Oncology Treatment Discovery, 2024, Volume 2, Issue 3
http://ojs.bbwpublisher.com/index.php/OTD
Online ISSN: 2981-8079

Tissue Engineering of Cornea via Type 1 Collagen Biomaterials – A perspective

Kirubanandan Shanmugam*

Independent Research Professional, Chennai, Tamilnadu, India

Corresponding author: Kirubanandan Shanmugam, ksh1005@yahoo.com

Abstract: Engineering corneal tissue has advanced significantly in recent years. Engineering a biocompatible, mechanically stable and optically clear tissue presents significant engineering hurdles. Two fundamental strategies have been explored by researchers to address these issues: (1) cell-based methods for controlling cells extracellular matrix and (2) scaffold-based methods for supplying dense, transparent matrices for cell growth. Both approaches have had considerable level of success. Additionally, new developments in innervating a tissue-engineered construct have been developed. Future research must concentrate on enhancing the mechanical stability of engineered constructions and the host reaction to implantation. Type 1 collagen biomaterial has been used for the construction of scaffolds or implantation for cornea repair. Various methods for the fabrication of the scaffolds have been described and mentioned tissue engineering applications for cornea repair and regeneration. Given this correspondence, type 1 collagen was a potential base biomaterial for tissue engineering scaffold fabrication for effective repair/restoration/regeneration of cornea.

Keywords: Cornea repair; Tissue engineering; Type 1 collagen; Biomaterial; Fabrication methods; Clinical practice

Online publication: October 2, 2024

1. Introduction

Tissue engineering offers a promising approach to cornea repair by utilizing a combination of cells, scaffolds and growth factors to regenerate damaged corneal tissue. In the context of corneal repair, researchers are exploring various strategies to develop bioengineered corneal substitutes that can mimic the structure and function of the native cornea. One common approach involves seeding corneal cells onto biocompatible scaffolds that provide structural support and guidance for tissue regeneration. These scaffolds can be made from natural or synthetic materials and are designed to degrade over time as the new tissue forms. In addition to scaffolds, growth factors play a crucial role in promoting cell proliferation, differentiation, and tissue remodeling during corneal repair. By incorporating growth factors into the engineered constructs, researchers can enhance the regenerative capacity of the cells and accelerate the healing process. Overall, tissue engineering holds great promise for cornea repair by offering customized solutions that can address the specific needs of individual patients. As research in this field progresses, it can be expected to see more advanced and effective

strategies for treating corneal injuries and diseases.

Tissue engineering of the cornea is a rapidly advancing field aimed at addressing the significant global need for corneal replacements due to disease, injury or congenital defects. The cornea is a critical component of the eye, contributing to vision by focusing light onto the retina. Damage to the cornea can lead to impaired vision or blindness. Tissue engineering offers promising solutions by developing biocompatible, functional corneal substitutes. Here's an overview of the current state, challenges, and future perspectives in corneal tissue engineering:

2. Current approaches in corneal tissue engineering: Scaffold-based techniques

(1) Biodegradable polymers: Polymers such as collagen, gelatin, and synthetic materials like polylactic-glycolic acid (PLGA) are used to create scaffolds that mimic the extracellular matrix of the cornea.

(2) Nanofibrous scaffolds: Electrospinning techniques produce nanofibrous scaffolds that closely resemble the natural structure of the corneal stroma, promoting cell attachment and growth.

(3) Hydrogels: Hydrogels, such as those based on hyaluronic acid or polyethylene glycol, provide a hydrated environment conducive to cell survival and proliferation.

(4) Cell-based approaches: Stem cells such as mesenchymal stem cells (MSCs), limbal stem cells, and induced pluripotent stem cells (iPSCs) are explored for their ability to differentiate into corneal cells (keratocytes, epithelial cells, and endothelial cells).

(5) Cell sheets: Culturing corneal cells into cell sheets without the use of scaffolds for transplantation to repair damaged corneal tissue.

(6) Decellularized corneal matrices: Natural corneal scaffolds involve the decellularization of donor corneas to remove cellular components while preserving the extracellular matrix. This scaffold can then be recellularized with patient-specific cells.

(7) 3D Bioprinting: Layer-by-layer printing can be carried out using bio-inks composed of cells and biomaterials to print corneal constructs layer-by-layer, replicating the complex architecture of the cornea.

3. Challenges in corneal tissue engineering

In corneal tissue engineering, several challenges need to be addressed for successful clinical translation. One major challenge is achieving proper cellular organization and alignment within the engineered tissue to mimic the native corneal structure accurately. Ensuring appropriate mechanical strength and transparency while promoting integration with the surrounding tissues is crucial for functional outcomes. Another challenge is developing biomaterials that are biocompatible, biodegradable and capable of supporting cell growth and differentiation. Achieving proper innervation and vascularization within the engineered corneal tissue is another hurdle as these are essential for maintaining tissue health and functionality. Controlling the immune response to prevent rejection of the implanted tissue is also a significant challenge in corneal tissue engineering. Additionally, scaling up production methods to meet the demand for corneal transplants worldwide is a logistical challenge that needs to be addressed. Overall, addressing these challenges through interdisciplinary collaborations, innovative biomaterials, advanced fabrication techniques and thorough preclinical testing will be essential for the successful development and clinical application of engineered corneal tissue.

(1) Biocompatibility and immunogenicity: Ensuring that engineered corneal tissues are biocompatible and do not elicit an immune response upon implantation is critical for their success.

(2) Mechanical properties: Replicating the unique mechanical properties of the native cornea, such as transparency, strength, and elasticity, remains a significant challenge.
(3) Cell source and viability: Finding reliable and ethically acceptable sources of corneal cells and ensuring their long-term viability and functionality in engineered tissues.
(4) Vascularization and innervation: Engineering corneal tissues that integrate well with the host's vasculature and nerve supply to maintain transparency and function.
(5) Scaffold degradation: Controlling the degradation rate of scaffolds to match tissue regeneration without compromising the structural integrity of the corneal construct.

4. Future perspectives

(1) Advanced biomaterials and smart materials: Development of smart biomaterials that respond to environmental cues to promote cell growth and tissue regeneration.
(2) Bioactive molecules: Incorporation of growth factors, peptides and other bioactive molecules to enhance cell proliferation and differentiation.
(3) Gene editing and regenerative medicine (CRISPR/Cas9): Utilizing gene-editing technologies to correct genetic defects in corneal cells or enhance their regenerative capabilities.
(4) Regenerative approaches: Combining tissue engineering with regenerative medicine techniques to improve outcomes.
(5) Personalized medicine (Patient-specific scaffolds): Using patient-specific cells and 3D printing technologies to create customized corneal implants tailored to individual patient needs.
(6) Clinical translation and trials (Regulatory approvals): Navigating regulatory pathways to bring engineered corneal tissues from the lab to clinical practice.
(7) Long-term studies: Conducting long-term clinical trials to assess the safety, efficacy, and durability of engineered corneal tissues.
(8) Interdisciplinary collaboration research: Encouraging collaboration between material scientists, biologists, clinicians and engineers to accelerate advancements in corneal tissue engineering.
(9) Funding and support: Securing funding and support from governmental and non-governmental organizations to drive research and development efforts.

