Tissue Engineering
Prof. Vignesh Muthuvijayan
Department of Biotechnology
Indian Institute of Technology, Madras

Lecture – 25
Challenges in Tissue Engineering

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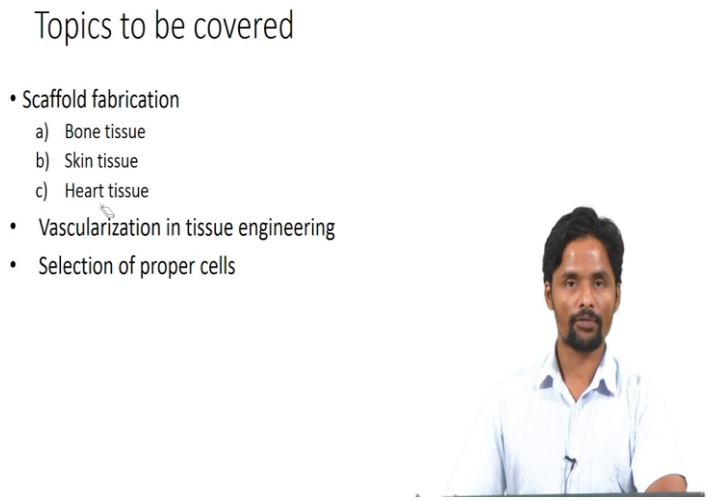

Hello everyone, in today's class, we will talk about a few of the Challenges in Tissue Engineering and a few of the developments done to tackle these challenges. The topics which will be covered are scaffold fabrication, the challenges associated with scaffold fabrication; we are discussing three different tissues that are bone tissue, skin tissue, and heart tissue. As a second challenge, we will talk about vascularization in tissue engineering, and also we will talk about the selection of proper cells.

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Scaffold fabrication; as we know, scaffolds are temporary support material that will enable cells to attach, grow, and form a 3D structure. Hence, scaffolds must meet the chemical and physical properties of the natural ECM. Also, the scaffold must degrade over a period of time so that the tissue regeneration to take place. As we know, different tissues have different regeneration times; for example, skin tissue regenerates faster than the bone tissue. Hence, the scaffold must meet the regeneration time of the tissue of our interest

Biocompatibility of the scaffolds; It is must that scaffold should not cause any toxicity to the host cells. In the 80s, when pioneers in the field, when they proposed polyanhydrides for biomedical applications, the scientific community thought that these synthetic materials would cause toxicity to the host cells. This belief was persistent until 1996 until FDA approves the first synthetic polymers for biomedical applications. Hence it is necessary to see the toxic compatibility of these scaffolds to the host cells.

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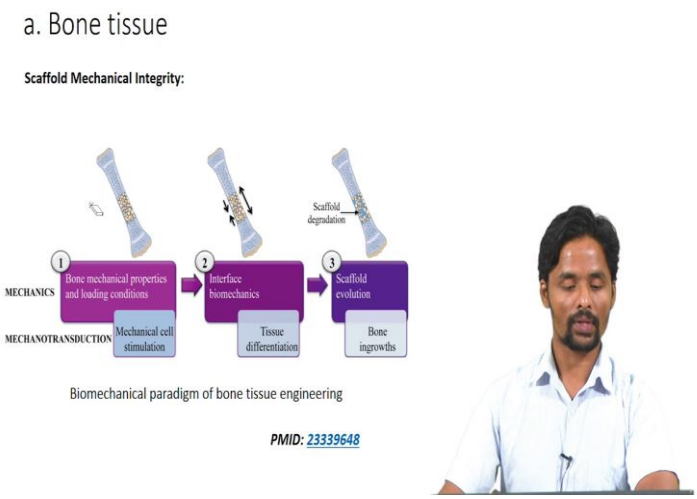

Let us begin with the Bone tissue; as we know, bone tissue bone is a hard tissue. Hence a scaffold, which we fabricate for bone tissue engineering, should have high mechanical strength. And it is explained in three different steps, which is known as the Biomechanical paradigm of bone tissue engineering. The mechanical strength of a scaffold should match with our natural bone tissue. At the same time, it should not induce a stress shielding effect, which is prevalent in the metallic implants.

