Tissue Engineering
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Lecture – 36
Corneal Tissue Engineering – Part I

Good morning everyone. This lecture is about Corneal Tissue Engineering. We are going to see why Tissue Engineering is necessary for corneal repair. There are going to be two videos on this. The first one will cover about the corneal anatomy, the physiology, the tissues, the layers between the cornea's layer, what is the function of the cornea, and why there is a damage or why there is a necessity for repair. We should also know what are the treatment approaches that are there and why do we need a tissue engineering approach for this corneal repair.

In the first video, we will be covering the corneal physiology and anatomy, and the next one will cover about the tissue engineering approaches of corneal repair.

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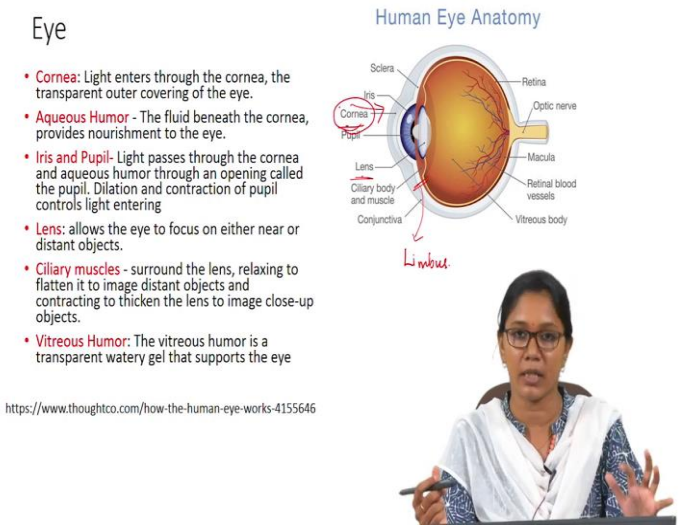

Taking through the parts of the eye, we have the cornea to be the first one. The cornea is the outermost layer of the eye, and this is where the light enters, and the transparency is the major reason or the major strength of what the corneal layer or the skin barrier is. Then we have the aqueous humor. So, the aqueous humor is the fluid that is behind the cornea that provides the nourishment to the eye. Then we have the iris and the pupil.

We all know what is a function of the iris and the pupil. The light that passes through the cornea enters and reaches the iris and the pupil, and that is where you get your vision. The dilation and the contraction help you in seeing the near and the far distant objects.

Then we have the lens that is behind the pupil, here this is the lens, and then we have the ciliary muscles surrounding the lens. Then we have the vitreous humor that is the transparent watery gel that supports the eye. Then we have the optic nerve that is where the nerve enters the eye, and then it moves on to different ciliary nerves and enters the corneal layer that is through the sclera. So, there is a connection between the cornea and the sclera, and this region is called the limbus and is where your nerves entered.

Out of all this, we are going to be studying about cornea why a tissue engineering approach is necessary for corneal repair.

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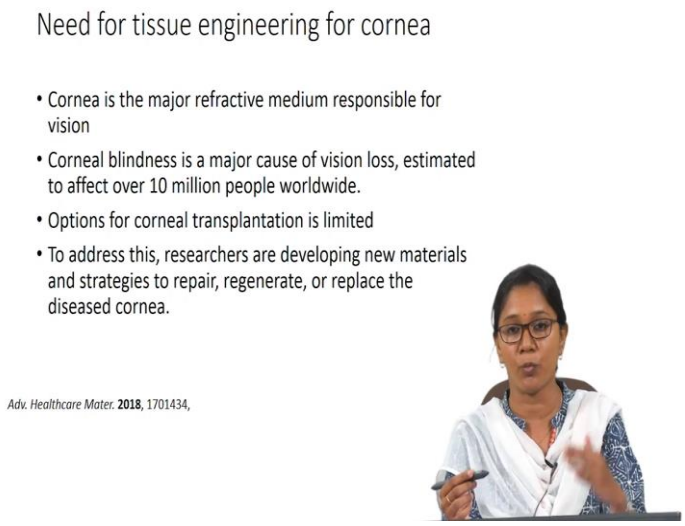

Why do we need a tissue engineering for corneal repair? As I have already mentioned, the cornea is an avascular membrane, and it gives you the strength and the necessary transparency, and it is a major region of why refraction of the light happens, and that is the reason why a vision is possible. Corneal blindness is a major cause of vision loss, which is affecting over 10 million people worldwide, and the options for corneal transplantation are there. So, either you can take an autograft or an allograft option.

In the case of an autograft, if there is one eye that is affected, the tissue from the other eye, which is fine, can also be used. The corneal layer or the corneal tissue is very extensive, which will we will be covering in the next few slides. So, the transplantation is not as easy as it can be explained. Another thing is the corneal donors, which can have a tissue rejection. So, to address all these issues, to avoid this, researchers are developing new materials and strategies to repair, regenerate, or replace the diseased cornea.

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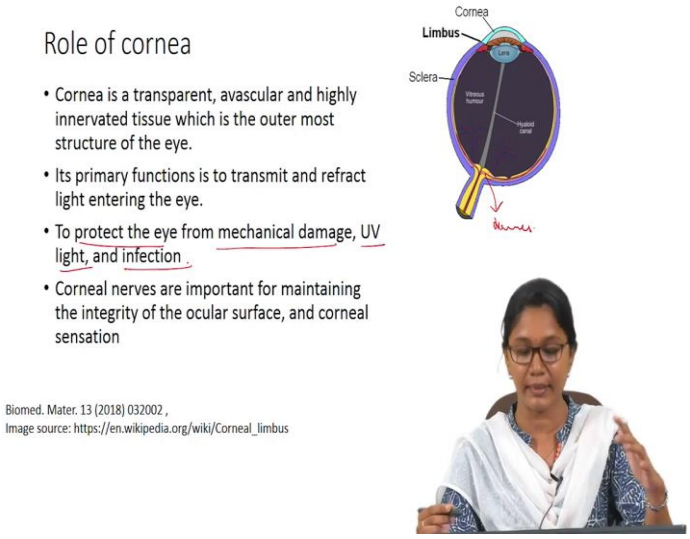

Here, in short, the same eye that we are looking at, we have the cornea that is the outer transparent region, which is the major refractive zone. Since it is avascular, but it is innervated. There are millions of nerves that go through the cornea, that is from the optic nerve when we see this red and the blue line here. So, these are the nerves. These nerves enter and through the limbus, enters through the lower regions of the cornea going up to the epithelial region.

The primary function of the cornea is to transmit and to refract light, and that is the reason we are able to see and visualize things. It also protects the eye from mechanical damage, UV light, and infection. The corneal nerves are very important for maintaining the integrity of the ocular surface and the corneal sensation.