Tissue engineering of the cornea holds great promise for addressing the global shortage of donor corneas and providing new treatments for corneal diseases and injuries. Advances in biomaterials, cell biology and engineering technologies are driving progress in this field. Overcoming the existing challenges through innovative research and interdisciplinary collaboration will be key to realizing the full potential of engineered corneal tissues and improving patient outcomes.

Corneal repair is a critical area in ophthalmology due to the importance of the cornea in vision. Several approaches have been developed to repair or replace damaged corneal tissue, ranging from traditional methods to advanced tissue engineering techniques. Here's an overview of various approaches for cornea repair.

(1) Traditional approaches: Corneal transplantation (Keratoplasty)
  (a) Penetrating keratoplasty (PK): Full-thickness corneal transplant where the entire damaged cornea is replaced with a donor cornea.
  (b) Lamellar keratoplasty: Partial-thickness transplant where only the affected layers of the cornea are replaced.
  (c) Anterior lamellar keratoplasty (ALK): Replaces the front layers of the cornea.

(d) Deep anterior lamellar keratoplasty (DALK): Replaces all layers down to Descemet's membrane.

(e) Descemet's stripping endothelial keratoplasty (DSEK/DMEK): Replaces only the damaged endothelial layer.

(f) Corneal cross-linking (CXL): A procedure used to treat keratoconus and other corneal ectasias by strengthening the collagen fibers in the cornea using riboflavin (vitamin B2) and ultraviolet light.

(g) Amniotic membrane transplantation: Use of amniotic membrane, which has anti-inflammatory and anti-scarring properties, to promote healing of the corneal surface.

(2) Advanced approaches: Tissue engineering and biomaterials

(a) Scaffold-based techniques: Use of biodegradable polymers, hydrogels, and nanofibrous scaffolds to create structures that mimic the corneal extracellular matrix.

(b) Decellularized corneal matrices: Removing cells from donor corneas to create a scaffold that can be recellularized with patient-specific cells.

(3) Cell-based therapies

(a) Stem cell therapy: Use of stem cells (e.g., limbal stem cells, mesenchymal stem cells, and induced pluripotent stem cells) to regenerate damaged corneal tissue.

(b) Cell sheets: Culturing corneal cells into sheets that can be transplanted to repair the cornea.

(c) Gene therapy (Gene editing): Using techniques like CRISPR/Cas9 to correct genetic defects in corneal cells or to enhance their regenerative capabilities.

(4) 3D bioprinting (Layer-by-layer printing): Utilizing 3D bioprinting technology to create corneal structures with precise architecture and cell placement, closely replicating natural corneal tissue.

(5) Artificial corneas (Keratoprostheses)

(a) Synthetic implants: Development of biocompatible synthetic corneas, such as the Boston Keratoprosthesis and the AlphaCor, to replace damaged corneal tissue.

(b) Hydrogel-based implants: Newer approaches use hydrogels that are similar to natural corneal tissue, promoting integration and reducing the risk of rejection.

(6) Nanotechnology

(a) Nanomaterials: Incorporation of nanomaterials to improve the mechanical properties and biocompatibility of corneal implants.

(b) Nanomedicine: Use of nanoparticles for targeted drug delivery to the cornea to promote healing and reduce inflammation.

(7) Bioactive molecules

(a) Growth factors: Incorporation of growth factors and cytokines in scaffolds or hydrogels to promote cell proliferation and differentiation.

(b) Anti-inflammatory agents: Use of bioactive molecules to reduce inflammation and enhance healing.

(8) Regenerative medicine

(a) Combination therapies: Combining cell therapy, gene therapy, and tissue engineering to create more effective treatments for corneal repair.

(b) Patient-specific treatments: Development of personalized medicine approaches, using patient-specific cells and materials to create customized corneal implants.

(9) Interdisciplinary collaboration

(a) Cross-field innovations: Collaboration between material scientists, biologists, engineers, and clinicians to develop novel materials and techniques for corneal repair.

(b) Clinical translation: Bridging the gap between laboratory research and clinical application through

robust clinical trials and regulatory approvals.

Various approaches for corneal repair offer a range of solutions from traditional methods like keratoplasty to cutting-edge techniques involving tissue engineering, stem cell therapy, and 3D bioprinting. The field is evolving rapidly, with promising advancements that aim to improve the outcomes for patients with corneal damage. Future developments will likely focus on enhancing the biocompatibility, functionality, and accessibility of these treatments, ultimately contributing to better vision health worldwide.

Corneal vision loss affects 10 million people worldwide, with approximately 40,000 corneal transplants performed annually in the United States. The growing need for corneal transplants is limited to allogenic and synthetic materials. Recently, autologous limbal stem cell transplantation has been an effective therapeutic option for corneal regeneration [1]. However, allogenic materials from human donors are the preferred choice, but they have limitations such as the limited availability of quality donor graft material with their consent and tissue rejection in the host person [2]. Traditional penetrating keratoplasty has been replaced by partial lamellar keratoplasty, which has increased implant success rates. The need for transplantable cadaveric corneas in developing countries is also increasing due to limited cadaveric donation [3]. Keratoprostheses, synthetic homologs, are used for full-thickness corneal replacement in severe ocular surface pathologies and eyes with limbal stem cell deficiency. The Boston type-1 keratoprosthesis is the most common, but its short-term visual recovery is limited by complications like glaucoma and endophthalmitis [4]. Osteo-odonto-keratoprosthesis has shown good long-term anatomical survival rates and is currently the most common treatment for end-stage inflammatory corneal diseases [5].

The human cornea is a transparent, avascular connective tissue that provides the optical interface, protection from infections, and transparency. It consists of three distinct cellular layers: (1) corneal epithelium, (2) stroma, and (3) endothelium, separated by Bowman's layer and Descemet's membranes. Figure 1 reveals the structure of cornea in the eye. The corneal epithelium is a stratified, non-keratinized squamous tissue with nociceptive nerve endings and a biological barrier function. It regulates water and soluble components transfer into or out of the stroma, allowing coherent light refraction. The tear film serves as a reservoir for antibacterial and growth factors, maintaining epithelial homeostasis, proliferation, and repair. Bowman's membrane, a 15 μm thick acellular layer, may act as a molecular barrier or contribute to corneal shape. The corneal stroma, comprising approximately 90% of the overall cornea thickness, consists of aligned collagen fibrils called lamellae. The keratocytes maintain the matrix components of the lamellar connective tissue. Descemet's membrane anchors the corneal endothelial layer, while the endothelium removes water from the stroma and maintains stromal hydration. The cornea's functions require a corneal substitute or tissue model to provide protection, transparency, and substantial refractive power. The stroma, a dense connective tissue, provides lateral tensile strength, while the epithelium protects the stroma and endothelium from chemical injuries. The cornea's transparency is determined by collagen interfibrillar spacing and tissue state of hydration [6].