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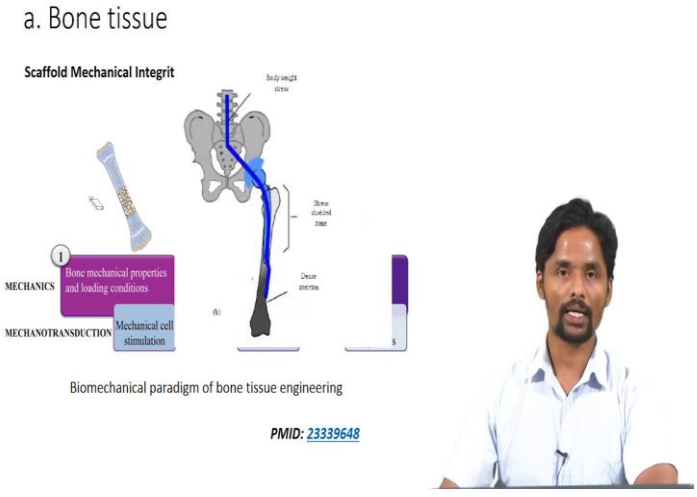

What happens in metallic implants is due to their high mechanical strength, all the load will be taken up by the metallic implants. As you see here, this is the metallic implant, and this metallic implant due to their high mechanical strength they take up all the load. The region, which is near to the metallic implant, fail to take the load, which is necessary for it to take. As you see, the region further from the metallic implant has higher bone mass compared to the region, which is near to the metallic implant. This region does not have sufficient bone mass, which is supposed to possess. Hence eventually, the regenerated bone will fail to take up the load.

Hence, it is necessary that the scaffold which we prepare should not induce this stress shielding effect. The second step is the mechanical property of the scaffold should be in such a way that it induces the scaffold to bone mechanotransduction.

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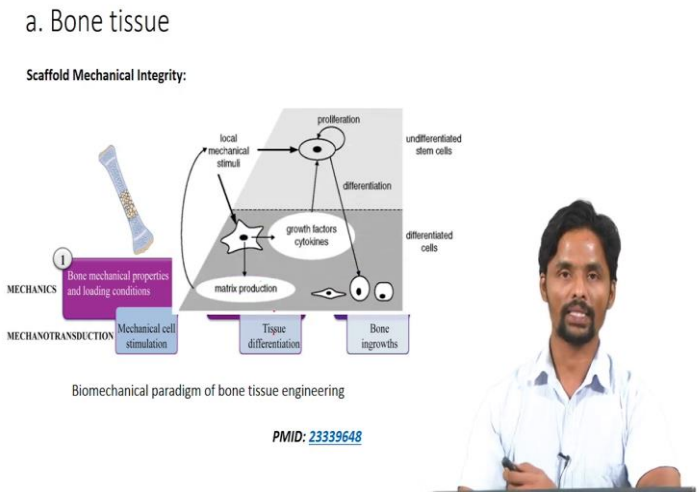

The mechanical stimulus provided by the scaffold induces tissue differentiation. In the undifferentiated stem cells, the mechanical stimulus causes the differentiation of the cells. Whereas in differentiated cells, it leads to matrix production by the differentiated cells. And in the third step, as the host bone tissue regenerates, it takes up the load as the scaffold degrades.

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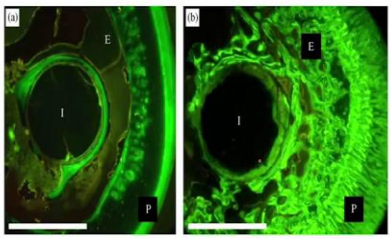

(a) Degradable polymer and **(b)** magnesium rod

E: endosteal bone formation; **P:** periosteal bone formation; **I:** Implant residual

PMID:15621246

A recent development is a degradable metallic implant. In this study, what they have done is they have used a magnesium alloy and to see their degradation profile. And this image is the degradable polymer, and this one is the magnesium alloy. In vivo staining using calcein green; as you see here, the region which is near to the magnesium alloy has sufficient bone growth formation compared to the degradable polymer.

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	Cancellous bone	Cortical bone
Compressive strength	4-12 MPa	50-150 MPa
Elastic modulus	100-500 MPa	12-18×10^3 MPa

Scaffold pore structure :

- Bone is highly porous structure
  - pore size
  - volume, and
  - interconnectedness.
- Balance between mechanical strength and porosity.

Here are the compressive strength and elastic modulus of cancellous bone and cortical bone are given. Second-most important parameter while designing the scaffold for bone

tissue engineering is the porosity. Bone is highly porous in nature. And it is defined in three different terminologies, pore size, pore-volume, and interconnectivity between the pores.

