Tissue Engineering
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Lecture - 35
Vascular Tissue Engineering

Today, we will talk about an application in tissue engineering; so, this is the Vascular Tissue Engineering. The idea for this is to try to bring together all the basic fundamentals which we have looked at. So, let us start to discuss vascular tissue engineering which is one of the applications. I am not going to discuss any particular paper. However, what I am going to do here is give an overview of how an application should be approached when we are talking about tissue engineering.

You will have to see and understand some of the biologies and then try to come up with a design for how you would tackle the problem. We will talk about vascular tissue engineering because that is one of the popular areas which people are researching.

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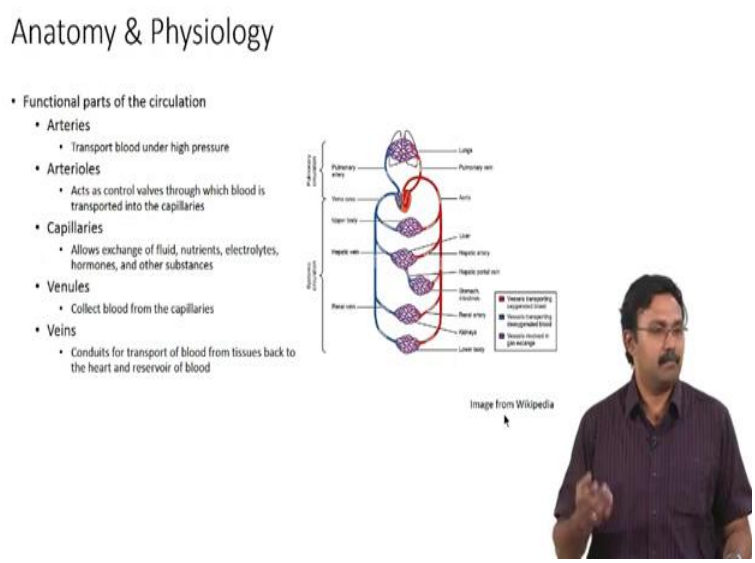

First, when we talk about vascular tissue engineering, we need to understand anatomy and physiology. There are different parts that are involved in the circulatory system. The functional parts which are involved are the arteries, arterioles, capillaries, venules, and veins. I have put them in this order because oxygenated blood first enters into the arteries, from where it branches out to enter into the arterioles. Then it goes into the

capillaries where oxygen transfer would happen, and the deoxygenated blood is collected by the venules, which then enters into the veins. This vein takes it back to the lungs and the heart, and so on. So, this is the overall structure.

The arteries transport blood at very high pressure. Arterioles act as control valves through which the blood is transported to the capillaries because, with that high pressure, it cannot enter into capillaries, which have very thin walls. Capillaries' job is to ensure there is an exchange of fluids, nutrients, electrolytes, hormones and any other substance that might have to be exchanged. Venules collect the blood from these capillaries, and veins are the conduits that transport the blood from these tissues back to the heart so that it can be pumped back again.

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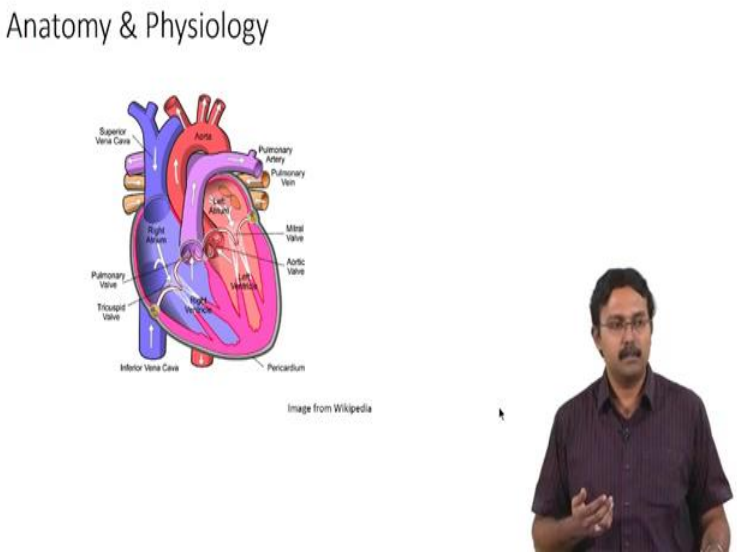

So, this is your heart. This is the detailed diagram which shows all the heart walls and all the arteries and veins which are coming out of the heart. We will not bother too much about heart anatomy and physiology. Here, we are only looking at vascular tissue engineering not really about cardiac tissue engineering. We are not trying to engineer the heart, but it is important to understand which blood vessels are actually coming out of the heart, and which blood vessels are supplying blood to the heart. Based on that, you might have to engineer them appropriately because they will experience different kinds of pressures and flow patterns.

