Tissue Engineering
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Lecture - 37
Corneal Tissue Engineering – Part 2

Hello everyone, in the previous session, we have learned about anatomy and other basic aspects of corneal tissue. In today's session, we will talk about the tissue Engineering aspects of the cornea.

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The topics that will be covered in today’s session will be; prerequisites for designing scaffolds for corneal tissue, and polymers and cells that have been already used in corneal tissue engineering, and commercially available corneal replacements.

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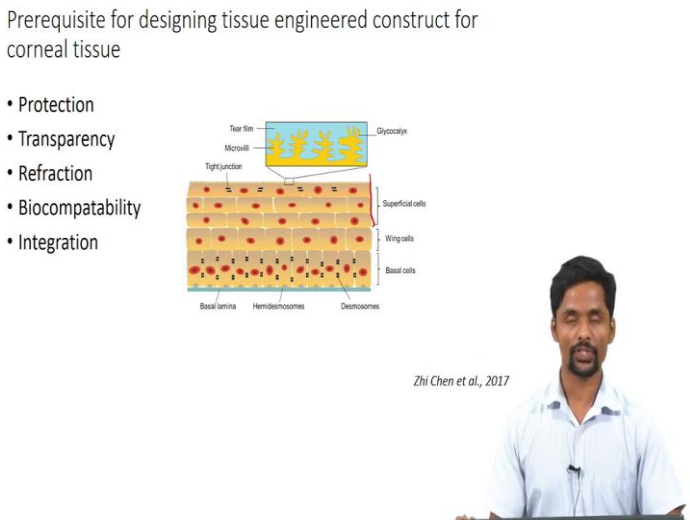

Let us begin with prerequisites for designing tissue engineering scaffolds for corneal tissue. The first prerequisite is protection. As we know, the eye is exposed to the external environment, and it has to be protected from bacterial and other microbial infections. The outermost epithelial layer contains tight junctions and microvilli, and it prevents from microbial invasion.

AS we know, the cornea is transparent in nature; the transparency of the cornea is maintained by the hydration level of the stroma layer. The excess of water in this stromal layer is removed from the bottom endothelium layer by using the metallic pump. Thereby it maintains the transparency of the cornea. Hence the tissue engineering construct must have transparency.

The cornea is responsible for 80 percent of the refraction of the eye; hence, the refraction is required for the cornea. Like any other tissues, the scaffold which we prepare for cornea should be compatible with the host tissue. And the most important thing is integration. The tissue engineering construct, which we prepare for cornea much integrate with the host tissue, thereby not allowing any type of microbial invasion.

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Here are the few of the work that has been done on various tissue layers of the cornea. As you see here, there are many types of polymers that have been used; both natural and synthetic polymers. As you see here, all five layers of the corneal layer are tried to reconstruct in these studies. As you see, for these studies on the cornea, collagen, chitosan, and synthetic polymers like PEG and polyacrylic acids have been used. Stromal layers of the cornea are also been explored to construct engineered tissue. As you see here, there are many types of polymers that have been used keratin, polylactic acid, gelatin and so on. We will talk about all these polymers in the coming slides.

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Various types of cell sources have been used for constructing the corneal tissue. For example, primary animal-derived corneal epithelial cells, and primary animal-derived corneal stromal cells, dorsal root ganglion cells. They have also used human corneal epithelial cells, human corneal fibroblast cells, endothelial cells, and human corneal stromal cells as a cell source for constructing the corneal tissue.

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Now, we will talk about the various polymers that have been used for constructing the corneal tissue. The primarily used polymer is collagen. As we have learned in the previous session, collagen is the most predominant polymer present in the cornea. It is the main component of the human stromal tissue.

The drawback of collagen is insufficient mechanical toughness and elasticity, that is required for corneal tissue. To overcome the drawback of collagen, they tried to crosslink collagen to improve the mechanical strength, and the type of collagen that is used has it is own role.

For example, type I and type III collagen hydrogels have adequate tensile strength and elasticity. Whereas a type III collagen hydrogels tend to be mechanically and optically superior. And crosslinking of collagen is done using various methods that are classified under physical, chemical, and enzymatic methods. Under the physical method, UV light has been used; the dehydrothermal method is also used.

Under the chemical method, formaldehyde, glutaraldehyde, and genipin are used to crosslink the collagen. In the enzymatic method, transglutaminase is used to crosslink the collagen and thereby overcome the drawbacks that are associated with the collagen.