Two primary strategies are being investigated to meet the increasing demand for corneal transplants: (1) allogenic and (2) synthetic materials. Currently, allogeneic tissue from human donors is the recommended option. However, donated corneal tissue is in short supply globally. Furthermore, tissue rejection frequently restricts this approach's long-term efficacy. On the other hand, because they have a high rate of graft failure, synthetic homologs to donor corneal grafts are generally regarded as temporary substitutes until appropriate donor tissue becomes available. To eliminate the necessity for animal testing of commercial items, tissue-created cornea analogues would offer efficient alternatives and substitutes for corneal tissue. This overview covers recent developments towards meeting these needs as well as outlooks for the future [7].

In addition to that, type 1 collagen from bovine tendons has been used in research and clinical trials for

cornea repair. Collagen is a key structural protein in the cornea, providing support and strength to the tissue. By using type 1 collagen derived from bovine tendons, researchers and medical professionals aim to provide a biomimetic scaffold that can support corneal tissue regeneration and repair. The use of type 1 collagen from bovine tendons for cornea repair involves creating a scaffold that mimics the natural extracellular matrix of the cornea. This scaffold can be used to support the growth and organization of corneal cells, promoting tissue regeneration and repair. In some cases, this scaffold may be combined with corneal cells or other growth factors to further enhance the repair process. Clinical trials and research studies have shown promising results for the use of type 1 collagen from bovine tendons in cornea repair. This approach has the potential to offer a biocompatible and effective treatment for corneal injuries, diseases and abnormalities. It is important to note that the use of bovine-derived collagen raises ethical and safety considerations, and alternative sources of collagen, such as recombinant or human-derived collagen, are also being explored for cornea repair applications. Additionally, regulatory approval and further clinical validation are necessary before this approach becomes widely available for cornea repair in clinical settings [8].

This paper deals with the tissue engineering of cornea via type 1 collagen biomaterial and various methods for scaffold fabrication which is used to reconstruct the damaged cornea. In addition to that, this paper reviews the tissue engineering strategies for damaged cornea into a regenerated cornea.

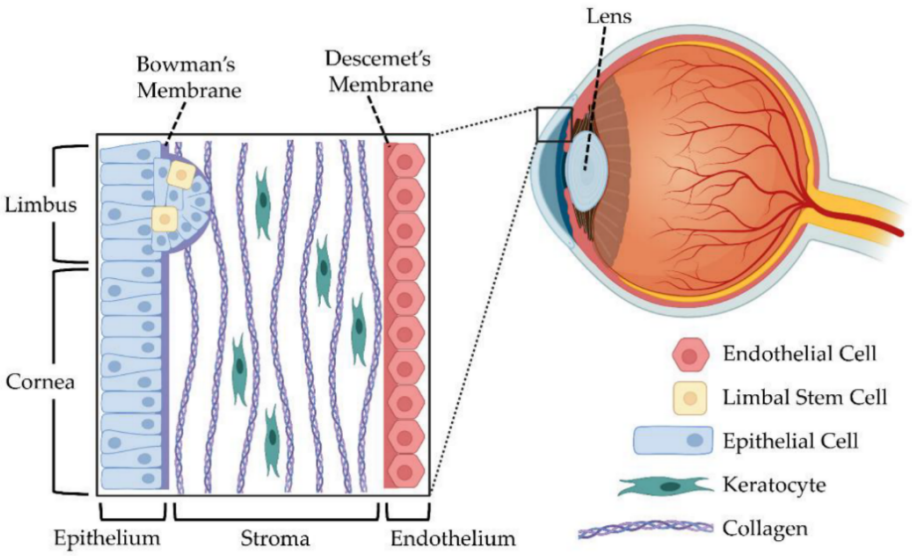

Figure 1. The outermost layer of the human eye is called the cornea. The stroma, endothelium, and epithelium are some of the layers that make up this translucent, avascular tissue. The limbus, Bowman's membrane, and Descemet's membrane are other elements [9]. Produced with Biorender.com.

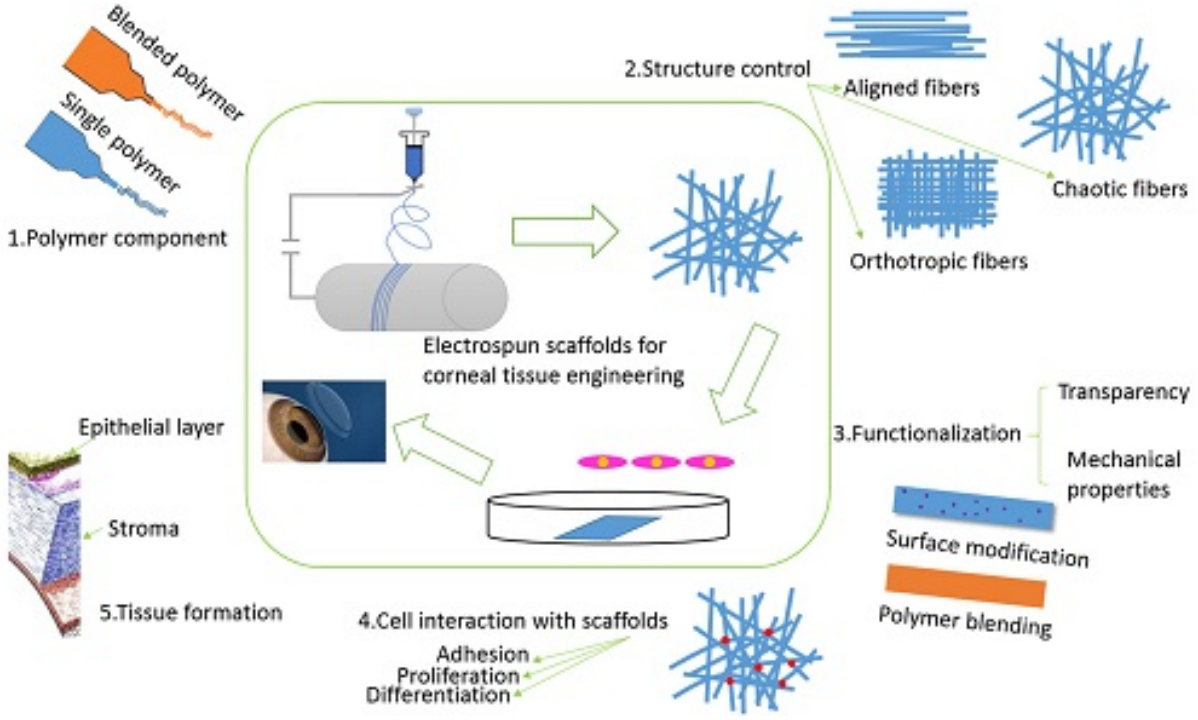

Figure 2. Corneal tissue engineering.