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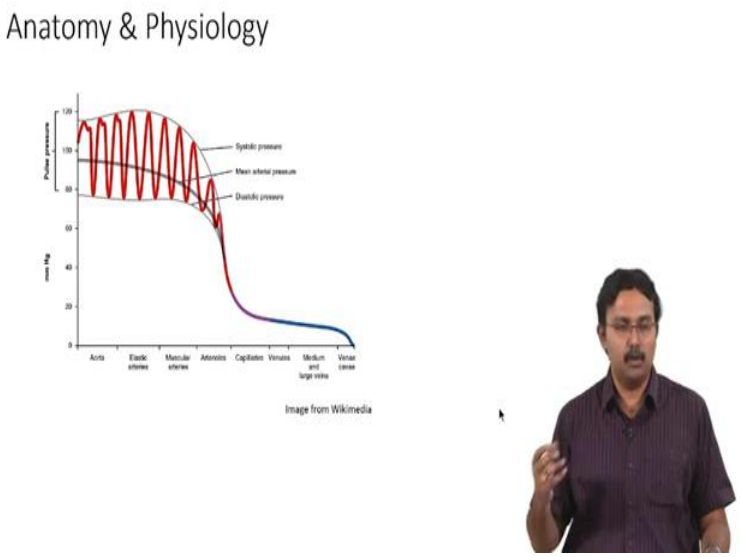

If you were to look at the pressure which these blood vessels experience, it is not uniform. It is not just because you have systolic and diastolic pressures; the pressure is also varying based on which blood vessel you are looking at. So, if you have to look at an aorta, the pressure is quite high. You are looking close to 80 to 120 mmHg, which is experienced by many arteries. As it comes into arterioles, you can see that the pressure is dropping and in capillaries the pressure is even lesser. Your venules and large veins and the venae cavae have much much lesser pressures.

So, Basically your heart is pumping the blood into the aorta, and because of this force, there is going to be a lot of pressure on the blood which is flowing into the arteries. As it goes into the arterioles which are smaller, they act as control valves reducing the pressure. Beyond that, there is no thrust which is pushing the blood; so, it is not being pumped in any way; it is just flowing along. Because of this, the pressure for these blood vessels is lower.

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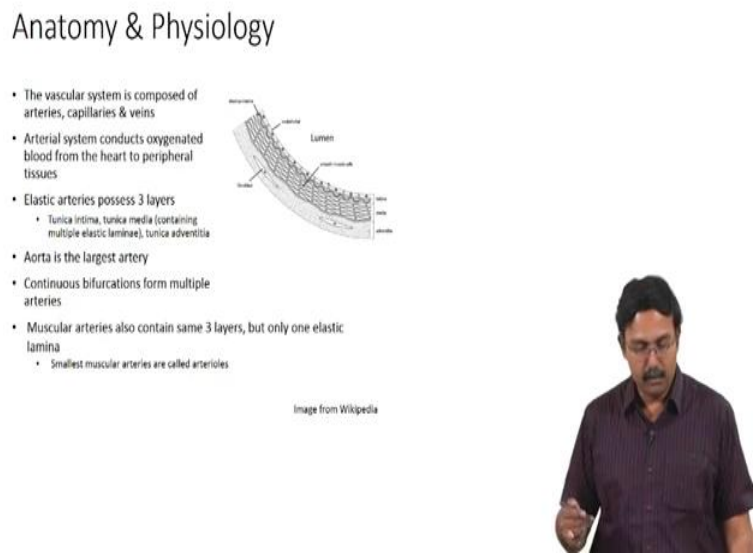

If you are talking about vascular tissue engineering, we need to understand what these blood vessels are, what they are made of. The vascular system is composed of arteries, capillaries, and veins. You mainly look at arteries when you are talking about the engineering of these vascular tissues. These arterial systems conduct oxygenated blood from the heart to the peripheral tissue. It contains three regions. So, the artery has the innermost region which is the tunica intima, and you have the middle region which is the tunica media and the outer region which is the tunica adventitia.

The tunica intima is what is exposed to the lumen, which means that is exposed to the flowing blood. Whereas your other tissues are not exposed to blood unless there is going to be damage to the tunica intima. The aorta is the largest artery, which is the one are which originates from your heart. This aorta then starts bifurcating, and it bifurcates repeatedly to form multiple arteries, which then form arterioles and so on.

Muscular arteries also contain the same three layers, but they have only one elastic lamina. Smaller muscular arteries are called as the arterioles.

Student: Muscular, muscular arteries they, do they have intima media?

Yeah, so, they have all the three layers. These three layers are present in everything. So, the intima, media, and adventitia are present in all arteries. However, in the aorta and these larger arteries, what happens is this media is much thicker. It has multiple layers of

smooth muscle cells, and it is very thick because that provides the ability for it to withstand higher pressures. As you move to muscular arteries and arterioles, this layer becomes thinner. So, you just have one layer instead of multiple layers of smooth muscle cells in your tunica media.

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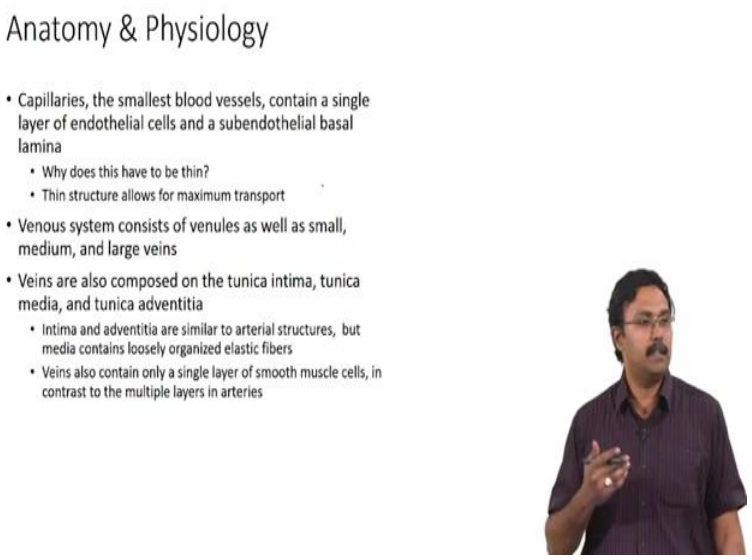

Capillaries are the smallest blood vessels that contain a single layer of endothelial cells, and a subendothelial basal lamina, it does not have anything else. Why do you think this is, this has to be thin?