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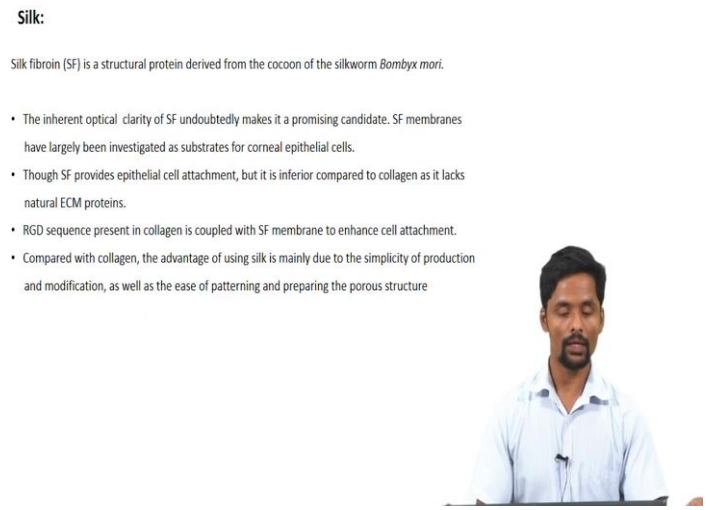

We will go on to silk. Silk fibroin is the structural protein that is derived from the silkworm Bombyx mori. The inherent optical property of silk fibroin makes it more suitable for corneal tissue engineering. This silk fibroin is used as a substrate for corneal epithelial cells. Though silk fibroin provides scaffolding material for epithelial cells, its cell attachment properties are not as superior as collagen, as it lacks the natural ECM protein.

To come that, what they have done is they have introduced the RGD domain into the silk fibroin membranes. Thereby, they tried to improve the cell attachment property of silk fibroin. Compared with collagen, the advantages of using silk fibroin are that it is simple to produce and modify, and as well as it is easier to produce porous scaffold using silk fibroin.

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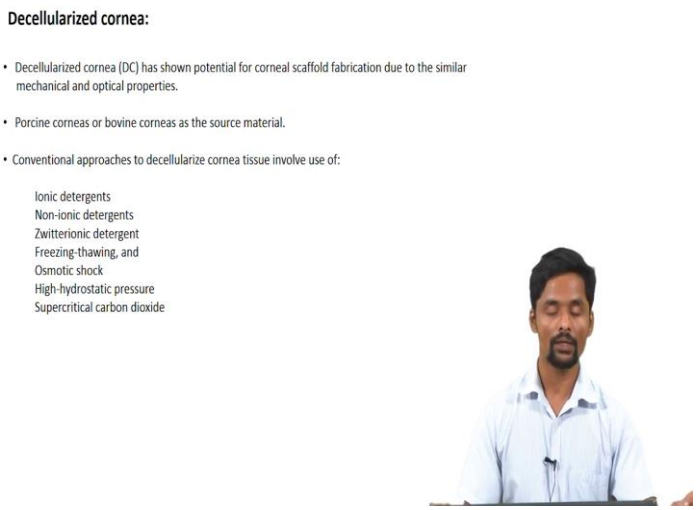

We will go on to the decellularized cornea. The corneal tissue, they decellularize; remove all the cells and the cell debris from the corneal tissue, and they use for corneal replacement purposes. But the problem is the availability of the human cornea tissue for preparing the decellularized cornea, hence are taken from porcine or bovine.

As it is a decellularized cornea, it is optical, and mechanical property are similar to the natural corneal tissue. The approaches to decellularize the cornea tissue are listed over here. They use the various types of detergents like ionic detergents, non-ionic detergents, zwitterionic detergents, and freezing-thawing and osmotic shock. Apart from these techniques recently, they have tried to use the high hydrostatic pressure and supercritical carbon dioxide.

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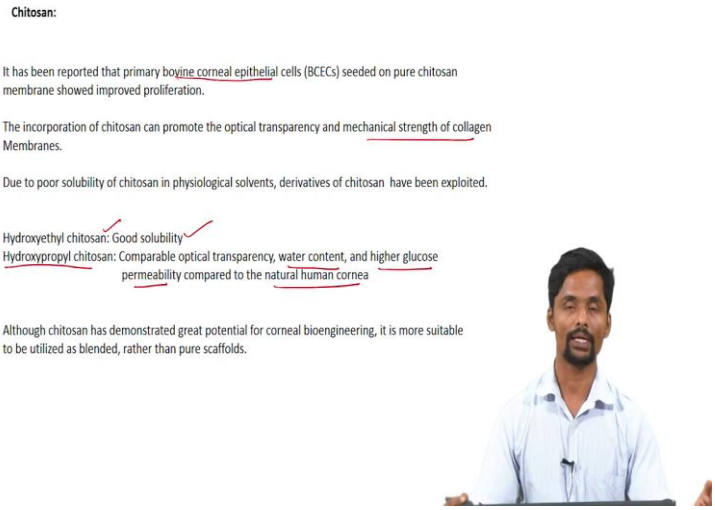

Chitosan is derived from the chitin by deacetylation process. It has been reported that primary bovine corneal epithelial cells seeded on the pure chitosan membrane, showed improved proliferation. The incorporation of chitosan can promote the optical transparency and mechanical strength of the collagen membrane. But the problem with chitosan is, it is very poorly soluble in the physiological medium. Hence, the derivatives of chitosan have been tried out. For example, hydroxyl ethyl chitosan, which has very good solubility.