While designing the scaffolds for bone tissue, we should consider all these three parameters. As we know, the bone has high mechanical strength, and at the same time, it is highly porous. So, balancing these two is a real challenge in bone tissue engineering.

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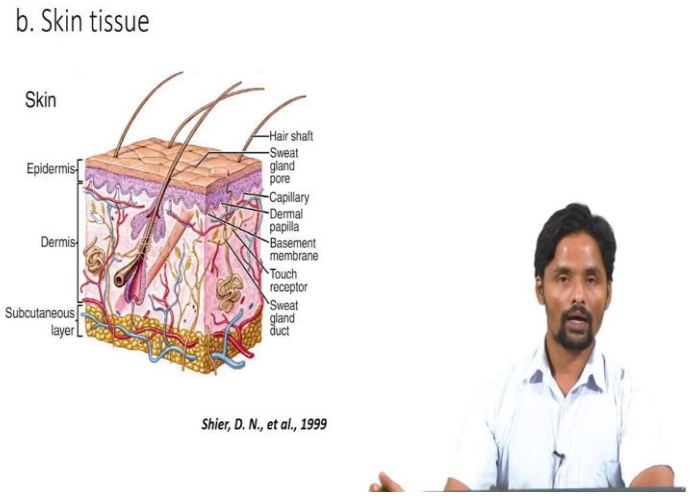

Let us go to the skin tissue. This is the general structure of skin tissue, the upper epidermal layer followed by the dermal layer. If the skin injuries limited to the epidermal layer, what happens is the fibroblasts present in the dermal layer migrate towards the epidermal layer. They start differentiating and secreting extracellular matrix, and thereby they fill up the gap. If the skin injury is very deep wherein the dermal layer is also lost, then the clinical interventions are necessary.

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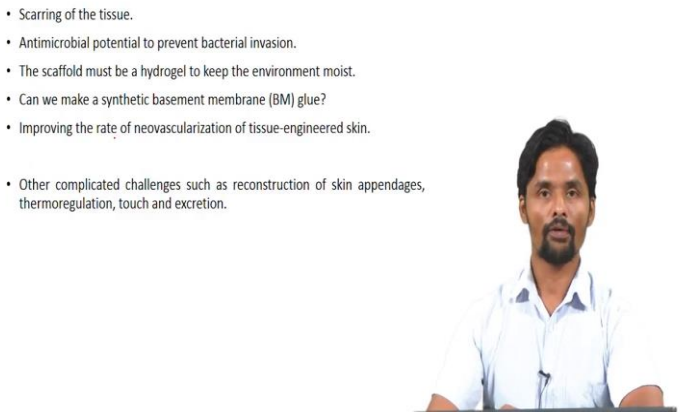

Here are a few challenges listed for skin tissue engineering. Scarring of the tissue; as I explained in the previous slide, the fibroblast present in the dermal layer migrates towards the injured skin site, and they start secreting the extracellular matrix. And, what happens is that they secrete collagen in excess then what it is required. This excessive collagen, it crosslinks, and contracts and that leads to the scarring of the tissue. Avoiding such scarring of the tissue is one of the challenges in skin tissue engineering.

As we know, the skin is the outermost layer of our body, preventing microbial infection. Hence, when we prepare a scaffold for skin tissue engineering, it is necessary that it should have antimicrobial properties. The scaffold which we prepare for skin tissue engineering should be a hydrogel so that they are able to keep the environment moist and at the same time should able to absorb the exudates from the wound. Let us go back to the previous slide, as you see here, the epidermal layer and the dermal layer they are connected with the basement membrane.

This basement membrane is rich with extracellular matrix secreted by the fibroblasts cells. This basement membrane is involved in several functions of the skin tissue. Reconstruction of this basement membrane is one of the challenges in skin tissue engineering. We will talk in great detail about vascularization, and other complicated challenges such as the reconstruction of the skin appendages, thermoregulation, touch, and excretions are a few of the challenges in skin tissue engineering.