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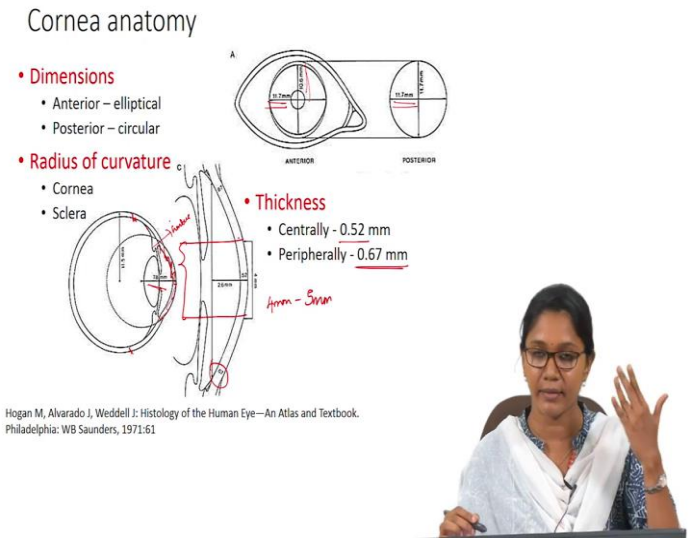

Let us go in detail about the corneal anatomy and that of physiology. Here, we will look at the dimensions of the cornea. This picture A, gives you the anterior position of the cornea and then a posterior view. When we see here, the anterior position is elliptical in shape with an 11.7 mm width and then a 10.6 mm of the vertical region. Then the posterior one is circular with 11.7 mm of the radius. All this is very necessary because the corneal curvature and the corneal shape is very important.

When there is a repair, that is either there is a bulge or a swelling or a decrease in the transparency; there is a change in these elliptical anterior and the posterior positions. When there is a change in your bulge, what happens is people when they use contact lenses, the contact lenses do not sit on their eye properly because the curvature of the eye changes. So, the radius of the curvature is also controlled by your corneal tissue.

The anatomy of the cornea is very important. Over age though, there are still variations from person to person, but with disease and with wound, there is a drastic difference that is associated with the corneal tissue damage. Then the radius of the curvature is given here. So, here we have the picture of the corneal radius of curvature, which is around 7.8 mm and the outer radius of curvature is around 11.5 mm, that is including the sclera.

The thickness is very important. The thickness is different in both the regions that are the central region of the cornea and the peripheral region of the cornea. So, the central region

is around 0.52 mm, whereas, the peripheral region is around 0.67 mm. This region, around 4 mm to 5 mm of this, is the central corneal portion that is of prime importance.

This is where your light enters, refracts, transmits, and this region is of importance, and different layers are what we will be looking at. Any disease or wound that we will be incurring in the corneal tissue will have a change in either their thickness, the radius of curvature or their size or shape.

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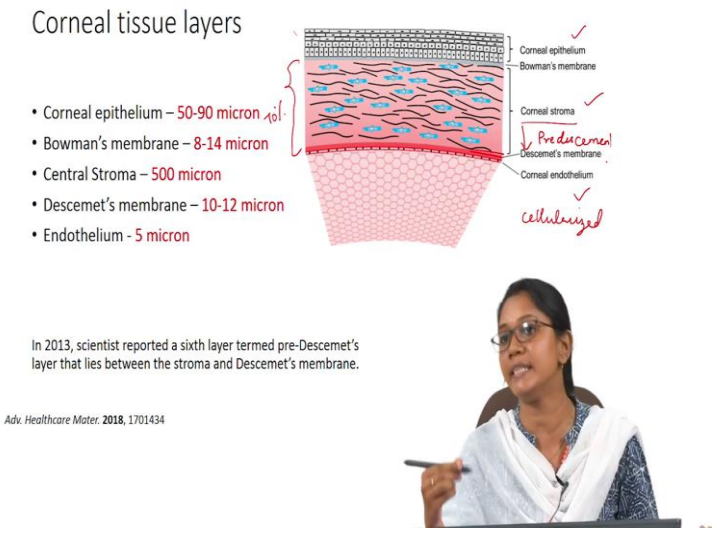

Now we know that there is a difference in the curvature radius or thickness, but we need to know what is the underlying process or what causes this to happen. Here, we have the corneal tissue layers. The figure here will show you an image of the different layers that are being in the corneal tissues. The corneal tissue for a long time has been divided into 5 layers. That is the corneal epithelium, the bowman's membrane, the central stroma, Descemet's membrane, and the endothelium.

Recently in 2013, scientists have reported that there is a 6th membrane that lies between the central stroma, which is here to here, that is called the pre-Descemet's membrane. So, it is pre-Descemet's membrane. So, this membrane is between the corneal stroma and the Descemet's membrane. Now there is a lot of critical thinking going on in evaluating whether there is a differentiation in the membrane between the Descemet's and the pre-Descemet's membrane. And it has found that the cells between them are different, and they also have a different function associated with them.

Now the scientists are classifying that we will have 6 different layers in the cornea layer of epithelial, bowman's, stroma, pre-Descemet's, Descemet's, and the corneal endothelium. The thicknesses of these respective mediums range from around the corneal epithelium to be 50 to 90 microns, which is huge. The 500-micron thickness of the central stroma accounts for around 70 percent of the overall region of your corneal layer, and then we have the Descemet's membrane and the endothelium, which are single-layered cells.

So, these are stratified squamous cells, the corneal epithelium, and then the endothelium. There are cellularized and decellularized cells. The corneal epithelium, this stroma, and the corneal endothelium are all cellularized whereas, the Bowman's membrane and Descemet's are acellular or de-cellular membranes.

Now we will be looking into further what is the functionality of these systems and how they repair, and the regeneration process of each of these layers will affect the corneal function.