Also, they have tried to use hydroxypropyl chitosan. The hydroxypropyl chitosan has comparable optical transparency, and it has comparable water content, and high glucose permeability compared to the natural human cornea. Although chitosan has demonstrated great potential for corneal tissue engineering, it is not used alone, it is blended with other polymer and hence used.

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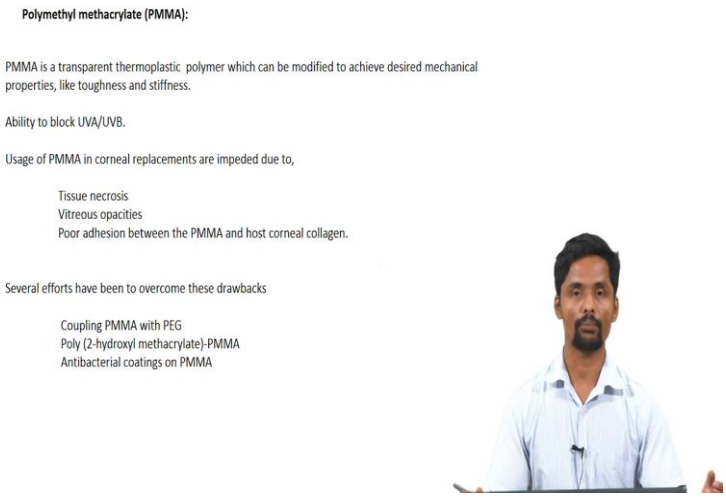

So far, we have discussed about the naturally occurring polymers; now, we will talk about a few of the synthetic polymers. The widely used synthetic polymer for corneal tissue engineering is polymethyl methacrylate (PMMA). Polymethyl methacrylate is a transparent thermoplastic polymer that can be modified to achieve the required mechanical properties like toughness and stiffness. PMMA has the ability to block UV light, and hence, it is more suited for corneal tissue engineering.

However, the usage of PMMA is impeded due to tissue necrosis, vitreous opacities, and poor adhesion between the PMMA and host corneal collagen. The vitreous fluid contains 98 percent of water and 2 percent of hyaluronic acid. What happens is, tiny clots are formed, and they form in the eyeball, and they move along with the eyeball movement, and that hinders the vision. This is known as vitreous opacities.

Several efforts have been taken to overcome these drawbacks. For example, coupling PMMA with the PEG, polyethylene glycol, and they also try to combine other polymers, PolyHEMA with PMMA. They also tried to improve the antibacterial activity of PMMA by using the antimicrobial agents into these PMMA polymers.

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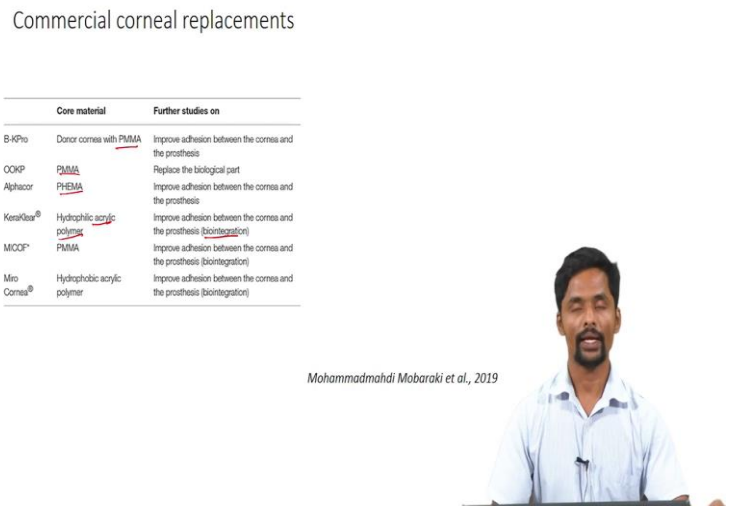

These are the few commercially available corneal replacements. As you see here, most of the core material that are used for this corneal replacements are synthetic in nature, PMMA, poly HEMA, acrylic polymer. As you see, the major drawbacks of these commercially available corneal replacements are integration. The poor adhesion between the cornea and the tissue engineering construct. Hence, future studies should focus on to improve the integration of these tissue engineering constructs with the host tissue.