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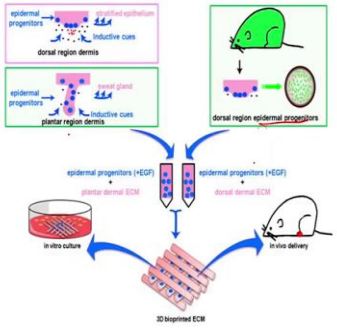

doi: 10.1016/j.actbio.2015.12.039

In the scaffold, they try to see whether they can able to produce the sweat gland. What they have done was, they have isolated epidermal progenitor cells from the dorsal region of the dermis, and they have mixed with the plantar region dermal ECM, which has cues for the sweat gland formation. As a control, they have used the dorsal region ECM which has cues for these stratified epithelium formations, but not for the sweat gland. And this mixture, they fed to the 3D bioprinter, and they try to see whether they could able to produce this sweat gland or not.

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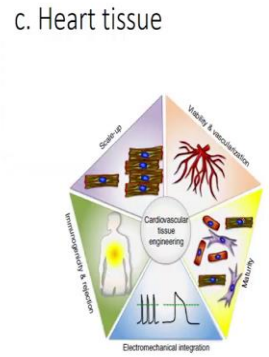

Ngan F. Huang et al., 2018

And the third tissue which we will be talking about is the Heart tissue. These are the few challenges listed in heart tissue engineering. We will talk about only those which are related to scaffold fabrication.

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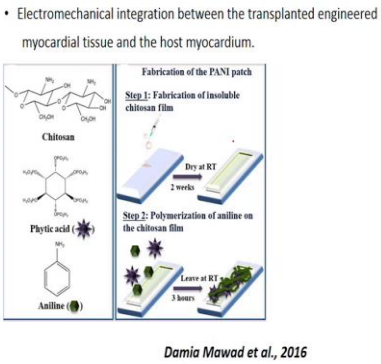

Electromechanical integration between the transplanted engineering myocardial tissue and the host myocardium is one of the challenges in heart tissue engineering. A recent development is a usage of conducting polymers. For example, polyaniline is a conducting polymer, and the conductivity of these polymers is dependent on the proton based doping process. Wherein what will happen is the imine, and amino groups of aniline are protonated in the presence of protonic acid.

There are a few challenges associated with this conducting polymer. When we implant these conducting polymers in vivo, the dopants which keep the imine and amino groups protonated they are lost in the process called dedoping. That leads to a decrease in the conductivity of these conducting polymers, and it will not last for a longer time. Another challenge associated with polyaniline is the amine groups of aniline are loses its physiological properties.

In this study, what they have done is they have used chitosan. Chitosan is a rich source of the amine group, and they fabricate it to chitosan film. On top of which, they polymerized aniline in the presence of phytic acid, and as we knew, the chitosan plays a role of amine group source, and they try to solve the problem of deprotonation.

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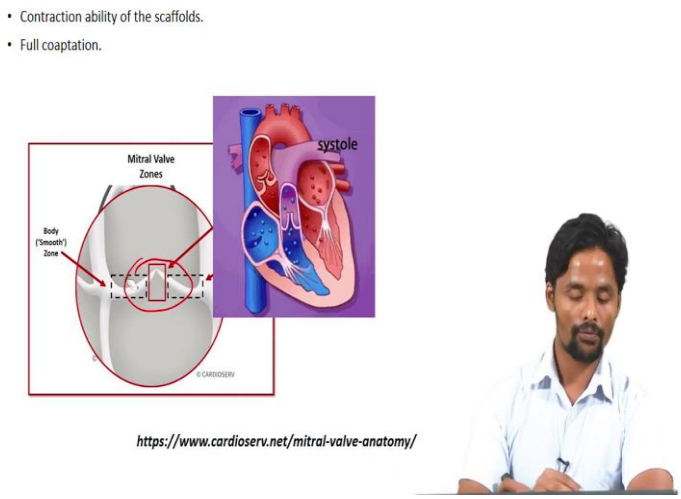

Another problem is the contraction ability of the scaffolds. As we knew, the heart is highly contractile in nature. Producing the scaffold, which has the contracting ability, is one of the challenges in heart tissue engineering.

And another challenge is full coaptation. As we knew, the heart is made up of walls; these walls make sure that the blood flows in the right direction, and these walls are able to contract and relax. Producing such a wall that can able to contract and relax is one of the challenges in heart tissue engineering.