Student: Easy exchange of.

Yeah, So, you want the nutrients and other things to be transported. So, you want to make sure that there is no diffusion limitation; for this reason, it has to be thin. The venous system consists of venules as well as small, medium and large veins. The veins are again composed of tunica intima, tunica media, and tunica adventitia; however, the compositions of these regions are different. Intima and adventitia are similar to arterial structures; however, the media contains loosely organized elastic fibers instead of tightly packed, as you would see in an artery.

Veins also contain a single layer of smooth muscle cells in contrast with the multiple layers which you see in arteries. This is all dependent on what kind of pressures these are facing. So, large arteries especially face very high pressures. So, the structure is designed

to be appropriate for that. However, the veins do not face that kind of pressure, or even smaller arterioles do not face that kind of pressure. So obviously, their structure and organization are going to be slightly different to fit the needs of that tissue.

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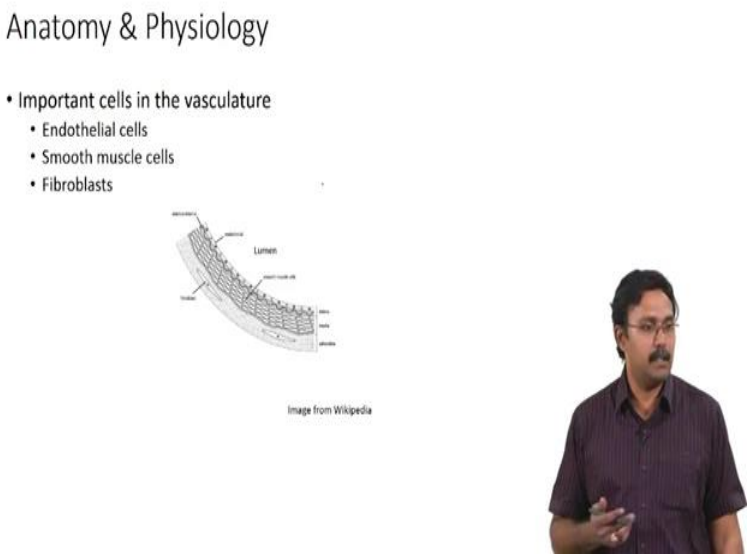

When you are talking about cells, there are three major cells that people look at. So, you have the tunica intima, which is the innermost layer, which is nothing but an endothelial cell lining. This endothelial lining prevents blood from getting activated, platelets from getting activated, it maintains blood homeostasis. Only when there is a rupture in this endothelial cell lining, you will have blood coagulation and wound healing cascades starting.

Smooth muscle cells are the cells which are aligned in the tunica media. Here, these are very tightly packed, and these cells constitute the major component in that region. Finally, you have the fibroblast, which is present in your tunica adventitia. This tunica adventitia has fibroblast, but it is not as dense as smooth muscle cells in your media. These are the three cells that people have to work with and you might have to arrange them in that particular structure to get your engineered tissue to emulate what the natural tissue is.

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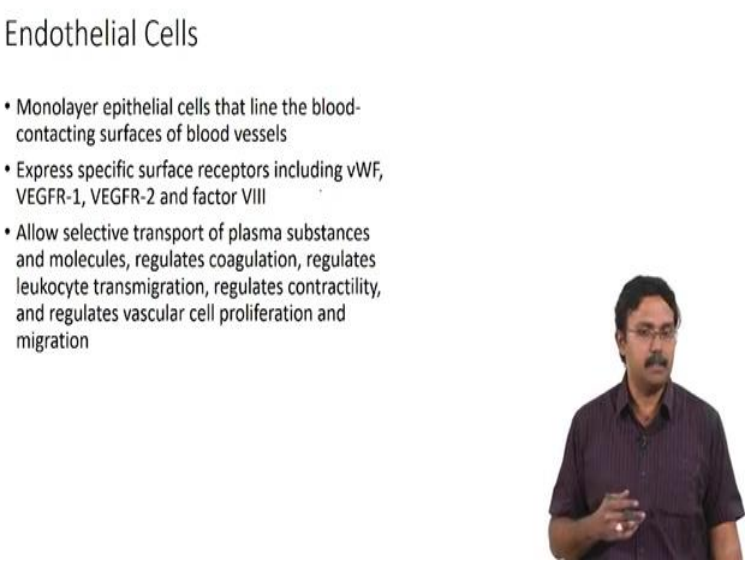

Endothelial cells are nothing but monolayer epithelial cells that line the blood-contacting surfaces of the blood vessels. They express specific surfaces receptors, which include von Willebrand factor which is vWF, VEGFR-1, VEGFR-2 and also factor VIII. These are all involved in different aspects of either angiogenesis or blood homeostasis. They allow selective transport of plasma substances and molecules, they regulate coagulation, they regulate leukocyte transmigration, contractility, vascular cell proliferation, and migration. So, these are the role of the endothelial cell lining in your blood vessels.