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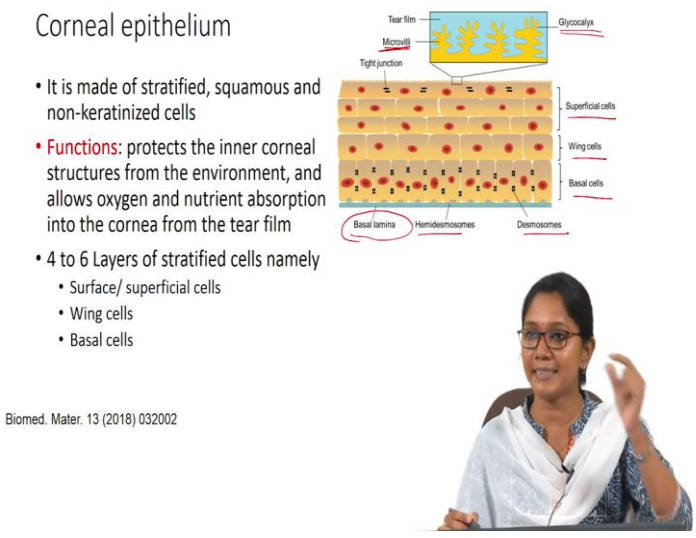

The first one is the outermost corneal epithelium that we are looking at. This is a more zoomed version of only the top layer of the stratified squamous epithelial cells. We all know what an epithelial cell is. It is the outermost cell layer, similar to how our skin cells are. These are the corneal epithelial cells that cover the outer surface. Similar to its function, the first thing is that it will give you the mechanical strength, and then it acts as

a barrier for any of the infections or microbes entering the cells. So, this is like the wall that is the first and the foremost region. It also allows the environment, such as oxygen and nutrient absorption into the cornea from the tear film.

Here, this corneal epithelium is further divided into 3 different layers, the superficial cells, the wing cells, and the basal cells. We have tight junctions within them. These cells are with tight junctions to have a compact layer, and the topmost superficial cells have microvilli. So, this is a zoomed portion. Similar to how a compound wall has broken horned glasses or something, we have the microvilli with the glycocalyx. This interacts with the tear film, and this is the outermost region.

This tight microvilli and glycocalyx are very important. Because when you have corneal transplantation from a donor, only when you have these microvilli and glycocalyx, it will start having the connection with the tear film and the tissue will start having the normal functions to be replaced. When the microvilli and the glycocalyx is damaged, we need to have different cell transplant for forming these microvilli and glycocalyx. Then, there are 4 to 6 layers of these cells, the superficial cells, the wing cells, the basal cells, and another important thing is the basal lamina, which is the bottom-most layer of these cells. Desmosomes and hemidesmosomes are new terminologies. The desmosomes and hemidesmosomes are similar to how tight junctions are, but the hemidesmosomes help in connecting the basal cells to that of the basal lamina.

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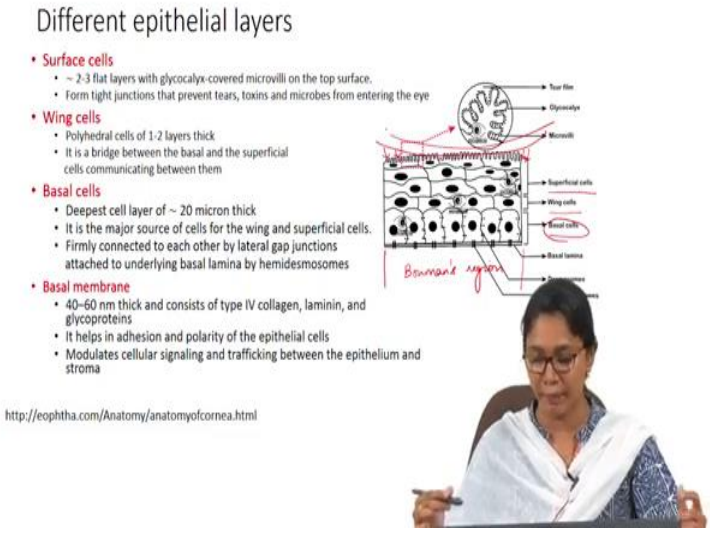

Going in detail about each of its functions and roles. First, as I have already told what a tear film; that is the outermost region. This is the tear film that is outside and will have a connection or interaction with that of the microvilli and glycocalyx, helping it as a barrier and allowing only a few things that are entering into the system. Then we have the superficial cells. The superficial cells are 2 to 3 layers of glycocalyx covered with microvilli. From these, the microvilli are starting up, and they prevent the tears, toxins from entering the eye. Then we have the wing cells. Wing cells are polyhedral cells. These form the second, which is the middle layer of the endothelial cells, which have a bridge between that of the superficial cells and the basal cells. This communicates between the superficial cells and the basal cells.

Then we have the basal cells. The basal cells are very important; it is a major source for the wing cells and the superficial cells. This is the one that balances the hemostasis of that of the corneal epithelial layer. These basal cells have tight gap connections, which is connected by the desmosomes or hemidesmosomes to that of the basal lamina. The basal lamina is the one that will connect to your lower region; that is your bowman's region. So, this is not cellularized. This will have glycoproteins, which will help in the adhesion to that of the Bowman's region. They have the collagen, laminin, and then the glycoproteins and help in adhesion. They also maintain the mechanical integrity of the corneal epithelium and avoids the influx of the keratocytes from the bowman's region to that of the corneal epithelial.

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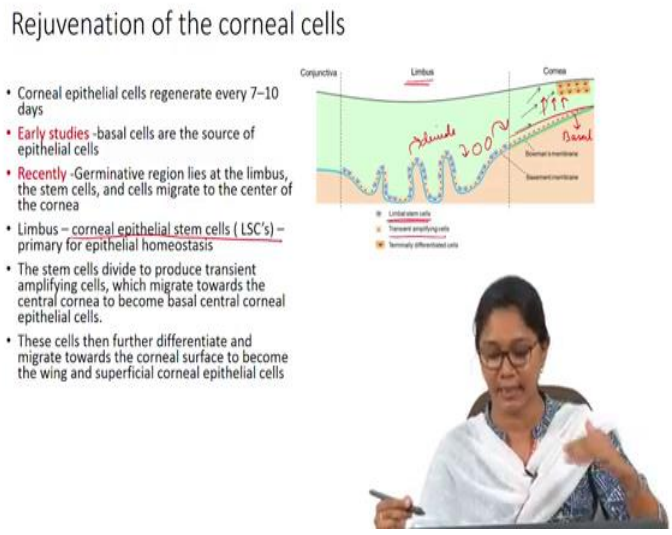

Now we were talking about rejuvenation. If there is a wound, it is going to repair itself. Similarly, the corneal wound or tissue damage will also have a repair on its own; the repair mechanism is known. Generally, it will take 7 to 10 days for the regeneration process to happen. From where this is happening? The earlier studies told that it would happen from the basal membrane. That is the cells that are known to have the wing cells and the superficial cells, the lowermost part of your epithelial.

Later, people have understood that this is just not the source. We have the Bowman's membrane the basement, and this is called as your basal membrane. This basal membrane cell is just not the source, but there is something else that is called as a limbus region. This limbus, which I have already mentioned, is between the cornea and that of your sclera, a connection. So, this is having limbal stem cells. The limbal stem cells are the stem cells that migrate. They first have asymptotic mitosis happening. The limbus corneal epithelial stem cells are called as LSCs. These LSCs divide to produce transient amplifying cells. First, the stem cells divide into many, and then they form cells. These cells then migrate, go to the Bowman's membrane, and from there, form the bigger layer. So, this is the principle. Now scientists have shown that both are necessary; that is, the limbal stem cell region, which is the major source of this transient amplifying cells. Their migration is an important process, and then the basal cells from which the cells are stratified into the wing cells and the superficial cells.