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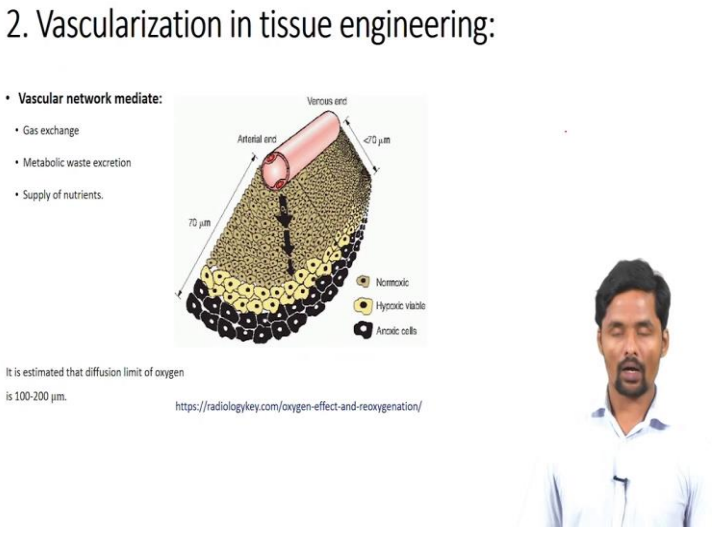

As we all know, vascular networks mediate gas exchange, they excrete metabolic waste, and they supply the nutrients. What will happen if we prepare a scaffold without vascular network within it, the diffusion process will take place. However, the diffusion is limited to 100 to 200 micrometers of distance. Beyond that, the cell starves of nutrients and oxygen, and they eventually die. Hence, it is necessary to include the vascular network when we design the scaffold.

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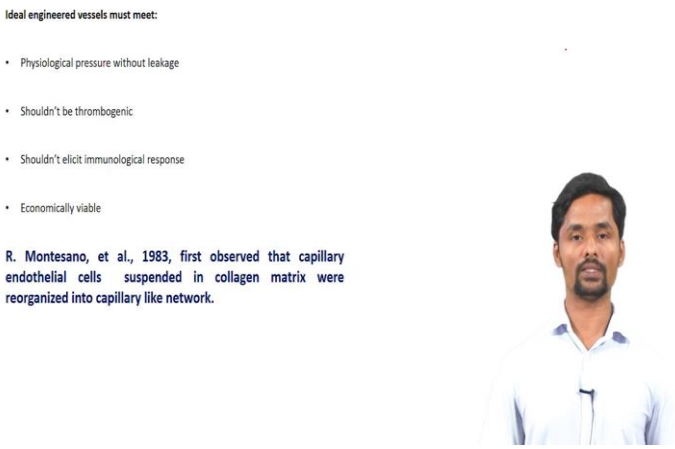

The ideal engineered vessels must be able to withstand physiological pressure without any leakage, and they should not be thrombogenic; they should not elicit any immunological response, and they must be economically viable.

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There are several strategies to introduce vascular networks into the scaffold and can be broadly classified under four categories. That is scaffold design, a supplement of angiogenic factors, in vivo prevascularization, and in vitro prevascularization. Let us discuss each in detail.

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The first one is the Scaffold design. One of the major prerequisites for introducing vascular networks into the scaffold is the porosity of the scaffold. The scaffold must be highly porous and highly interconnected. And there are several strategies through which

we can prepare the porous scaffold, and few of the techniques are listed over here. For example, gas foaming, electrospinning, particulate leaching, freeze-drying, phase separation, and microfabrication.

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b. Supplement of angiogenic factors

Growth factor	Role in angiogenesis
PlGF	1. Inflammatory cell recruitment 2. Proliferation and differentiation of MSCs into osteoblasts and osteoclasts
IGF	1. Promote neovascularization 2. Chondrocyte hypertrophy
SHH	Proliferation and differentiation of bone marrow-derived MSCs into an osteoblastic lineage
VEGF	1. Vascular invasion 2. Stem cell recruitment
FGF-2	1. Angiogenesis 2. Proliferation of MSCs and osteoblasts
Ang-1	Blood vessel remodeling and maturation
PDGF	1. Chemotactic factor for osteoblasts 2. Proliferation of osteoblasts 3. Increases the expression of VEGF on endothelial cells

S. Almubarak et al., 2016

The second approach is a supplement of the angiogenic factor. Just by supplying the growth factors which are involved in angiogenesis, we can achieve the vascularization in tissue engineering scaffold. Few of the growth factors which are involved in angiogenesis are listed over here.