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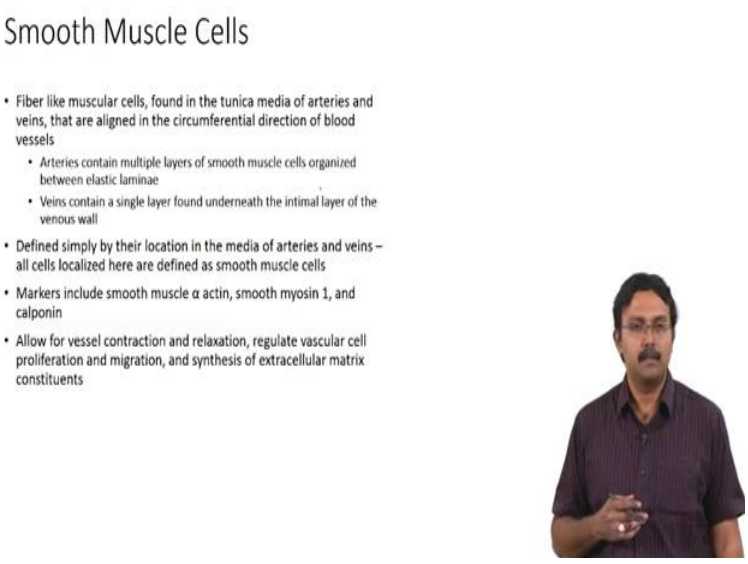

Smooth muscle cells are fibre like muscle cells that are found in the tunica media of arteries and veins. They are aligned in a circumferential direction of the blood vessels. Arteries contain multiple layers, as I was saying, whereas veins contain a single layer. These multiple layers in the arteries are organized between the elastic laminae. So, that provides the elasticity for the tissue. Veins contain a single layer which is found right underneath the intimal layer of the venous wall. Just below the endothelial cell lining, you have another layer of smooth muscle cells.

This is just defined by the location in the tissue because they are present in the media they are the smooth muscle cell. All the cells which are localized in this region are classified as smooth muscle cells. Some of the markers which are used to identify smooth muscle cells are α actin, smooth myosin 1, and calponin. They allow for vessel contraction, relaxation, and regulate vascular cell proliferation and migration; they are also involved in ECM secretion and production.

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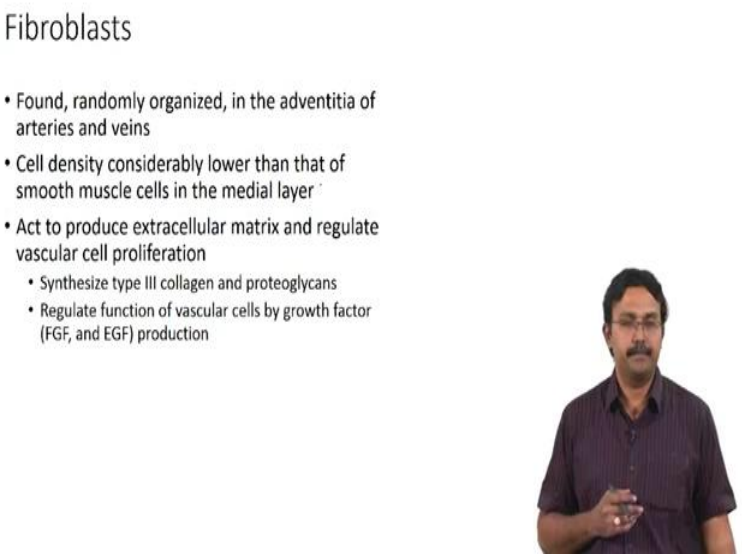

Fibroblasts are the last set of cells that are found in the tunica adventitia. These are found as randomly organized cells. The cell density is significantly lower as I was saying. They act to produce either ECM and regulate vascular cell proliferation. They can synthesize type III collagen and other proteoglycans; they also regulate the function of vascular cells through growth factor production. Some of the growth factors which are produced are fibroblast growth factors and epidermal growth factors.

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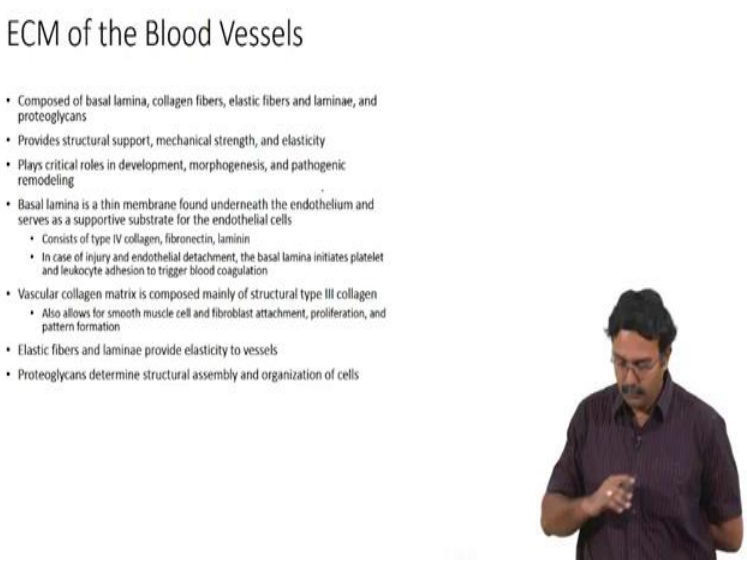

This ECM of the blood vessel is just like many other ECMs; you have collagen and other proteoglycans, but they also have a lot of elastic fibers to provide the elasticity. This ECM provides the structural support, mechanical strength and the elasticity for the tissue. It plays a critical role in development, morphogenesis, and pathogenic remodeling.