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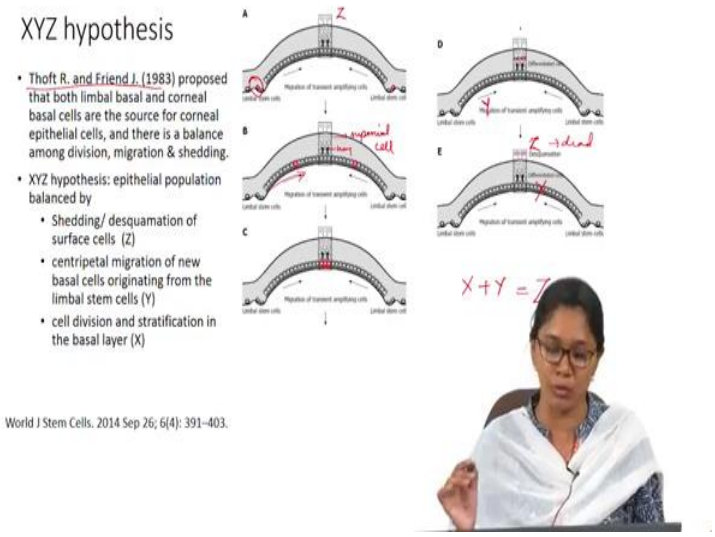

This theory was explained by an XYZ hypothesis. This picture will tell us what is happening. Thoft R and Friend J, these are the two scientists who first proposed this theory of the XYZ hypothesis in 1983. This showed that there is a corneal balance between the basal cells and the limbal stem cells, which helps in maintaining the corneal epithelial homeostasis.

The first thing is, please note this red color small stem cells that we are looking at. The limbal stem cells initiate it, and then they migrate. That is, they are forming transiently amplifying stem cells which can be differentiated into any stem cells. Here they migrate, this is by the centripetal force, migrate to the basal layer. This lowermost region is a basal layer, and as I said, the corneal layer is this. So, this is how we are looking at the eye, and these are the different regions. We will have a wing cell here, and then we have a superficial cell.

First, it migrates, goes to this place. After the migration, then in this step, they are differentiated into the different cells; either their wing cell, which is the second layer or the superficial cell, that is the third layer or the topmost layer, and they are squamized. So, this is the entire process.

So, what is this XYZs theory? The Z is the shedding. That is when the cells are worn out, with the tissue damage or by age, they become dead cells. So, that is desquamation. The rate at which the cells are worn out or when the process is being damaged. Then we have the centripetal migration. So, this migration is termed to be Y. Then we have the cell division or the stratification, which is X. All these 3 are combined, that is for this to happen,

$$X+Y=Z$$

Thus homeostasis is maintained by this theory. That the limbal stem cells, along with the basal stem cells, the differentiation, the mitosis process, and the centripetal movement, all play a role in maintaining this process. This happens upon a normal tissue repair and when there is a wound is created.

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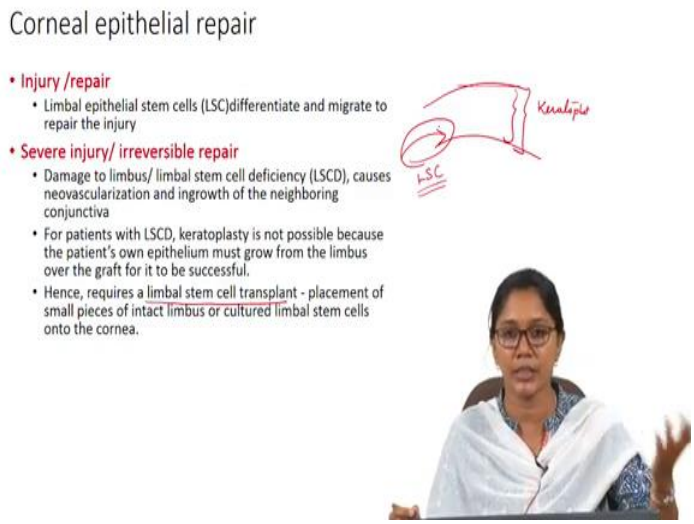

When an injury happens, limbal epithelial stem cells differentiate and migrate to repair the injury. But what happens when there is a severe injury to your epithelial cell? When the damage occurs to the limbus, so when there is a difference in your layers. In your corneal layer, when you have damage to any one of them, we can have the repair process happening. When there is damage to that of your limbus systems itself, what will happen? In that case, corneal transplantation will not be helpful.

Keratoplasty is a normal term for corneal transplantation. When any one of these tissues is damaged, you can either take it from your own eye as an autograft taken from the other eye or from an external donor that can be used for any of the corneal tissues. When there is damage to the limbal stem cells, a keratoplasty will not work. Because, only when these cells migrate, there will be no tissue rejection that will come as a problem.

For this, what we do is, there is a limbal stem cell transplant that is to be done. Here, we will have a limbal stem cell transplant or placement of the small limbal stem cell itself into the recipient's place for tissue engineering. For these, this is the normal approach. So, for this, we need a tissue engineering approach to have the use of the epithelial layer of mass.

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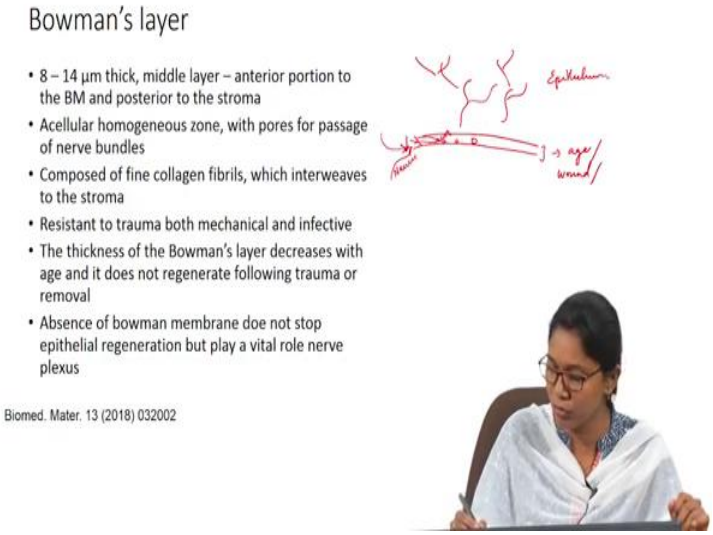

Now, we are moving into the second layer of the epithelium; that is the Bowman's layer. The Bowman's layer is around 8 to 14 micrometers thick. This is the region that lies between that of your epithelial and that to the stroma. Here, they do not have cells; they just have proteins, glycoproteins, and collagen. So, they get infused to the stromal layer. The stroma consists completely of collagen fibers. So, this is the border between that of your stroma and that of the corneal epithelium.