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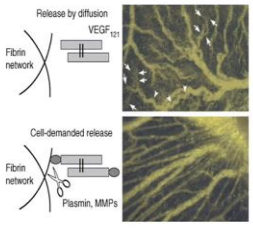
- Release by diffusion
- Release by cell demand



J Rouwkema et al., 2008

There are two ways through which we can supply these growth factors. One is the release by diffusion, which is a direct method, and the second one is the release by cell demand, which is an indirect method. Let us talk about these methods in brief. Release by diffusion, in this method, we supply the growth factors which are directly involved in angiogenesis. For example, VEGF, but we do not know how much the exact amount of these growth factors need to be supplied for vessel formation. Hence what happens is, that leads to the unhealthy vessel formation as you see in the upper image.

The second method is release by cell demand. In this method, you supply the growth factors to the cells. These growth factors, in turn, stimulate the cells to secrete the angiogenic growth factor. Thereby, you can control the release of these angiogenic growth factors; thereby, we can achieve a healthy vascular network. As you see in the lower image.

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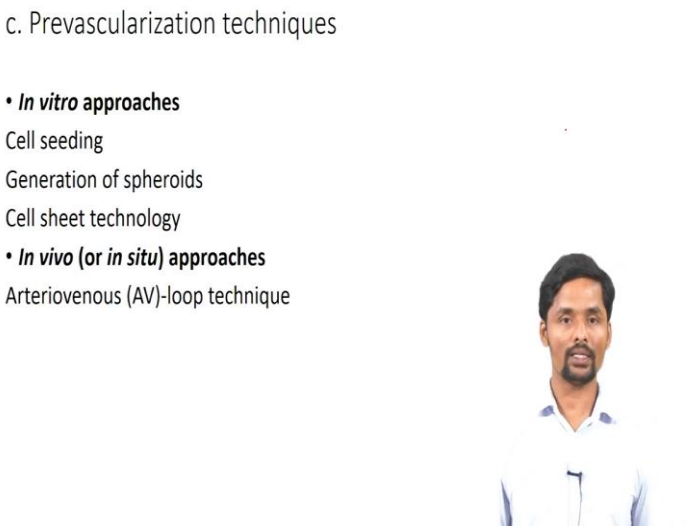

The third approach is prevascularization technique; there are two approaches. One is in vitro approaches, and the other is in vivo approaches. Let us discuss each in detail.

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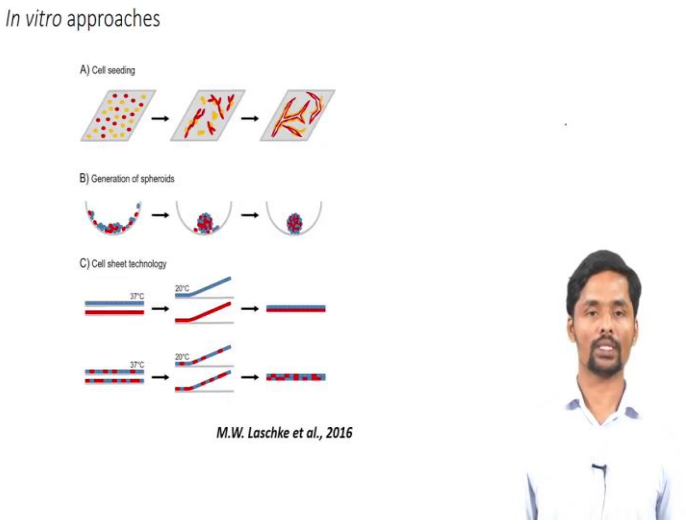

The first one is in vitro approaches. There are three approaches, cell seeding, generation of spheroids, and cell sheet technology. The first one is cell seeding. In this approach, you seed the endothelial cells into the porous scaffold and incubate it over a period of time. Eventually, what will happen is there will be vascular network formation within this porous scaffold, and then you implant at the site of interest.

The second approach is the generation of spheroids; spheroids are a structurally three-dimensional arrangement of cells with intensive cell to cell and cell to matrix interaction. There are several ways through which we can achieve the formation of these spheroids. If we choose endothelial cells and tissue-specific cells for the formation of spheroids, what will happen is over a period of time, there will be a vascular network formation within these spheroids. Once the vascular network forms within these spheroids, then we can transfer these spheroids into the scaffold, and then thereby, we can achieve the vascular network into the scaffold.