5. Treatment of cornea repair via tissue engineering

Corneal repair through tissue engineering involves creating bioengineered corneal tissue to replace damaged or diseased corneas. This process typically begins by obtaining cells, such as corneal epithelial cells, stromal cells and endothelial cells, from the patient or a donor. These cells are then grown and expanded in culture to develop into functional corneal tissue. Various approaches can be used in corneal tissue engineering, including scaffold-based techniques where cells are seeded onto a scaffold that mimics the structure of the cornea, or scaffold-free techniques where cells are grown into sheets or layers without a scaffold. These bioengineered tissues can then be transplanted onto the damaged cornea to promote healing and restore vision. Advantages of corneal repair through tissue engineering include a reduced risk of rejection compared to traditional corneal transplants, as well as the potential for customized treatments tailored to the individual patient. Ongoing research in this field aims to improve the effectiveness and availability of tissue-engineered corneal replacements for patients in need of corneal repair. Figure 2 demonstrates the process of corneal tissue engineering for the repair/regenerate/restoration of the cornea.

The cornea is the transparent, dome-shaped surface that covers the front of the eye. It plays a crucial role in focusing light into the eye and protecting the eye from dust, debris, and other harmful particles. The cornea also helps to shield the eye from harmful UV rays. It is made up of highly specialized cells and proteins that help maintain its clarity and shape. When the cornea becomes damaged or diseased, it can lead to vision problems and require medical treatment or surgery [10].

The repair of the cornea involves various treatments and procedures, depending on the extent and nature of the damage. Some common methods for repairing the cornea include (1) Medications: For minor injuries or infections, topical medications such as antibiotics, antiviral drugs, or corticosteroids may be prescribed to help the cornea heal and reduce inflammation. (2) Corneal transplantation: In cases of severe damage or disease, a corneal transplant may be necessary. This involves replacing the damaged corneal tissue with healthy donor

tissue from a deceased individual. (3) Phototherapeutic keratectomy (PTK): This laser procedure is used to remove damaged or diseased corneal tissue and promote the growth of healthy tissue. (4) Corneal collagen cross-linking: This procedure uses ultraviolet light and riboflavin eye drops to strengthen the cornea and prevent further deterioration in cases of conditions such as keratoconus. (5) Amniotic membrane transplantation: This involves placing a piece of amniotic membrane over the damaged area of the cornea to promote healing and reduce scarring. (6) Intacs: These are small plastic rings that are surgically inserted into the cornea to reshape it and improve vision in cases of conditions such as keratoconus. It's important to consult with an ophthalmologist or corneal specialist to determine the most appropriate treatment for your specific condition [11].

At various levels of complexity, tissue engineering principles have been used to build functional corneal tissue equivalents. These range from techniques to replicate the natural innervation to engineering of the epithelium, stroma, and endothelium layers. By utilizing a wide range of biomaterial systems and cell types various techniques have resulted in full-thickness cornea tissue equivalents that are used in clinical settings. Specifically, the primary methods can be separated into four categories: (1) structures based only on cells, (2) decellularized, (3) synthetic, and (4) natural polymers combined with various cell types [12].

5.1. Tissue engineering of corneal epithelium

Tissue engineering of corneal epithelium involves creating functional corneal tissue in the lab for potential transplantation or research purposes. This process typically involves seeding corneal epithelial cells onto a scaffold that mimics the natural environment of the cornea, providing structural support and cues for cell growth and differentiation. Various techniques like bioprinting, cell sheet engineering, and scaffold-based approaches are utilized to create the engineered corneal tissue. The goal is to develop a tissue that closely resembles the native corneal epithelium in terms of structure and function. One key challenge in corneal tissue engineering is achieving proper cell organization and alignment to promote healthy tissue formation. Researchers also need to ensure that the engineered tissue is transparent, biocompatible, and promotes proper wound healing when transplanted. By advancing tissue engineering techniques and optimizing cell culture conditions, scientists aim to develop more effective treatments for corneal diseases and injuries, ultimately improving patient outcomes and reducing the reliance on traditional donor tissue for transplantation.

Epithelial and endothelial layers are crucial for maintaining corneal deturgescence (dehydrated cornea) and transparency. Cell sheet engineering, an advanced concept of tissue engineering has successfully generated the corneal epithelial layer in vitro, promoting functional stratified epithelium growth. However, high variability and extended culture time are major drawbacks. Human amniotic membranes have been used for corneal epithelial-derived expansion and reconstruction, but their high variability limits their use in clinical settings. Human donor corneal stromal tissues are proposed for human corneal epithelium growth, but the lack of corneal tissue donor availability affects their clinical potential. Reconstituted type I collagen hydrogels can encapsulate human corneal limbal epithelial cells, resulting in functional stratified epithelial layers. Chemically cross-linked collagen hydrogels are explored as corneal epithelium scaffolds due to their enhanced mechanical and optical properties. In addition to that, there are some biopolymers also in tissue engineering practice for cornea repair. For example, Silk has been exploited as a substrate for human epithelial cell growth and functional organization due to its optical properties, mechanical robustness, and versatile processability. Keratin-based substrates have been used for ocular surface reconstruction due to their good optical properties and ability to support epithelial cell growth in vitro [13].

5.2. Regeneration of corneal stroma

The reconstruction of the corneal stroma is still challenging due to its complex structure, mechanical strength, and transparency. To address this, researchers have been exploring the development of functional corneal stroma substrates using synthetic polymers, natural-derived materials, and silk films. Synthetic polymers have been used for stromal corneal substrates due to their tuneable mechanical properties and the ability to direct stromal cell organization and differentiation in vitro. However, these materials lack adequate optical properties. Hydrogel films prepared from chitosan blended with poly(ethylene glycol) and poly(l, D-lactic acid) nanofibers have shown improved mechanical, optical, and biological performances. Silk films have also been developed to combine mechanical, biological, and optical properties, supporting corneal stromal cell differentiation in 2D and 3D film architectures [14].

5.3. Corneal endothelium

Natural polymer substrates, including type I collagen, gelatin, decellularized tissues, chitosan, and chondroitin sulfate, have been the primary focus of contemporary research on endothelial layer engineering. These substrates have demonstrated signs of endothelium development. In a lamellar keratoplasty model, in which the endothelium and a portion of Descemet's membrane were removed, the clinical evaluation of decellularized aminiotic membrane in conjunction with human corneal endothelial cells was conducted. This membrane was found to be capable of serving as an equivalent to corneal endothelium [14].