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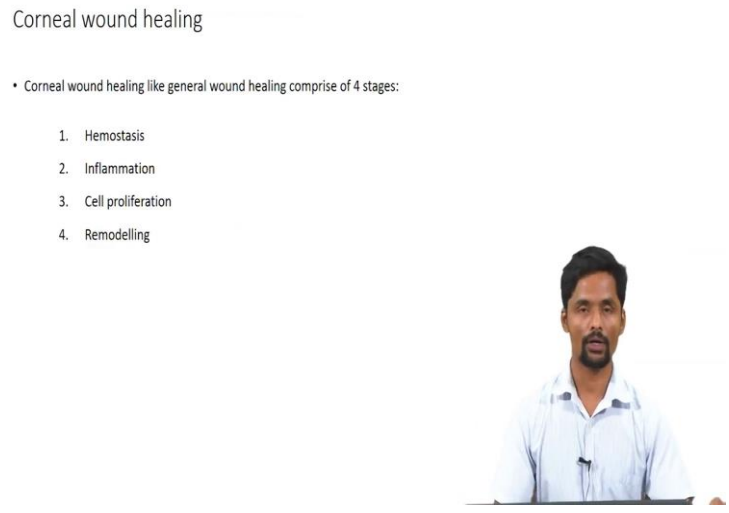

One more major aspect of the cornea is corneal wound healing. Like any other wound healing process that happens in our body, even corneal wound healing involves four stages like hemostasis, inflammation, cell proliferation, and remodeling.

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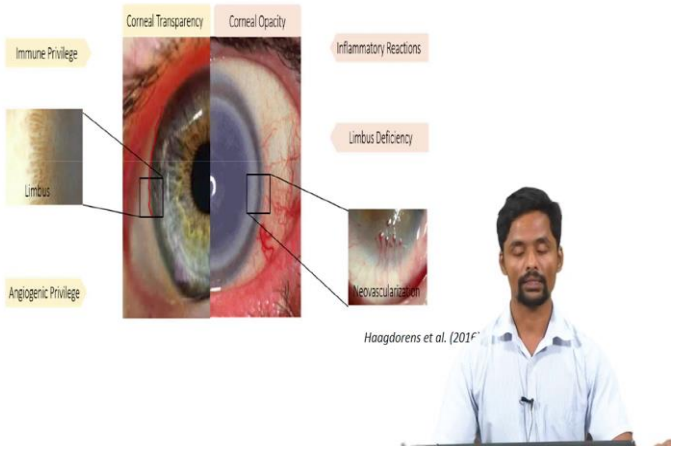

But the problem with the corneal wound healing is cornea contains limbus region. This limbus region is an immune privilege and angiogenic privilege. That means, it naturally lacks neovascularization and immune cells in the limbus region, and that is required for the transparency of the corneal tissue.

As you see here, the wound healing process involves the step called inflammation, and due to this, the immune cells march into the limbus region. They destroy the limbus region, and there will be neovascularization takes place; as you see over here, and that leads to the corneal opacity. As in any other wound healing process, wound healing leads to the scaring of the tissue, which ultimately leads to the loss of vision. There are many attempts to prevent angiogenic privilege and immune privilege of cornea. For example, amniotic membrane.

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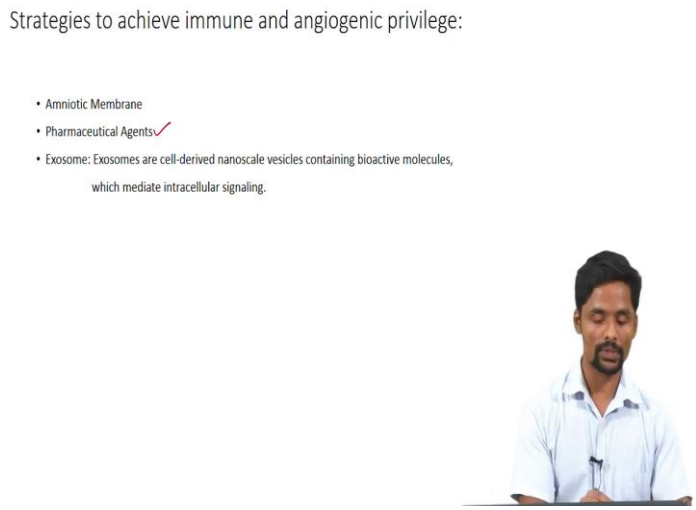

The amniotic membrane naturally contains antiangiogenic, anti-inflammatory compounds. Using this amniotic membrane for corneal wound healing prevents or lessens the inflammation stage. Many pharmaceutical agents, that are antiangiogenic or anti-inflammatory agents are used for corneal wound healing to achieve the immune and angiogenic privilege.

Exosomes are cell-derived nanoscale vesicles that are secreted out. These exosomes contain several bioactive molecules that have various functionalities. The exosomes that have been released by corneal epithelial cells are known to involve in the wound healing process. Usage of these exosomes for the corneal wound healing is proven better.

Thank you.