The third approach is cell sheet technology; this technique is devoid of any scaffolds. In this technique, what we use is thermosensitive polymer. For example, poly isopropyl acrylamide; at 37 °C, these thermosensitive polymers allow the cell adhesion. So, at 37 °C, you seed endothelial cells and tissue-specific cells on to these thermosensitive polymers and incubate it over a period of time until it reaches the monolayer formation. Once the monolayer is formed, you decrease the temperature to 20 °C, what happens is at

20 °C, this polymer they swell. And the cell adhesion property of this polymer is lost, which leads to the peeling off of these monolayers from the polymer surface. And these monolayers are stocked and then transfer to the site of interest. Thereby we can achieve the vascular network formation at the site of interest.

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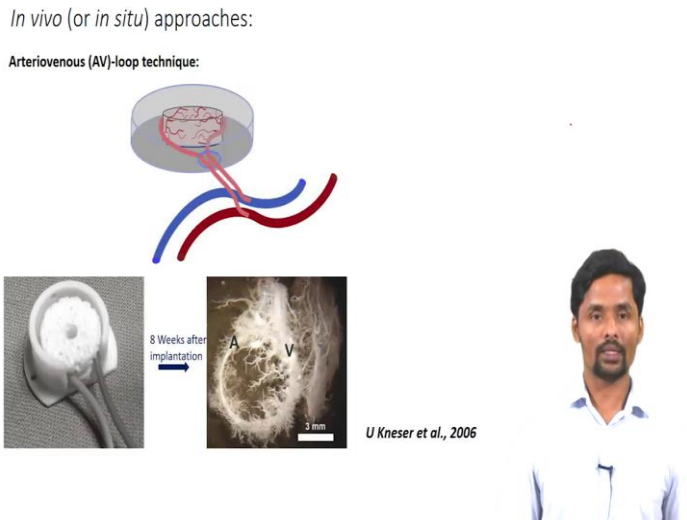

The next approach is In vivo approaches; in this, there is a technique called Arteriovenous loop formation. As you see here, the synthetic vessel is connected to the artery and venous surgically. After surgically connected to the artery and venous, this scaffold, this setup is implanted in vivo. As you see here, the synthetic vessel is looped inside this scaffold once we implant inside. Over a period of time, there will be sprouting of capillaries into this scaffold as we see over here.

Once there is a sprouting of capillaries into the scaffold, you surgically remove from that site and implant it to the site of your interests, that is injured site. Thereby, we can achieve the vascularization into the scaffold.

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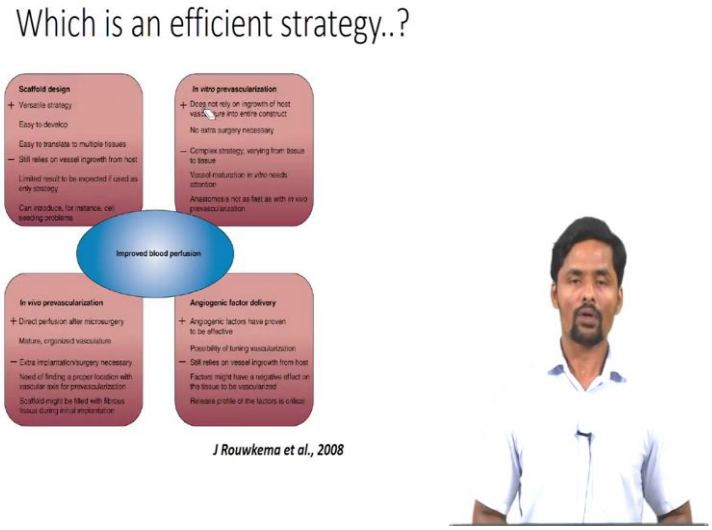

Which is an efficient strategy? As you know, all these techniques come with certain advantages and disadvantages. For example, in the case of scaffold design, it is easy to fabricate, but again the disadvantages are it relies on the ingrowth by host vasculature. In the second method in vitro prevascularization, it does not rely on the ingrowth by host vasculature, but again the perfusion rate is low compared to the in vivo prevascularization.

But in case of in vivo prevascularization, though it does not rely on ingrowth by host vasculature and it involves very rapid perfusion once after implantation. But again, this method involves the surgery. The fourth method is angiogenic factor delivery; this approach has given a promising result. But again, it depends on ingrowth by host vasculature. So, it is difficult to say which strategy is an efficient one.