The basal lamina is the thin membrane that is found underneath the endothelium, which serves as a supportive substrate for the endothelial cells. So, it cannot just be cells, right? We looked at three layers. The tunica intima, we said, had endothelial cells, but these endothelial cells have to adhere to something. So, that is your basal lamina.

Basal lamina primarily consists of type IV collagen, fibronectin, and laminin. When there is an injury or endothelial detachment, the basal lamina initiates platelet and leukocyte adhesion, which triggers the blood coagulation cascade. The vascular collagen matrix is composed of primarily type III collagen. It also allows for the smooth muscle cells and fibroblasts to attach, proliferate and create the required patterns.

The elastic fibers and the laminae, which is present, provide the elasticity. Proteoglycans provide the structural assembly and organization of the cells. So, each of these has its own importance, and in the way, the tissue is developed.

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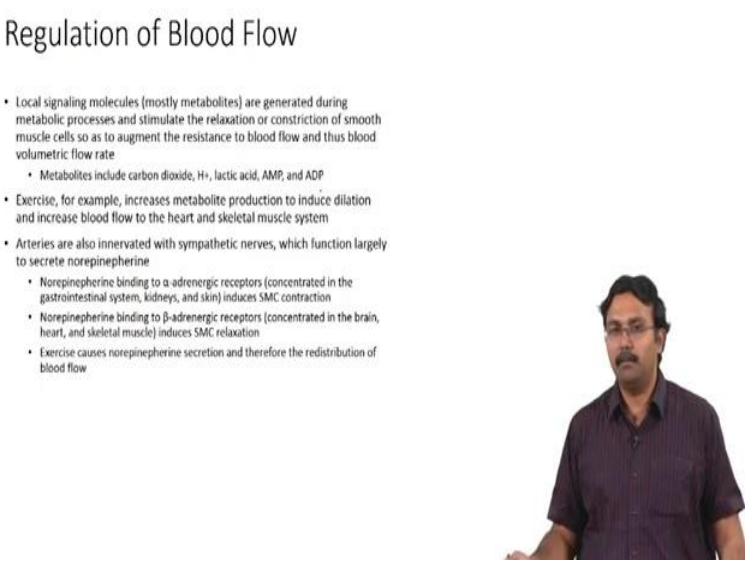

There is regulation of blood flow when it comes to these tissues. So, you have to have blood flowing in one direction. There are local signaling molecules that are generated during the metabolic processes, which stimulate relaxation or constriction of the smooth muscle cells.

This provides resistance to the blood flow, thereby regulating the blood volumetric flow rate. Some of these metabolites include carbon dioxide, hydrogen ions, lactic acid, AMP and ADP. When you exercise, you can actually increase these metabolite productions. Thereby, you can induce dilation and increase blood flow to the heart and other skeletal muscle systems. Arteries are also innervated with sympathetic nerves; their main function is to secrete norepinephrine.

Norepinephrine, which binds to receptors can induce smooth muscle cell contraction. When it binds to some other receptors, like the beta receptors, it can induce smooth muscle cell relaxation. So, this helps in regulating the blood flow; it helps you make sure that there is a pulsatile effect which causes the blood to flow. Exercise can again cause norepinephrine secretion. Therefore, create a redistribution of blood flow because you might have more pulsatile moment of your blood vessels.

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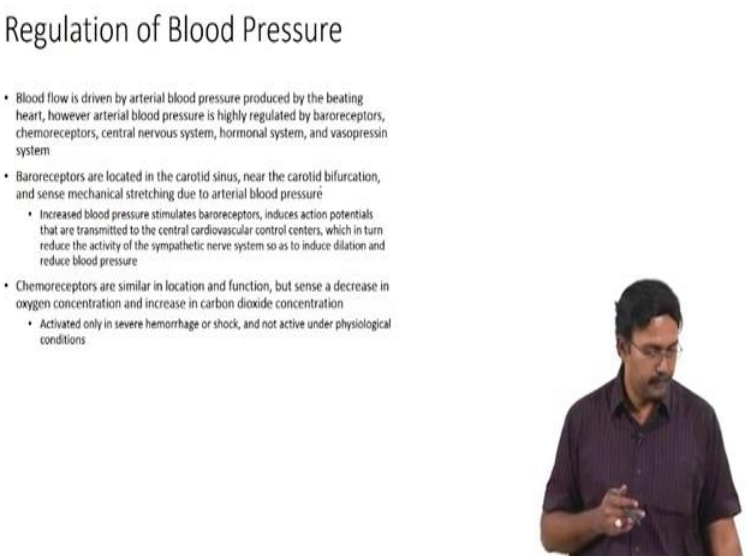

The blood flow itself is driven by the arterial blood pressure, which is produced by the beating of the heart. However, this pressure is also highly regulated by other receptors like baroreceptors and chemoreceptors, central nervous system, hormones, and the vasopressin system and so on.

Baroreceptors are located in the carotid sinus, near the carotid bifurcation. They sense mechanical stretching due to arterial blood pressure. And increased blood pressure can stimulate baroreceptors, inducing action potentials that are transmitted to the central cardiovascular control, which will then reduce the activity of the sympathetic nerve system so that there is dilation and reduced blood pressure.