5.4. Corneal innervation

With nociceptive nerve protrusions that terminate in the epithelium layer, the cornea is one of the most innervated tissues in the human body. To preserve the general health of the cornea, the corneal nerves serve as mechanical and temperature sensors. The pathological diseases known as dry eye are caused by a progressive absence of innervation, which eventually leads to diffuse corneal ulcers and a decrease in corneal sensitivity. Few attempts have been made to stimulate peripheral nerve proliferation within corneal tissue-engineered constructions, despite the crucial role that innervation plays in ocular functioning. According to in vitro research, the application of peptides generated from laminin to a substrate stimulated the proliferation of neurons and epithelial stratification. Additionally, employing cross-linked collagen replacements, functional nerve regeneration was demonstrated in a pig model undergoing deep lamellar keratoplasty, regaining prior nerve density one year after surgery [14].

5.5. Replacement of full-thickness cornea via tissue engineering

Tissue engineering offers a promising approach for the replacement of full-thickness cornea by creating functional corneal equivalents. Using a combination of biomaterials, cells, and growth factors, researchers aim to develop corneal constructs that mimic the structure and function of native corneas. Various techniques such as decellularization of donor corneas, bio-printing, and cell seeding onto scaffolds are being explored to generate these corneal substitutes. One common strategy involves seeding corneal cells such as keratocytes, epithelial cells, and endothelial cells onto a biocompatible scaffold that mimics the native corneal architecture. By providing the necessary cues for cell growth, differentiation, and extracellular matrix production, these constructs can potentially integrate with the host tissue and restore vision. While significant progress has been made in the field of corneal tissue engineering, challenges such as achieving optimal transparency, biomechanical strength, and long-term integration remain to be addressed. Further research is needed to improve the functionality and clinical outcomes of engineered corneal replacements. Overall, tissue engineering holds great promise for the development of innovative solutions for corneal regeneration and transplantation.

In vivo implantation of corneal equivalent biomaterials without cells has been studied to study the integration of implanted biomaterials with native corneal tissue. Efforts have been made to mimic the three-layer structure of the cornea, using decellularized biological material. However, the use of acellular porcine cornea in rabbit lamellar keratoplasty led to the degradation of the tissue-engineered cornea. Further efforts have been made to develop in vitro corneal stroma equivalents, promoting nerve ingrowth, and engineered full-thickness cornea for tissue replacement. Biosynthetic corneas from cross-linked recombinant human collagen type III were implanted in human patients to enhance endogenous tissue regeneration. The implants were stably integrated, innervated, and vascularized for up to 2 years. Epithelial and stromal cell lines are extensively investigated for in vitro 3D cornea tissue models and surgical replacements. Primary human epithelial and stromal cells are mainly used in in vitro 3D tissue models and preclinical approaches. Human stromal stem cells have been used to repopulate mouse corneas and restore stromal thickness, fibril deficits, and transparency in cloudy corneas. These cells were found to be stably integrated into the mouse cornea for over 10 weeks without eliciting an immune response. This suggests that a bioengineered cornea populated with immune-privileged cells could supplement corneal replacements and in vitro models [14,15].

6. Pros and cons for existing treatment for repair of cornea

There are pros and cons for existing treatment available for the treatment for the repair of cornea. The following pros and cons have been reported.

Pros: (1) Effective at treating corneal damage and diseases such as on as keratoconus, corneal ulcers, and corneal dystrophies. (2) Can improve vision and overall quality of life for patients. (3) Treatments such as corneal transplants have a high success rate. (4) Many treatment options are available, including medications, contact lenses, and surgical procedures.

Cons: (1) Some treatments may have risks and potential complications, such as infection or rejection of transplanted tissue. (2) Surgical procedures can be expensive and may require a long recovery time. (3) Availability of treatment options may be limited in certain areas. (4) Some treatments may only provide temporary relief and require ongoing maintenance.

Tissue engineering for the repair of the cornea involves the use of bioengineered materials and techniques to restore the structure and function of the cornea. The cornea is the transparent outer layer of the eye that plays a crucial role in vision, and damage or disease to the cornea can lead to vision impairment or loss. One approach to tissue engineering for corneal repair involves the use of synthetic or natural biomaterials to create a scaffold that mimics the structure of the cornea. This scaffold can be seeded with corneal cells, such as corneal epithelial cells or corneal stromal cells, and then implanted into the damaged area of the cornea. The cells can then grow and proliferate on the scaffold, eventually integrating with the surrounding tissue and restoring the function of the cornea. Another approach involves the use of stem cells to regenerate corneal tissue. Stem cells can be isolated from the patient's body, such as from the bone marrow or the limbus of the eye, and then expanded and differentiated into corneal cells. These cells can then be transplanted into the damaged cornea to promote tissue regeneration and repair. In addition to these approaches, tissue engineering for corneal repair may also involve the use of growth factors, gene therapy, and other advanced techniques to enhance the regenerative potential of the cornea. Overall, tissue engineering holds great promise for the repair of the cornea and the restoration of vision in individuals with corneal damage or disease. Ongoing research and development in this field continue to advance the potential for tissue-engineered corneal repair to become a standard treatment in the future.

The transplantation of full-thickness human corneas through penetrating keratoplasty is one current

strategy for addressing the scarcity of corneal donors. However, this operation requires access to eye banks, which delays the rapid recovery of corneas in emergency cases. Advances in corneal regenerative medicine are required to offer patients undergoing such high-risk grafts with alternatives.

Keratoprostheses (KPros), which consist of an optical core that softly communicates with the host's eye, are used as substitutes for donor corneas in high-risk corneal grafts. The Boston KPro and osteo-odonto-keratoprosthesis are two popular KPros that are not the best options for replacing the cornea because of problems with their irreversible insertion and related complications like infection that can require continuous use of antibiotics and immunosuppressants. The developments in KPros aim to maintain native corneal functions to facilitate epithelial growth. In addition to preventing infection and implant extrusion, this is required to maintain tear film. These kinds of developments have recently become possible because of enhanced lithographic and surface chemistry changing methods [16].

7. Collagen biomaterials for tissue engineering of the cornea

Collagen biomaterials have shown great potential for tissue engineering of the cornea. The cornea is a transparent, avascular tissue that plays a crucial role in vision. When damaged or diseased, the cornea can result in vision impairment or blindness. Tissue engineering aims to develop biomimetic materials that can replace or regenerate damaged corneal tissue. Collagen is the main structural protein in the cornea and provides it with its unique properties of transparency and strength. Therefore, collagen-based biomaterials are an attractive choice for corneal tissue engineering. These biomaterials can be derived from natural sources, such as animal tissues, or produced synthetically using recombinant technology. One common approach to using collagen biomaterials for corneal tissue engineering is to create a scaffold that mimics the structure and properties of the native cornea. This scaffold can then be seeded with corneal cells, such as corneal epithelial cells, keratocytes, or endothelial cells, to promote tissue regeneration. Collagen-based scaffolds can provide a suitable microenvironment for cell attachment, proliferation, and differentiation, ultimately leading to the formation of new corneal tissue. In addition to serving as a scaffold for cell growth, collagen biomaterials can also be used to deliver bioactive molecules, such as growth factors or drugs, to promote tissue regeneration and modulate the inflammatory response. Furthermore, collagen-based hydrogels have been investigated for their potential to serve as a carrier for corneal endothelial cell transplantation, which is a promising therapy for corneal endothelial dysfunction. Overall, collagen biomaterials hold great promise for tissue engineering of the cornea. Their biocompatibility, bioactivity, and ability to mimic the native corneal tissue make them an attractive choice for developing new therapies to treat corneal diseases and injuries. Continued research and development in this field are likely to lead to further advancements in corneal tissue engineering using collagen biomaterials.