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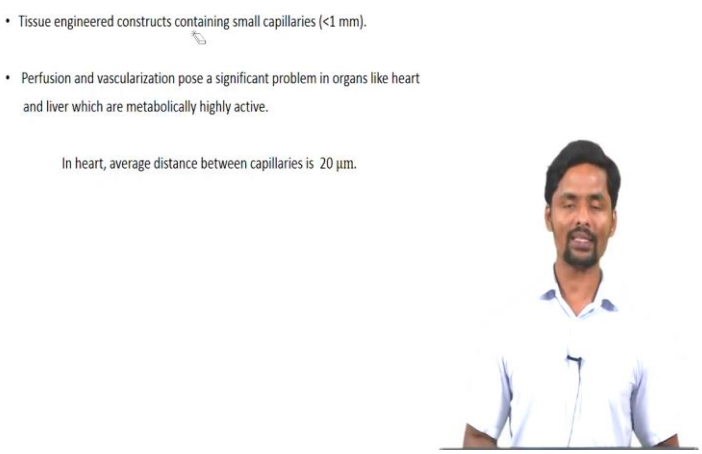

Again, there are few other challenges in vascularization; for example, it is difficult to construct a tissue engineering scaffold with smaller capillaries whose diameter is less than 1 mm. Perfusion and vascularization is a significant problem in metabolically highly active organs like heart and liver. For example, in the heart, the inter capillary distances is around 20 meter; achieving such are highly vascularized tissue is a challenge.

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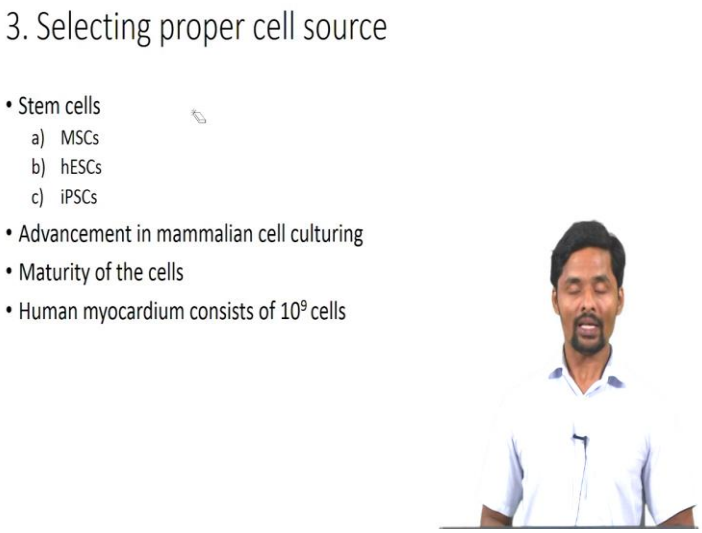

We next move on to the third challenge that is Selecting Proper Cell Source. In this section, we will talk about different types of stem cells, which we have explored in tissue

engineering applications. As we know, stem cells have the potency to differentiate into many numbers of cells, and we will talk about a few of these stem cells which have been exploited in tissue engineering.

The first one is mesenchymal stem cells. Mesenchymal stem cells are multipotent adult stem cells. These stem cells are able to differentiate into several cell types of the body, but not all. These mesenchymal stem cells can be isolated from different parts of the body, for example, bone marrow, adipose tissue, and dental tissues. But the problem with mesenchymal stem cells, they are multipotent but not pluripotent.

The second stem cell which we are talking about is human embryonic stem cells. These embryonic stem cells are isolated from the inner mass of the blastocysts. These stem cells are pluripotent in nature, which means they are able to give rise to any cell types of the body. But the problem is associated with the embryonic stem cells is the ethical concerns as we are extracting this stem cell from the embryo.

And the recent development is induced pluripotent stem cells. What they have done is they induce pluripotency in the normal somatic cells. What they have done is they have introduced four genes into the mouse fibroblasts cells. And thereby they could able to reprogram these somatic cells into these stem cells.

But the problem with stem cells is their proliferation capacity is very low in a two-dimensional cell culture plate. So, it is difficult to harvest the required number of cells that are required for our tissue engineering application. There are again certain advancements in conventional cell culture techniques. For example, exposing stem cells to the hypoxic condition led to the improvement in their survival. So, such advancements have been done quite recently.

Another problem is the maturity of these stem cells; they will largely differentiate into immature cells with variability in structure and function. And, another problem which we face in cells is producing a clinically relevant number of cells. For example, human myocardium consists of 10^9 cells. Generating such a huge number of cells is again a challenge in tissue engineering.

Thank you.