Chemoreceptors are similar in location and function, but they sense a decrease in oxygen concentration and an increase in carbon dioxide concentration instead of looking at mechanical stretching. So, the signals which they read are different between chemoreceptors versus baroreceptors.

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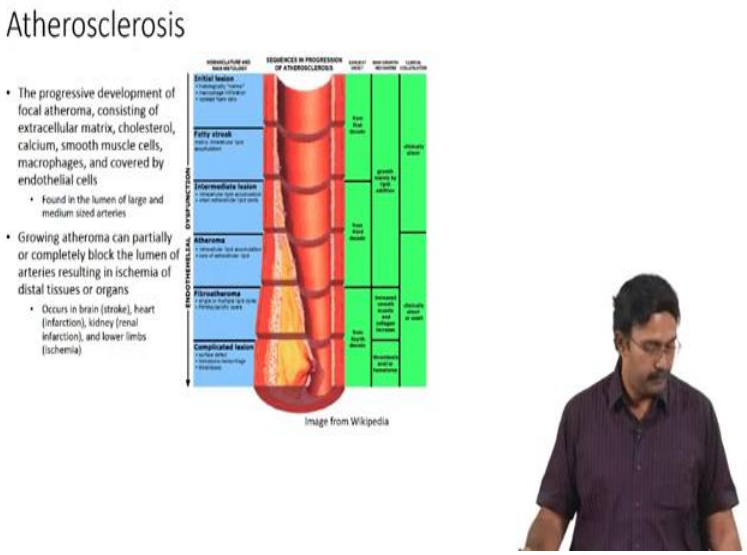

Why do we need to look at vascular tissue engineering? Atherosclerosis is a very common disease condition. This is the progress of development of atheroma, which consists of ECM, cholesterol, calcium, smooth muscle cells, macrophages, and it is covered by endothelial cells. This is a plaque that gets deposited, and it is found in the lumen of the large and medium-sized arteries. So, this will create a block which prevents blood flow, which can cause serious complications.

The growing atheroma can partially or completely block this lumen, depending on how big it is and at what stage you are looking at; this can happen in different parts of your body, which can cause different kinds of complications. If you have it in your brain, you might end up with a stroke. If you have it in your heart, you end up with the myocardial infarction. If it is in the kidney, you might have a renal infarction. If you have lower limbs, it can lead to ischemia. All of these can cause serious tissue damage and even loss of life.

Student: Does not this happen in small diameter vessels?

Yeah, it does; See in small diameter vessels, if one vessel is blocked, it is usually not that bigger problem. Because you would have multiple capillaries, and even if one of them is blocked, it may not cause very serious ramifications. However, if your large blood vessels are blocked, larger arteries are blocked, it will be a serious problem because supply to many regions will get affected.

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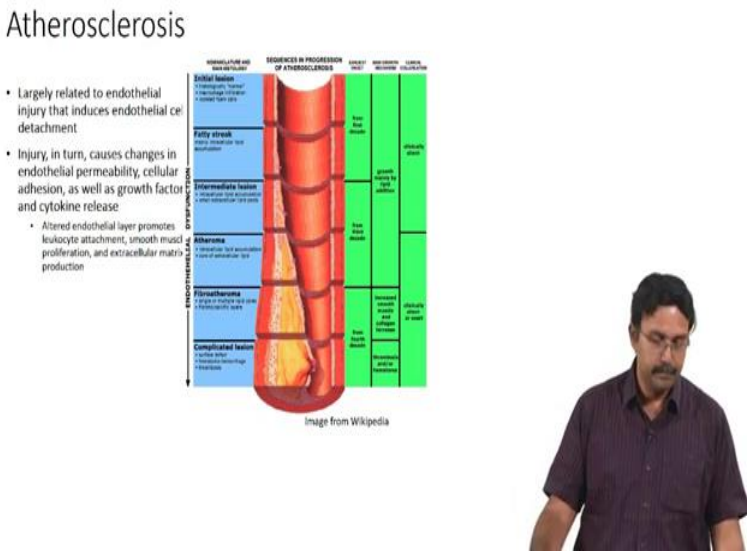

Atherosclerosis has been identified to have a lot of correlation with endothelial dysfunction. Even when there is endothelial damage, what happens is endothelial permeability changes, and cellular adhesion also changes. So, you have more fibroblasts and smooth muscle cells adhering there causing this kind of plaque deposition. That is why a lot of interest has been growing with respect to understanding endothelial dysfunction and atherosclerosis and looking at how diabetes affects endothelial dysfunction which can, in turn, cause heart disease. All these correlations are being studied to try and understand what is the cause? So, that is the basic biology side of it.

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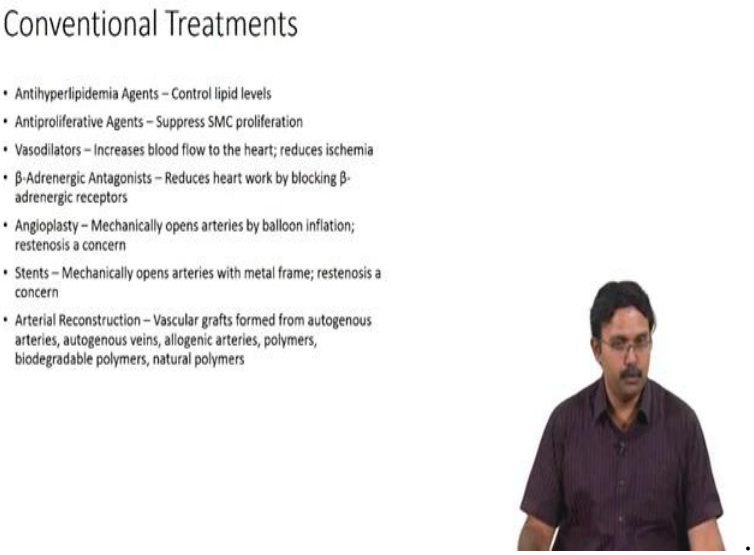