Here, it is resistant to both mechanical and infective legion. We know that the corneal region upon damage can repair on its own. Only when there is severe damage, it cannot repair. Whereas, the Bowman's, the thickness of this will decrease over age or with any wound or damage; so this keeps decreasing. But this will not affect your corneal epithelial layer because only above this is the entire process that is happening, and the Bowman's will not have a region. But there is something else that the Bowman is responsible for, that is the influx of nerve impulses.

I have already mentioned, that within this Bowman region is where from your optic nerve, your nerves enter the eye. So, from this, the nerve enters and then gets into smaller nerves and goes up to the epithelium. When there is damage to the Bowman, the corneal epithelial repair is not stopped, but there would be a problem in the nerve plexus that can have a major role to be played. So, this will have pores for the influx of nerves. So, this is the Bowman's layer.

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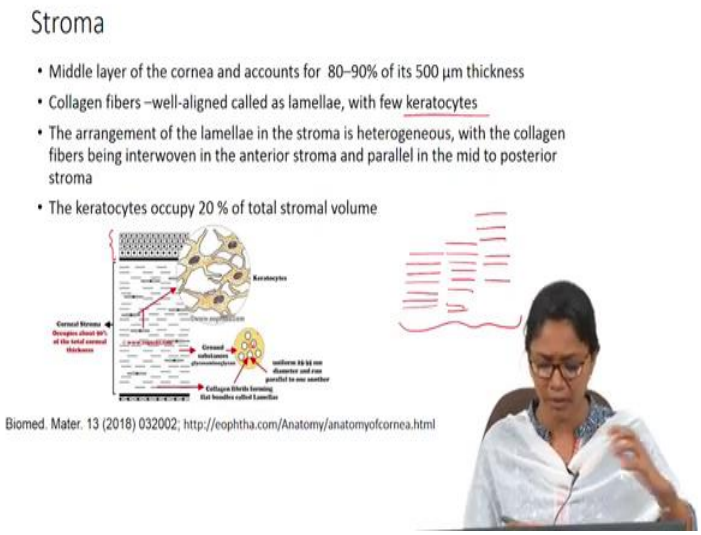

Then, we have the stroma. We have already spoken about what these layers are; the corneal layer, and then we have the Bowman's layer, and now, we move down to the major corneal function that is called the stromal region. The stromal region is the middle layer, accounts for 80 to 90 percent of this entire thickness. They have collagen fibers associated. So, the collagen fibers are well aggregated, and they are arranged in a parallel fashion called lamellae with a few keratocytes

There are two things here primarily maintained, that is the collagen and the keratocytes. The arrangement of the lamellae is heterogeneous; How a lamellae is formed is, these fibers are aligned in a parallel fashion in bundles, and these bundles are then further oriented. Here, they form nice good lamellae and they are differentiated to the anterior position and then to the posterior position. In between them, we have the keratocytes that occupy them to giving them the proper periodic arrangement and the patterning of the system.

Looking at this yellow substance is where these are the small bundles of your fibers. Looking them at a lateral view, these are the collagen fibers as bundles, and they are arranged into a specific diameter of 25 to 35 nanometer.

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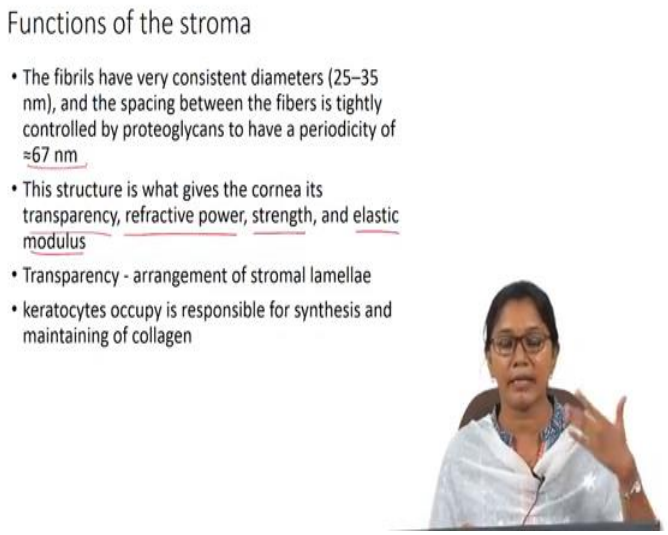

What are the functions of this big stromal layer? This stromal layer is known to associate the transparency of the corneal tissue. Whenever corneal tissue comes into your mind, transparency is one of the major functions, because that is the reason you are able to transmit and refract light and have a vision. So, these give you that property and then the elasticity and also the strength; refractive power, elastic modulus and the transparency.

The fibers are very consistently arranged as I have said and the spacing between the fibers is tightly controlled to give you a periodicity of around 67 nanometers. These numbers are very important because these numbers give you the periodic arrangement similar to how a crystal structure lattice has periodicity in the arrangement of the lattices. And then, the keratocytes occupy is responsible for the synthesis and maintaining of the collagen, giving you the extracellular matrix.

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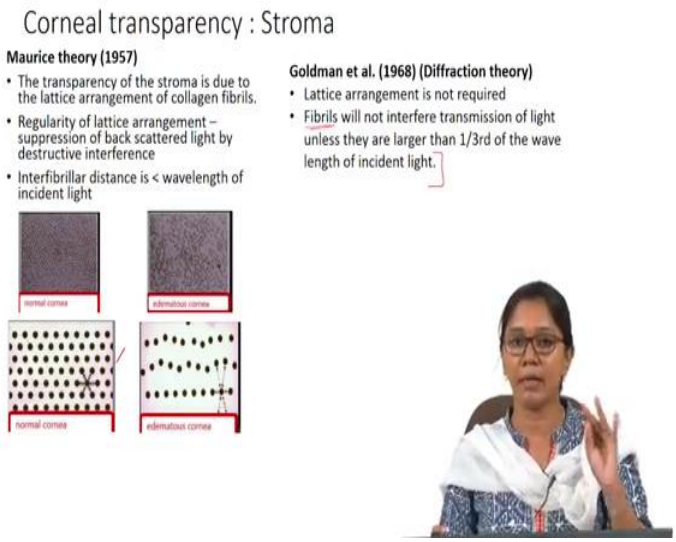

This transparency, as I have said, is the major function of what the stroma is. But why will a collagen fiber arranged in a lamellae position give you a transparent membrane? What is the functionality? For this, two people have developed their theories. The first theory was by Maurice, known as the Maurice Theory, in 1957. So, the transparency of the stroma is here that he has mentioned is because of the periodic lattice arrangement. As I have mentioned, in the crystal lattice, the periodicity is a major role. When the light comes and passes through, because of the lattice regularity of the arrangement, there is the separation of the backscattered light by destructive interference. So, there is the vision, there is proper refraction that is coming up, and this arrangement is the one that is the reason. It is what his claim is.