Because collagen resembles native tissue and is the most abundant component of the cornea, using it in corneal implants improves implant grafting potential and promotes implant success. The tripeptide arginine-glycine-aspartic acid (RGD) sequence that makes up the collagen microstructure is recognized by neighbouring integrin receptors and is crucial for controlling cell activity. To produce a unique optical transparency, small leucine-rich proteoglycans (SLRPs) control the thickness of the corneal collagen fibrils during development. It is essential to comprehend how these developmental processes add to intricate corneal micro-anatomy to maintain corneal functionality as much as possible when creating corneal implants.

Collagen's capacity to functionalize robust chemical linkages with neighbouring fibrils is known as cross-linking. Collagen cross-linking in the cornea happens spontaneously as a result of an oxidative deamination event that happens inside the collagen's end chains. It has been suggested that keratectasia, or corneal ectasia,

frequently advances most quickly in youth or early adulthood but tends to stabilize in patients beyond middle age due to this natural cross-linking of collagen. Although crosslinking usually happens spontaneously over time, there are alternative mechanisms that can cause crosslinking to happen sooner than expected. Glycation is a process that occurs more frequently in diabetics and can result in the formation of new collagen connections. It has been demonstrated that oxidation can cause corneal cross-linkage by releasing oxygen free radicals in the pathway that is most pertinent to our subject. Researchers at the University of Dresden in Europe created the foundations for the corneal collagen cross-linking methods that were currently in use in the late 1990s. Via the oxidation process, collagen cross-linking was induced in the riboflavin-soaked corneas of rabbits and pigs using UV radiation. It was demonstrated that the resulting corneas were more rigid and resistant to enzymatic breakdown. The study also demonstrated that, as a result of fibril crosslinking, treated corneas had greater molecular weight collagen polymers.

According to safety studies, if the corneal thickness was greater than 400 microns and appropriate UV irradiation was maintained, the treatment did not harm the endothelium. Early findings from Dresden's human trials on UV-induced corneal cross-linking were encouraging in 2003. 16 patients with fast-developing keratoconus have participated in the original pilot research, and following therapy, all of the patients' progression stopped. Furthermore, 65% of patients showed an improvement in visual acuity, and 70% of patients experienced flattening of their steep anterior corneal curvatures (decreases in average and maximum keratometric values). No issues were reported. The FDA granted Avedro orphan drug status for its riboflavin ophthalmic solution formulation in late 2011, to be used in conjunction with the company's specific UVA irradiation equipment. On April 18, 2016, the FDA approved riboflavin and UV-induced corneal collagen cross-linking. The dearth of adequately conducted Randomized Controlled Trials has reduced the data supporting the use of CXL in the therapy of keratoconus, according to a 2015 Cochrane systemic review that examined the drug's efficacy in treating the condition [17].

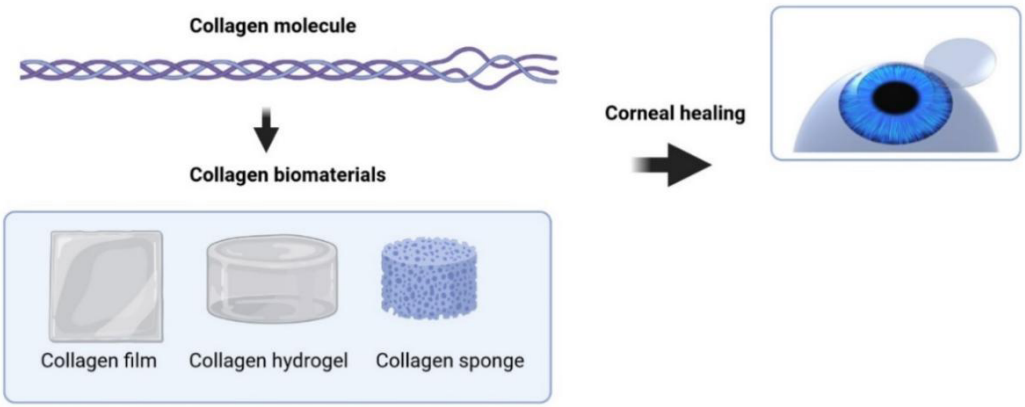

Figure 3. Application of collagen biomaterial in tissue engineering of cornea [18].

8. Type 1 Collagen for tissue engineering scaffolds

Collagens are a significant class of structural proteins that are expressed in various tissues and have unique characteristics. Collagen gives transparency to the cornea and crystalline lens of the eye, although it is opaque in the skin. There are 28 different forms of collagen, and while they all have a triple helix structure, their α-chain compositions vary, giving each type of collagen unique features. The diverse arrangement of collagen fibers also plays a role in the alteration of tissue shape. The discovery and use of collagen as a biomaterial

is a result of its significant capacity to create various tissues. The two main types of collagen present in the tissues of the cornea and lens are collagen type I (Col-I) and collagen type IV (Col-IV). For a clear vision, both colleges offer structure and transparency. The utilization of these two forms of collagen as novel biomaterials in bioengineering distinct tissue to cure a range of ocular conditions that could result in blindness is examined in this article. Figure 3 shows the application of Type 1 collagen in corneal tissue engineering.

9. Fabrication Methods

Biofabrication methods for tissue engineering of the cornea involve utilizing advanced technologies to create functional corneal tissue for transplantation. Some key methods include:

(1) 3D bioprinting: This technique involves layer-by-layer deposition of bio-inks containing corneal cells and biomaterials to create a 3D corneal structure with precise control over cell placement and architecture.

(2) Electrospinning: Electrospinning is used to fabricate nanofibrous scaffolds that mimic the natural extracellular matrix of the cornea, providing a suitable environment for cell growth and tissue regeneration.

(3) Decellularization and recellularization: This method involves removing cells from a donor cornea to create a scaffold that retains the structural integrity of the tissue. The scaffold can then be repopulated with patient-specific corneal cells to generate a functional cornea.

(4) Self-assembly techniques: By providing the necessary cues and environment, corneal cells can self-organize and form a corneal tissue structure without the need for external scaffolds.

Overall, these biofabrication methods offer promising avenues for creating corneal tissue constructs that closely resemble the native cornea, with the potential to revolutionize corneal transplantation and improve outcomes for patients with corneal diseases or injuries. There are many fabrication methods for tissue engineering scaffolds for cornea repair and regeneration. The following methods have been reported and these methods also utilized Type 1 collagen as biomaterials [19].