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Our goal is to look at treating this right. Conventionally the treatments are using anti hyperlipidemia agents, which control the lipid levels; you just take tablets. Or anti-proliferative agents, which will prevent smooth muscle cell proliferation. You can take vasodilators, which will improve blood flow to the heart and reduce ischemia. You can also take beta-adrenergic antagonist which will reduce the heart's work by blocking these receptors.

You can end up having angioplasty, where you mechanically open the arteries using balloon inflation. But restenosis is usually a concern; people nowadays do not do just balloon angioplasty. They go and place stents after doing a balloon angioplasty. Stents mechanically open the arteries with the metal frame and keep it open. As long as the stent does not collapse, it will stay opened. Again here, restenosis a concern. You have arterial reconstruction which is possible where vascular grafts from autologous sources can be reconstructed; you can also use polymers and other materials.

People have even tried using biodegradable polymers as for temporary fix, while the actual plaque is being treated. So, there have been many strategies. With angioplasty, people had initially also looked at taking a fan blade kind of a mechanism and cleaning out this plaque. But, what people realized was when they do that, there is aggravation with plaque deposition. So, they have stopped doing that now; they primarily do only stent placements. So, these are some of the conventional treatments

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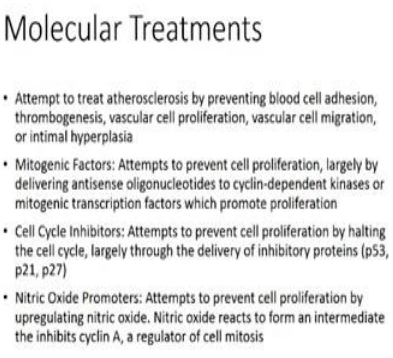

There are also molecular therapies that are done, which attempt to treat atherosclerosis by preventing cell adhesion, thrombogenesis or vascular cell proliferation, migration or intimal hyperplasia. There are different molecules that have been tried. People try to use mitogenic factors or cell cycle inhibitors and nitric oxide promoters, which have been used to try to prevent this formation of atherosclerosis or even reduce this problem.

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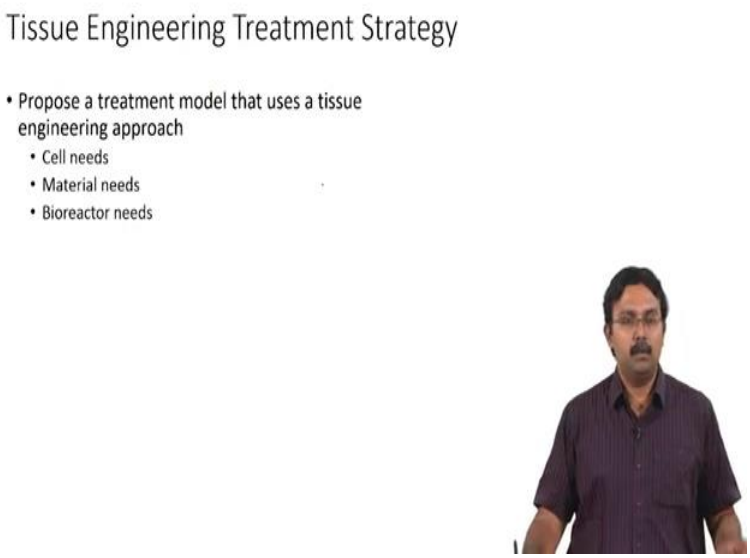

Our goal for today is to propose a treatment model that will use a tissue engineering approach. So, we need to engineer vascular tissue that would be useful in treating this condition. If that is the goal, we need to identify what would be the cells that are required, what would be the materials that are required, what would be the signals you would want to provide to this tissue which you are creating to make sure that it is conditioned for the application you are looking at.

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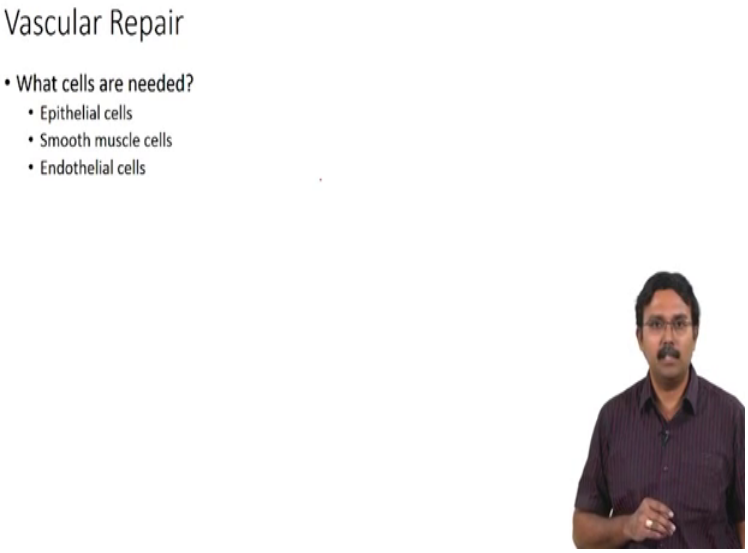

So, this is basically what I had. I will just quickly go through because we are running out of time, so epithelial cells, smooth muscle cells, endothelial cells.