There is one more reason, that is the interfibrillar distance. The interfibrillar distance, as I have said, are all completely arranged. The bundles will be around 25 to 35 nanometer, and the interfibrillar distance is around 67 nanometer. So, these numbers are less than that of the incident light. The incident light is the light that we are seeing, which is around 400 to 700 nanometer. So, whenever there is a lesser than the wavelength of that light, there is going to be no interference that is being done. Two factors are responsible; one is the arrangement. The arrangement has a specific pattern packing, which is helping in responsibility for the transparency.

Later, after the Maurice theory in 1957, Goldman, based on the diffraction theory, proposed another statement. Here, we have a picture of the normal cornea, and then we have an edematous cornea, that is when there is edema. When swelling or something that happens, there is a bulge in your corneal layer and your transparency is reduced. In the normal cornea, there is a periodic arrangement that is happening, whereas, in this, there is no periodicity that is seen. So, this shows that the periodicity plays a major role in the transparency that is bulging or the clouding of your vision.

Goldman later said that the lattice arrangement is not the only requirement. He said that fibrillars do not interfere when the transmission of light to the transmission of light until they are larger than one-third of the incident light. So, the fibrillar diameters are very small. Similarly, the transmission of light, that is the light entering through is around 400 to 700 nanometer. Based on the diffraction principle, he says that since the fibrillars the dimensions are very small, they would not interfere with the transmission of light. They would interfere only when they are more than one-third of the wavelength of the incident light. So, periodicity is not a matter, but the fibrillar, as such, will give you transparency.

These are two theories that are ongoing, but both would say that the stromal layer is made up of the collagen fibers and the fibril arrangement, their fibril type, and the bundle packing and the lamellar packing is important in giving you the transparency, elasticity, and the mechanical strength.

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What happens when there is a stromal injury or a repair? As we know, there are two major components, the keratocytes, and the collagen fibers. The keratocytes help in increasing the collagen source. When they are injured, they activate and synthesize the new extracellular matrix. So, this is upon the normal injury and the repair process.

Upon severe disease or injury, cell death occurs, and then we have a scar tissue that is being formed, which is reflected as scarring of your cornea. Here, we have an entire keratoplasty that is to be done. So, keratoplasty, as I mentioned, is corneal transplantation. Either, it is penetrating keratoplasty or anterior lamellar keratoplasty.

Penetrating keratoplasty is when the scar is deep. Penetrating keratoplasty means when the entire corneal region is to be changed; that is to be replaced. Like, the stromal region as such or the entire epithelial along with a stromal. When we have an anterior lamellar keratoplasty, only when few of the regions are damaged, you can have a partial replacement of the corneal tissue. So, these are the ongoing current treatment methods for corneal transplantation.

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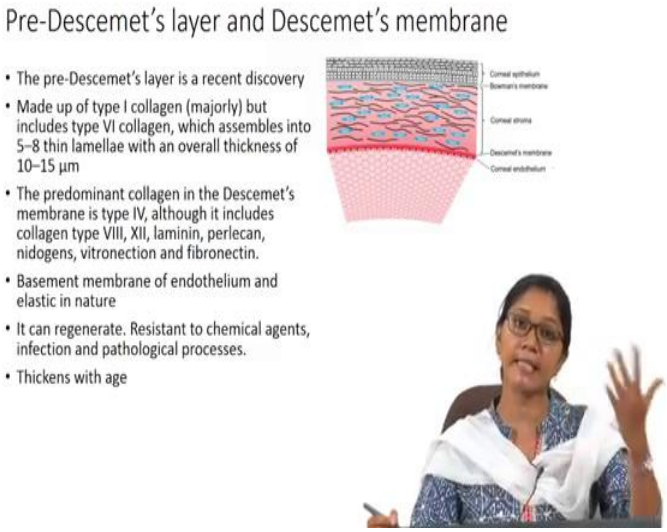

Now, we move on to the pre-Descemet's and the Descemet's membrane. As I have already told, the Descemet is the 4th membrane, but since from its inception in 2013, the pre-Descemet is the layer, that is before the Descemet's membrane. Both these membranes are acellularized membranes, so they contain collagens, laminin, lectins, and different types of collagen. Like before this, we will have the pre-Descemet membrane,

and it is made up of type I collagen that is the pre-Descemet, which assembles into the lamellae, and the overall thickness is around 10 to 15 micrometer.

After that, we will have the Descemet membrane. In this, we have the type IV collagen laminin, nidogens, vitronectin, and the fibronectin. This is the basement membrane of your endothelium. This membrane will form the bases for the endothelial cells to attach and have tight junctions. This membrane can regenerate and is assistant to chemical agents and infections.

So, we have looked at Descemet and Bowman's; both are acellularized membrane. This cannot regenerate whereas, a Descemet membrane can regenerate, and they are resistant to chemical agents infections and pathology. The other difference is over age, the Bowman's membrane reduces in thickness whereas, the Descemet membranes increase in its thickness over the age.

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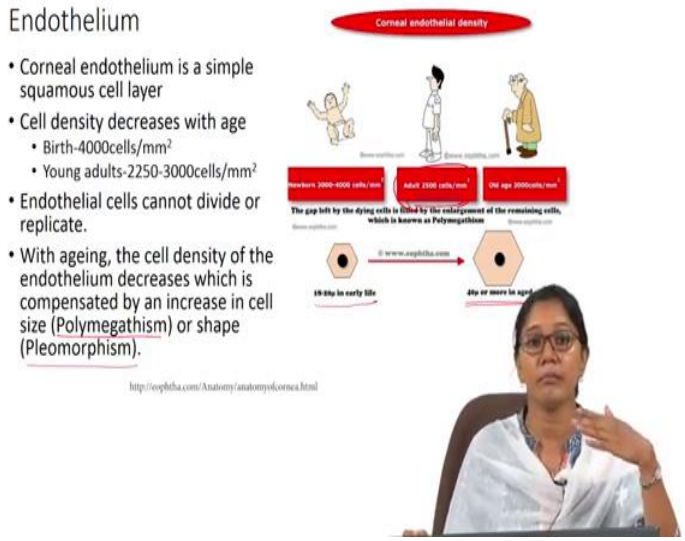

Then, we have the endothelium. The corneal endothelium is the bottom-most cell that we are looking at. So, this is the layer. It is a single layer, or one to two layers of cell thickness is what forms your corneal endothelium, the innermost cells of the corneal tissue. It is a simple squamous cell layer, and the cell density is a major role. So, the cell density of these endothelial cells decreases overage. For example, this picture will tell you that.