10. Bioprinting

Additive manufacturing includes bioprinting, which is a subset of 3D printing. Here, the basic method used is layer-by-layer material stacking to fabricate a scaffold or framework using computer images. With its advantages over traditional molding techniques, including the capacity to produce customized structures from recorded images and the repeatability of cell printing, bioprinting has gained attention recently. Recent research on in situ printing, which uses handheld printers to print biomaterials or cells directly onto wounded regions to repair wounds and speed healing, has provided us with an idea of what future surgeries might entail. Materials used in bioprinting relate to biomaterials, which are often organic materials like collagen, gelatine, and alginate, or bio-ink with a cell-laden ability, as opposed to materials utilized in regular 3D printing, such as plastics. Extrusion-based bioprinting, laser-assisted bioprinting, and inkjet bioprinting are the three techniques most commonly utilized in 3D bioprinting. In bioprinting, other technologies such as vat photopolymerization might also be employed.

Inkjet bioprinting is a fast and cost-effective method for fabricating various tissues, including bone, cartilage, blood vessels, and retinal layers. It involves depositing micro-drops of bio-inks onto a substrate, with techniques such as continuous inkjet printing (SIJ) and drop on demand inkjet printing (DOD). DOD has been used to fabricate corneal-like structures with corneal stromal cells, achieving good cell viability. Laser-assisted bioprinting involves a pulse laser applied to a laser absorption layer, allowing thermal expansion to

eject micro-droplets onto the substrate. This technique can precisely control the type and density of cells during printing, making it suitable for scaffold-free cell structures. However, it has high cell death rates, which may impact tissue long-term survival. Stereolithography (SLA) is a 3D bioprinting technique that uses a laser beam to initiate photo-polymerisation or photo-crosslinking in a selected area of bioink. This technique has been successfully used to print human corneal-like stroma and artificial cartilage and liver. Extrusion bioprinting is the most common bioprinting method, with shear-thinning bioink extruded from a syringe using pressure from air, pistons, or screws. Extrusion 3D printers, including single, multi-syringe, and joint syringes, are used in tissue engineering and surgery. They can print human skin with different layers, such as dermis and epidermis. Coaxial extrusion bioprinting has been used to crosslink alginate with calcium ions. Handheld devices like bio-pen and double syringe extrusion printers have been developed for treating cartilage injuries. For low viscous bioinks, a method called "freeform reversible embedding of suspended hydrogels (FRESH)" has been developed, printing low-viscosity bioinks in a supporting material for higher resolution and structural support. This technique has been used to print various human tissues [20].

10.1. Type 1 collagen as bio-ink

Col-I, a key component of the human corneal stroma, is often combined with other materials for corneal applications. Recent bio-inks have shown improved printability and transparency with increased collagen concentration and sodium alginate. However, the alginate structure could potentially inhibit cell proliferation. Overall, these bioinks offer potential for corneal applications. Collagen-based bio-ink has been explored using temperature-sensitive biomaterials and natural cross-linkers to facilitate the liquid-to-gel transition. A bio-ink primarily incorporating decellularised cornea was used to print a corneal model, with over 75% light transmittance and compatibility with human turbinate-derived mesenchymal stem cells. The study focuses on 3-D printing of corneal tissue using various printing methods, including FRESH, extrusion printing, DoD, and laser-assisted printing. Crosslinking methods used in these studies include natural biomaterials like alginate-calcium, gelatin, thrombin, and low-temperature agarose. Most studies use lower concentrations of Col-I to maintain transparency but require additional gentle crosslinkers. The only bioink with a higher Col-I concentration is 20 mg/mL of decellularised cornea with 86% collagen. The collagen sources are either animal-based or human tissue. Current studies are primarily proof-of-concept and focus on corneal stromal layers and cells. None of the projects use photo-crosslinking, which could be cytotoxic. However, studies have shown that cell viability can be enhanced by lowering the strength of the curing light. This could lead to the development of cell-encapsulating collagen-based bio-inks for cornea bioengineering [21].

11. Electrospinning

Globally, corneal disorders impact over 10 million individuals and are the second most common cause of visual loss. Since there is a significant scarcity of newly donated corneas and there is an uncertain danger of immunological rejection with conventional heterografts, it is imperative to replace pathologic corneal tissue with a corneal counterpart. The construction of a scaffold with mechanical characteristics and transparency akin to the natural cornea is critical for the regeneration of corneal tissues. Corneal tissue engineering has become a viable approach to the development of corneal tissue substitutes. High surface area-to-volume ratios and porosity in electrospun nanofibrous scaffolds mimic the structure of native extracellular matrix (ECM) protein fibres. Electrospinning polymer components, fibre architectures, and functionalization are versatile processes that have made it possible to fabricate nanofibrous scaffolds with the right mechanical strength, transparency, and biological characteristics for corneal tissue engineering. The current state of electrospun scaffold

development for corneal tissue engineering is reviewed in this paper. The main topics covered are electrospun materials (single and blended polymers), fibre structures (isotropic or anisotropic), functionalization (better mechanical properties and transparency), applications (corneal cell survival, phenotype maintenance, and corneal tissue formation), and future development prospects.

A high voltage is applied between a syringe and a deposition target (or collector) in the versatile fabrication process known as electrospinning, which draws nano- or micro-scale fibers from the material the syringe dispenses. The basic components of electrospinning equipment are a grounded collector, a spinneret (usually a hypodermic syringe needle) linked to a high-voltage power supply, and a high-voltage direct current power supply (5 to 50 kV). According to the theory underlying this technology, a droplet suspended on top of the pipe will form a charged jet when the repulsive force between charged particles in polymer solutions overcomes the surface tension of the solution. The droplet will then repeatedly split in the electrostatic field and the solvent will eventually evaporate, forming fibres on the collector. Three-dimensional (3D) fibrous scaffolds can be created by depositing these fibres in a designated collector. More than 200 polymers, both synthetic and natural (e.g., polycaprolactone (PCL), poly-l-lactic acid (PLLA), poly(lactide-co-glycolide) (PLGA), polyethylene oxide (PEO), collagen, gelatin, hyaluronate (HA), chitosan, silk fibroin (SF), etc.), have been successfully electrospun to nano- or micro-scale fibres to date. The chemical makeup of the polymer solution (molecular weight, concentration, and solvent), the applied voltage, the distance between the spinneret tip and the collector, the feeding rate, the capillary diameter, the humidity, and the temperature are some of the processing variables that can impact the fibre morphology. The morphology can be changed to create fibres with spindles on a string, uniform fibres, or beaded fibres, depending on the applied parameters.