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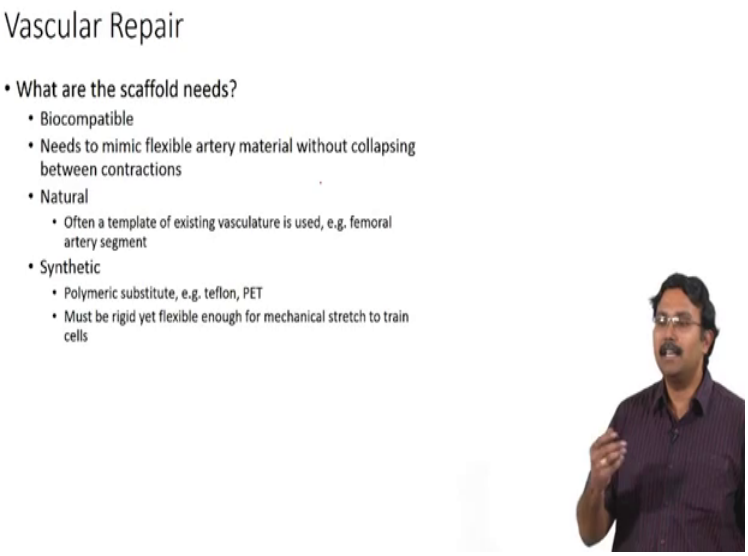

Scaffolds, obviously, biocompatible, flexible, and should be able to withstand contractions, and at the same time, it should not collapse because it needs to be stable enough to avoid collapse. If you are going to use something natural, then using a template of existing vasculature, which should be your decellularized matrix, or if you are going to use synthetic PET and Teflon would be the alternate which are commercially looked at.

It must be rigid and flexible enough for mechanical strength to train the cells. Because the cells which you are seeding may not have experienced the same kind of mechanical pressure. So, you need to condition them.

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The reactor should have a pulsatile flow system that can have a flow rate of about 5 litres of blood per minute, and it should be able to withstand pressures in the range of 1 to 5 kilo Pascal. It should also have media exchange for long term growth because you are going to have multiple layers.

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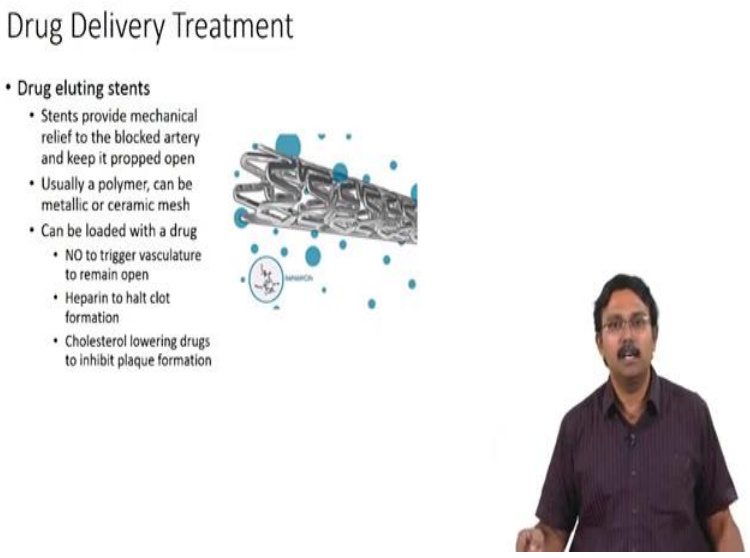

The other aspect is people usually work with drug eluting stents. You could also look at loading drugs, drug molecules to these scaffolds, which could help in anti-inflammatory properties and ensure there is better host integration. So that is also an aspect which people can look at.

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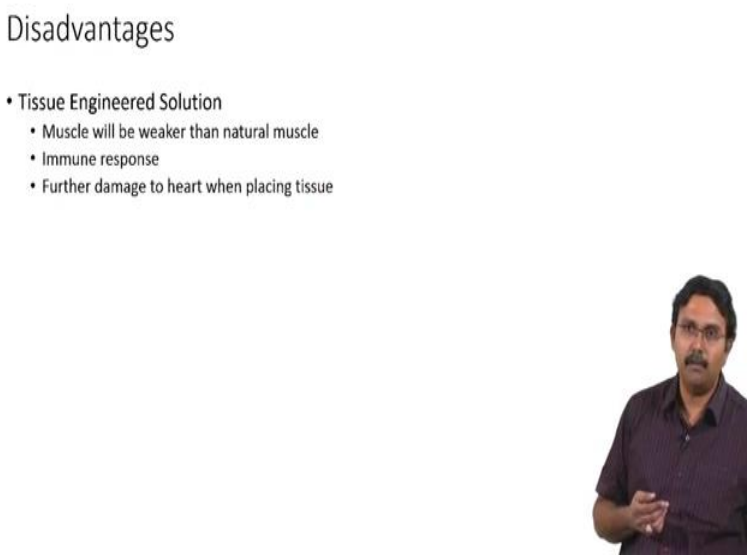

But there will be challenges with respect to this being weaker than the natural muscle, there can be immune response and there can be further damage to the tissue while it is being placed. So, these are some of the challenges which have to be accounted for.