A newborn baby would have around 3000 to 4000 cells/mm^2. Whereas, as we all grow, the adult would have 2500 cells/mm^2. Whereas here with age, it further reduces to 2000 cells/mm^2. So, what happens? When these cells are reducing in number, we are going to have gaps in between them. So, to occupy that space, what happens is there is an increase in the size and shape. So, compensated by the increase in cell size and cell shape, that is polymegathism and pleomorphism.

In early life, it is 18 to 20 micron; over age, it becomes 40 or more; this is the characteristic of what endothelial cells is. As we know, epithelial cells will act as a barrier; the endothelial cells will perform the major functions for the cells. So, they will be metabolically very active for the passage of the flows. Similar to that, the corneal endothelium is also like that.

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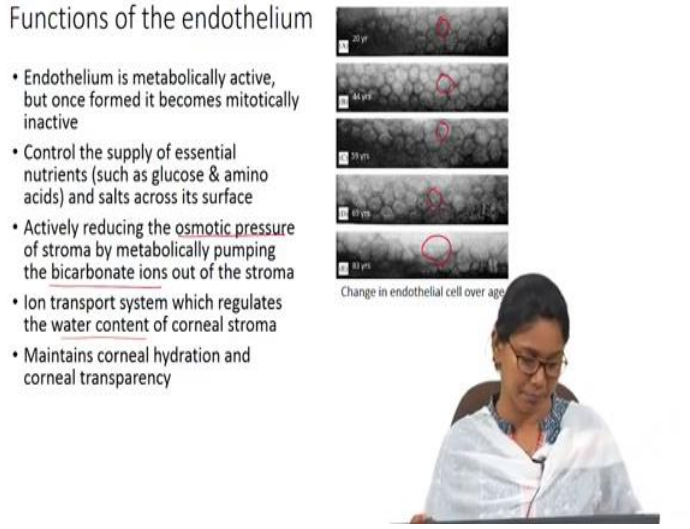

So, it is metabolically active; it has mitochondria, but it is mitotically inactive. So, that is the reason they will not regenerate. Once it is formed, it just degenerates over age, but it is not going to be replenished. The major functions are they are metabolically active, so they control the sources; that is the nutrient inflow and outflow from the cells within the rears. It controls what goes into the stroma and what is being pumped out of this stroma.

The stroma is a hydrated layer. So, that is also why it gives you transparency. This endothelium is acting as the pump. It has the efflux pumps pumping out the excess water and the fluids outside, maintaining the osmotic pressure. So, it maintains the osmotic

pressure and then the bicarbonate ions. So, it acts as the pump and then trans regulates the water content to the system.

This is another picture showing how over age, your endothelial cells size and shape changes. With 20 years, over 40 years, the shapes are increasing, though the cell number that is density is decreasing. To occupy those spaces, we have the size to be bigger. So, here if it is this, so over age we have found them to be bigger in cell size and shape.

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What happens when there is an injury to this layer? Arrested in the G1 phase. So, they have a G1 phase that is arrested. That is the reason they are mitotically not active, so they would not have cell division. With age and with any repair, they are going to reduce cell numbers. They also result in the thickness of the basement membrane and the endothelial cell density decreases.

But when there is a severe density decrease, that is less than 500 cells/mm. At that time, the endothelial cells will not be able to function; that is, they will not be able to pump out the excess fluid from the stroma. So, which will lead to a swelling of the corneal layer, there will be swelling or a bulging of the layer; the transparency would be minimized, and there would be a cloudy appearance, therefore, distorting your vision.

This is the region when there is a basement membrane issue; that is when there is a problem in your endothelial cells, your entire corneal cells will be affected and it has to

be replaced, corneal transplantation would be done. Viable stromal and epithelial tissues and endothelial keratoplasty or this endothelial keratoplasty will be done and the transplantation with minimal rejection can be performed. Only an endothelial cell layer can be transplanted if there is no damage to that of the other superficial layers.

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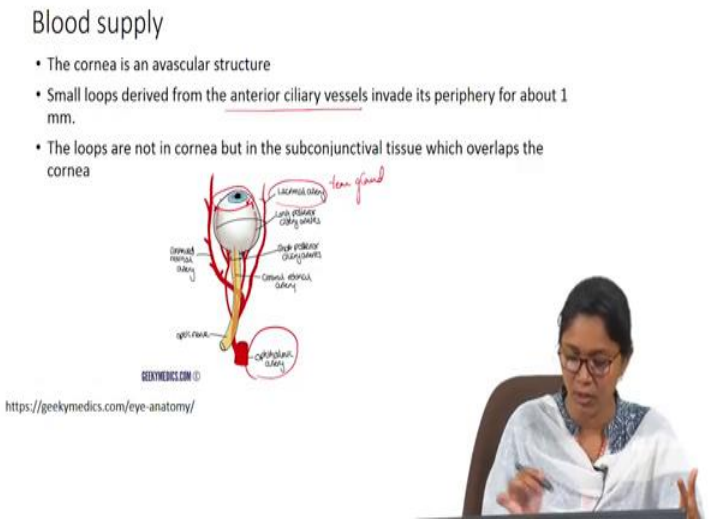

And what is the blood supply? So, the cornea is an avascular structure. So, here we have the optic nerve. So, the ophthalmic artery is here. This has got its blood vessels from the carotid artery. From here, it enters. The cornea is one of the tissues which are avascular, so it will not have any blood vessels within the corneal region. But only at its outermost junction, it would have few ciliary vessels; the anterior ciliary vessels invade at its periphery, not towards the center of the corneal regions.

The loops here what happens is, the ophthalmic artery comes, and it enters along with the optic nerve and then branches out the central retinal artery and the short posterior ciliary arteries. From these posterior arteries and these arteries, we are going into the lacrimal artery or the tear gland. Within them, only towards the sides, we are having a small touch, through the posterior hinges we will have the blood supply that is reaching through the cornea, which is only at the sub-conjunctival tissue.