Due to its close structural resemblance to native extracellular matrix (ECM), high surface area-to-volume ratio, and good porosity. All of which support cell adhesion and movement, proliferation, and differentiation, as well as its excellent mechanical properties, great material handling, suitability for implantation, and scalable production. Electrospinning has recently attracted increased interest for fabricating biomimetic engineering functional corneal tissue [22].

12. Corneal implants

Corneal implants are medical devices used to treat various eye conditions affecting the cornea, the transparent front part of the eye that covers the iris and pupil. These implants are designed to improve vision by reshaping or replacing damaged corneal tissue. There are different types of corneal implants available, each serving a specific purpose. One common type of corneal implant is the intrastromal corneal ring segments, which are small, clear, arc-shaped devices inserted into the cornea to correct conditions like keratoconus or irregular astigmatism. Another type is the artificial cornea or keratoprosthesis, which is used when traditional corneal transplant surgery is not an option. Corneal implants can help improve vision, reduce dependence on glasses or contact lenses, and enhance the overall quality of life for individuals with corneal disorders. However, like any surgical procedure, there are risks and potential complications associated with corneal implants, so it is important to consult with an ophthalmologist or eye surgeon to determine the best treatment option for each case.

The basic collagen cross-linked cornea substitute was fabricated. N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) were combined with 10% porcine type I collagen at pH 5. Lamellar keratoplasty was used to implant the final homogenous solution into rabbits and minipigs after it had been molded to the dimensions of the cornea and allowed to cure. Following surgery, the implants were monitored for up to six months. Detailed slit lamp biomicroscopy, in vivo confocal microscopy, topography,

and esthesiometry for nerve function were all used in the clinical assessment of the cornea. Additionally, histopathologic analyses were carried out on rabbit corneas that were removed after six months. The optical clarity of cross-linked collagen was higher than that of human corneas (refractive index: 1.35). Just one out of every twenty-four surgical corneas implanted into rabbit and porcine corneas displayed a minor haze six months following surgery. Every other implant stayed optically clear and did not respond negatively. The topography revealed a smooth surface and a profile resembling the nonsurgical contralateral eye. The matrices that were implanted encouraged the regeneration of neurons, tear film, and corneal cells. There was a return of touch sensitivity, suggesting partial function. The implanted corneas displayed steady host-graft integration and did not exhibit any appreciable decrease in thickness. Water-soluble carbodiimides can be used as protein cross-linking reagents to stabilize collagen enough for use in the creation of implantable corneal matrix substitutes. The straightforward cross-linking technique would make it simple to create transplant matrices in locations where corneas are in scarce supply or where emergency perforation repairs require temporary patches.

Nowadays, the second most common cause of blindness worldwide is corneal illness. Currently, corneal transplantation is thought to be one of the most popular therapies for visual loss. In the development process, this work proposes a novel strategy that uses dual-crosslinked membranes made of N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and polyrotaxane multiple aldehydes (PRAs). EDC/NHS and PRAs crosslinked collagen, respectively, to create imine groups and stable amide linkages. When compared to membranes crosslinked with a single crosslinker, dual-crosslinked (Col-EDC-PRA) membranes demonstrated improved resistance to collagenase degradation and higher mechanical qualities due to the creation of a double interpenetrating network. Col-EDC-PRA membranes also have good water content and light transmittance properties. Col-EDC-PRA membranes were demonstrated in cell studies to be noncytotoxic and to differ very slightly from other membranes. The presence of stromal cells and neo-stroma, as shown in hematoxylin–eosin-stained histologic sections and optical coherence tomography images of the anterior segment, indicates that corneal stromal repair occurred in a rabbit keratoplasty model at 5 months. In addition, the surgical site showed no signs of inflammation, corneal neovascularization, or corneal rejection reaction. Overall, the findings showed that corneal tissue regeneration following a corneal defect was successfully facilitated by the dual-crosslinked membranes [23,24].

13. Clinical challenges and limitations

Clinical challenges and limitations in cornea repair include the following. Addressing these challenges requires ongoing research into novel treatment modalities, improving surgical techniques, increasing donor awareness, and expanding access to specialized care for patients with corneal disorders.

(1) Inadequate donor availability: There is a shortage of corneal donors, leading to delays in corneal transplant surgeries.

(2) Graft rejection: The risk of immune-mediated rejection of the transplanted cornea remains a major challenge, requiring lifelong monitoring and management.

(3) Complications post-surgery: Complications such as infection, inflammation, and graft failure can occur following corneal transplant surgery.

(4) Corneal scarring: Severe corneal scarring due to trauma or infection can limit the success of corneal repair procedures.

(5) Limited treatment options: Current treatment options for corneal diseases such as keratoconus or corneal ulcers are limited, with some cases requiring more advanced interventions like keratoprosthesis.

(6) Surgical expertise: Performing corneal transplant surgeries requires specialized training and expertise, limiting access in certain regions or healthcare settings.

(7) Cost of treatment: Corneal repair procedures can be costly, posing a barrier to access for some patients.

Several obstacles need to be cleared before bioengineered corneas are accepted as implant forms. The mechanical toughness and elasticity necessary for a longitudinally functional optical system are inherent to bioengineered collagen. Even though collagen cross-linking techniques have improved these inferior qualities, it is still a difficult task to determine the ideal blend ratios of biomaterials to build a corneal implant that is identical to the natural cornea. While both type I and type III collagen hydrogels are flexible and have tensile strength that is suitable for handling, type III collagen hydrogels alone provide a more accurate mechanical and optical replica of the original cornea in implants. Similarly, employing natural biomaterials increases the light transparency of corneal implants. Nevertheless, these materials are more challenging to electrospun, a regularly utilized fibre manufacturing technique used to improve the mechanical strength of biomaterials. As a result, corneal implants lose mechanical strength when they are designed without the electrospinning process. Therefore, it is necessary to produce implants that have as many properties as human corneal tissue if corneal implants are to be as useful in a clinical context as possible. Eventually, it will also be necessary to overcome the high costs linked to these intricate production processes. Ultimately, the best way to recreate native corneal tissue will depend on our ability to comprehend the molecular intricacy of corneal development, which is mostly represented by the stromal architecture.

14. Conclusion

Recent years have seen tremendous advancements in ocular tissue engineering. There are several engineering challenges in creating a biocompatible, mechanically stable, and optically transparent tissue. To tackle these problems, researchers have looked at two basic approaches: using cells to manage their extracellular matrix and using scaffolds to provide thick, transparent matrices for cell development. Each strategy has had some degree of success. Furthermore, novel advancements in tissue-engineered construct innervation have been created. Enhancing the mechanical stability of engineered constructs and the host response to implantation must be the focus of future studies. Biomaterial made of type 1 collagen has been utilized to implant or build scaffolds to restore corneas. A variety of scaffold-building techniques have been discussed, along with tissue engineering applications for corneal regeneration and repair. In light of this correlation, type 1 collagen was a viable biomaterial for the creation of tissue engineering scaffolds for corneal regeneration that works.

Disclosure statement

The author declares no conflict of interest.

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