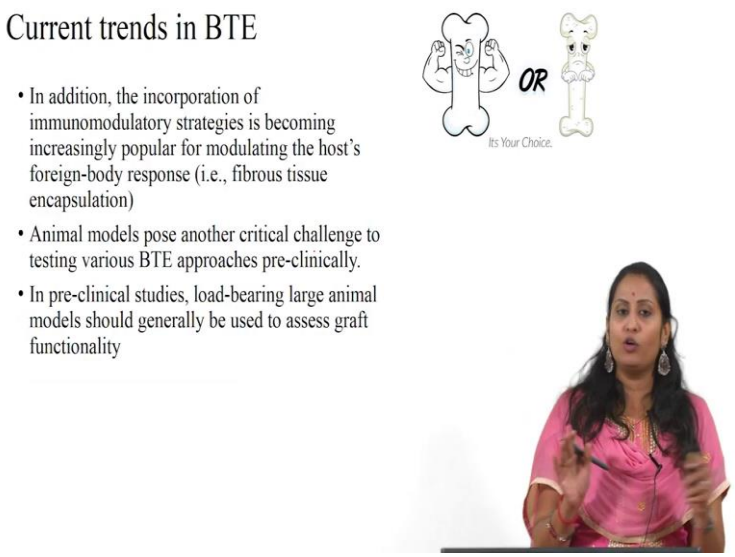

Now immunomodulatory strategies are becoming increasingly popular for modulating the host-foreign body response; that is, fibrous tissue encapsulation. See once the construct has been placed, immediate reaction will be the inflammatory response, then followed by the fibrous tissue encapsulation. So, we need to modulate that.

So, incorporation of immunomodulatory strategies, it is becoming popular to avoid the kind of encapsulation. That is one of the current trends where researches are ongoing in bone tissue engineering. One is mainly on vascularization techniques and scaffold design and the incorporation of immunomodulatory strategies, then the main important challenge or a critical challenge is the availability of animal models.

Animal models pose another critical challenge to test various bone tissue engineering graft approach preclinically. Preclinically load-bearing large animal models should be used to access graft functionality and this is one of the major pitfalls we face in order to check for the bone graft properties.

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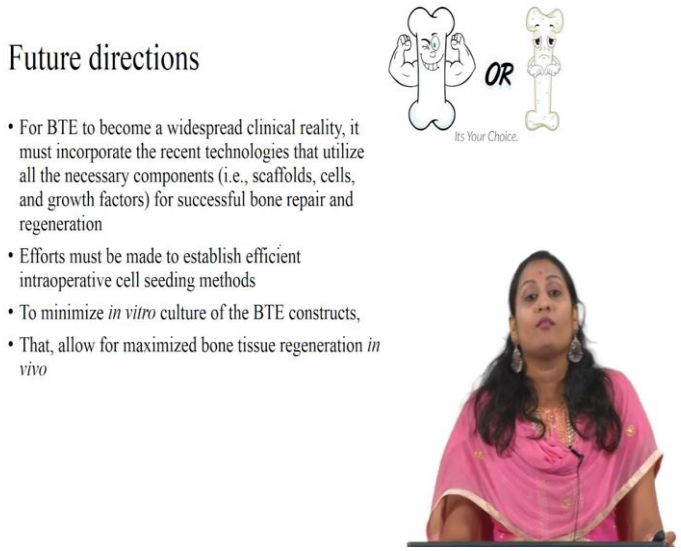

The future direction for bone tissue engineering to become a widespread clinical reality. It must incorporate the recent technologies that utilize all the necessary components like scaffolds, cells and growth factors. So, we need to utilize all the new recent technologies in order to develop the ideal scaffold, and also seeded with the cells and the growth factors for successful bone repair and regeneration.

Some of the efforts must be made to establish efficient intraoperative cell seeding methods, as well. To minimize the in vitro culture of the bone tissue engineering constructs, that allows bone tissue regeneration in in vivo studies. So, these are the few future directions where we need to focus mainly on to develop an ideal bone repair and regeneration.

So, thank you. So, overall picture where we studied about the bone its basic functions and its anatomy of the bone and the modeling and remodeling of the bone and the tissue engineering strategies, scaffolds, growth factors, cells and the commercially available products and the current trends and the future directions.

Thank you.