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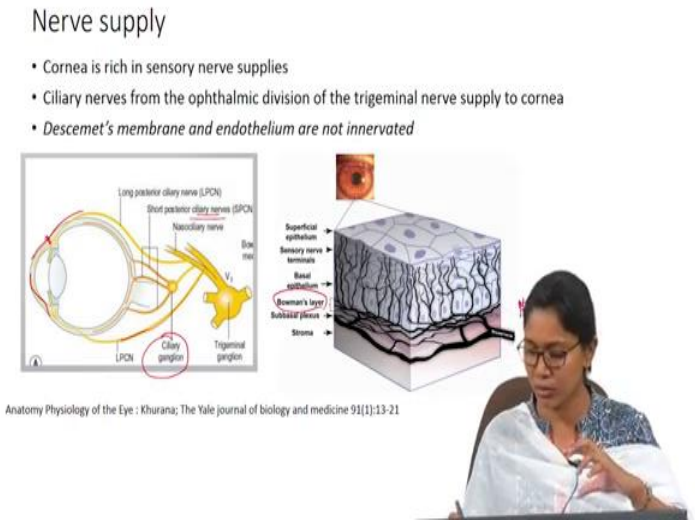

And the nerve supply; we have already mentioned where is the region at which there is a nerve supply that is happening. The cornea is a highly innervated tissue. So, it has a lot of nerves in the corneal tissue, and the nerves enter through the Bowman's membrane. The ciliary nerves from the ophthalmic division enter through this. Here, we have the ciliary ganglion. First, from this ganglion, we have our ciliary nerves that are all coming off, and before this, we have this channel going in up to that of the cornea.

Long posterior ciliary nerve and the short posterior ciliary nerve. So, if this is the case, we have the Bowman's membrane. On a clearer picture, this is our entire stromal structure; we have the stroma. Below this, we have the basement membranes. So, the endothelial and the Descemet membrane do not have nerves. Then we have only the stroma coming in, and here we will have the nerves going inside them, and from the Bowman's layer, the nerves are being made further down. So, they are further branched and then go up through the different layers. That is the basal cells, then wing cells, and then the superficial cells, up to that of the sensory nerve fibers, that go through the topmost corneal epithelial.

This Bowman's layer is a major region of your nerve plexus. So, this will have pores for the nerves to enter and this gives you the nerves for the entire epithelial region of the corneal. Stromal has just then a nerve entering, and below this, that is the Descemet membrane, and the endothelium does not have nerves.

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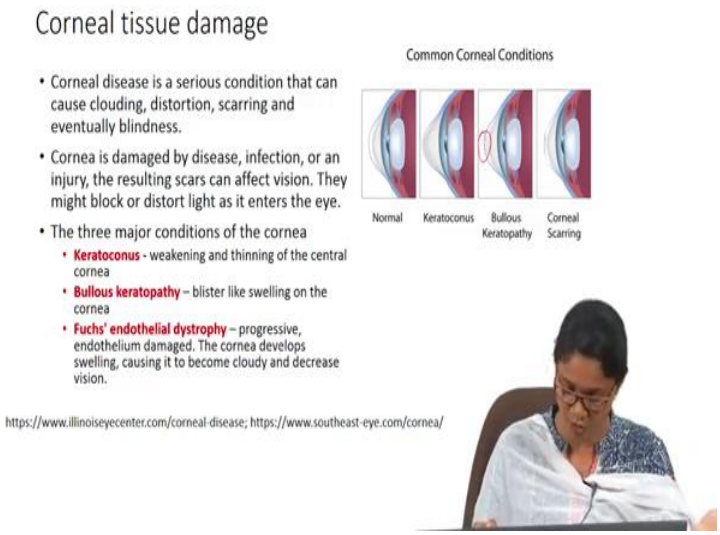

What happens when there is corneal tissue damage? We have now studied that there are different sections of the cornea and different layers and regions; each has its own tissue, repair, damage. That is, whenever there is a wound or damage, there is a repair system that happens. Only in severe cases, when there is complete damage, it cannot repair on its own. So, that is the time when there is an intervention by all these corneal transplantations is to be done.

So, what happens? How do we see when there is an underlying phenomenon that is happening? The first thing is a blurry vision, cloudiness, because your transparency is lost; so you are not able to see and bulging, all this would happen. So, there are 3 major conditions of the cornea that are commonly studied; the keratoconus, the Bullous keratopathy, Fuchs endothelial dystrophy.

This is the picture of a normal corneal structure. When there is keratoconus, there is a bulge. So, the cornea protrudes out, and there is a bulge so that the anterior and the posterior elliptical position is changed. This can also be seen when there are people who use contact lenses; the contact lenses will not be in position. So, this disease is called keratoconus.

Then we have the bullous keratopathy, where you have blisters like swelling on to your corneal outer layer that is happening, that is the bullous keratopathy. Then we have the Fuchs endothelial dystrophy or the corneal scarring, which is a progressive disease where

the corneal endothelium is damaged, that is as I have said the bottommost endothelial cells are being damaged. So, the cornea develops swelling, causing it to become cloudy, and there is vision delay or vision loss. So, this happens when the endothelial cells are being distorted.

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So, for these, what are the treatment options? The current treatment options tell you the full penetrating keratoplasty. The entire donor cornea is transplanted to the recipient cornea. Then we have the endothelial keratoplasty. In the endothelial keratoplasty, we have a few layers that are similar to what anterior lamellar keratoplasty is. When there is an endothelial keratoplasty, only the endothelial corneal tissue is being replaced.

When there is an anterior lamellar keratoplasty, only a stromal region. So, knowing the names, you will be able to see which region of the cornea is being damaged and what needs to be repaired or what needs to have transplantation. Whichever regions from your stroma to your epithelium to the endothelium, if there is a minimal change or very few layers that are being damaged, those layers can be transplanted, and then it will be taken up by your host system and will have a minimal rejection.

But if there is entire damage, then new corneal transplantation has to be done, which is called a full penetrating keratoplasty. And why is this a difficulty? The first is the donor corneas are limited worldwide, with only 1,30,000 donated annually. The problem with

the cornea donors is that tissue rejection, which is very common with any skin tissue donation; the corneal tissue donation also has this problem.

To overcome these, we would have to have other tissue engineering approaches worldwide, so that the minimal levels of tissue donors can be used and we will have a better regeneration, repair for these systems of the different layers. We have studied that there are different layers and each layer is significant of its own. Taking into consideration, the stroma which gives you the transparency, epithelium which will give you the mechanical barrier, the endothelium which will give you a metabolically active state. All these cells of different kinds and tissues of different layers are being formed which has a different function and structure. Based on that, if tissue engineering approaches are used in the form of scaffolds, cells, or by stem cells, that is the limbal stem cells or corneal stem cells that can be grown outside and then be infused again. If these are all done, then the avoiding of corneal transplantation is possible where tissue engineering approaches can overcome.

Thank you.