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Functional biomaterials for corneal tissue regeneration

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Abstract: The cornea, a pivotal component of the eye, plays a critical role in maintaining visual acuity through its mechanical strength and transparency. Approximately 90% of its thickness is derived from collagen lamellae, essential for its structural integrity. Alterations in corneal pathology, often resulting from injuries or age-related degeneration, significantly impair its function in light transmission and focusing, subsequently affecting visual quality. Traditional approaches to managing corneal diseases primarily include conservative therapies and donor tissue transplantation. However, these methods are frequently limited by risks of infection, rejection, and a scarcity of donor tissues.

The inherent regenerative capabilities of corneal cells present a promising avenue for research into corneal tissue regeneration. Challenges such as inflammatory responses, neovascularization, and limbal stem cell deficiency pose significant threats to corneal clarity and function. The delicate balance between anti-angiogenic and proangiogenic factors is crucial.

This review delves into the intricacies of corneal repair, examining the multitude of factors influencing this process. We provide an in-depth analysis of current advancements in corneal regeneration, highlighting the role of various functional biomaterials. These biomaterials, both synthetic and natural, offer innovative solutions for enhancing corneal regeneration, potentially revolutionizing the treatment of corneal diseases and injuries.

Keywords: cornea; regeneration; eye; transplant; biomaterial

1. Introduction

Anterior and posterior segments are the two compartments of the complex organ eye one-third of the eye was occupied with the anterior segment and the remaining portion occupies the posterior segment. Various tissues occupies the different compartments of the eye. There are many life threatening diseases affecting the eye and few diseases are holding high prevalence globally. Age-related macular degeneration and diabetic retinopathy are the most frequently affected diseases of the eye [1]. Topical route is the non-invasive route. Around majorly the eye drops paves a significant role. A ocular bioavailability of less than 5% was accounted by the topical administration. The corneal permeation is suffered with various factors. The therapeutic drug concentration to reach the posterior segment of the eye is the major problem by overcoming the various barriers associated with the eye [2–4]. In case of ocular drug delivery the topical route is preffered compared to the other ocular routes. The repeated intravitreal injections may leads to several complications. Static and dynamic barriers (ocular barriers) restricts the entry of drugs in to the eye [5]. The ocular barriers protects the cornea from dehydration and preserves the transparency for transmission of light.

The ocular barriers play a crucial role in protecting the eye from corneal injury, acting as a defense mechanism against external threats. These barriers include the various obstacles restricting the entry. Each layer serves a specific function in protecting the eye.

1.1. Tear film

The outermost barrier, the tear film, is composed of three layers. The oily layer prevents evaporation of the underlying water layer, the aqueous layer provides nutrients and carries away waste, and the mucous layer ensures even distribution of tears on the eye surface. This film provides defense against foreign particles.

1.2. Corneal epithelium

The multilayered tissue beneath the tear film is the corneal epithelium, which serves as a mechanical barrier. It prevents the entry of microorganisms and foreign substances into the eye. The epithelial cells containing the tight junctions restrict the movement of substances between cells, maintaining a controlled environment.

1.3. Anterior ocular barriers

The cornea and conjunctiva holds innate immunity features like lysozymes and immunoglobulins in the tear film. The cornea itself, despite its avascularity, has immune privilege due to the expression of anti-inflammatory factors and the lack of blood vessels, which reduces the risk of immune-mediated damage.

1.4. Blood-aqueous barrier

The blood-aqueous barrier is located deeper in the eye, which is composed of the endothelium of the iris blood vessels and nonpigmented ciliary epithelium. It controls the exchange mechanism, and prevents the entry of toxic substances and pathogens into the eye's anterior chamber.

1.5. Blood-retinal barrier

Retinal pigment epithelium and the endothelium of the retinal vessels covers the innermost ocular barrier, the blood-retinal barrier. This barrier maintains the homeostasis of the retinal environment, essential for optimal visual function. It regulates the movement of molecules between the retina and the bloodstream, protecting the retina from blood-borne pathogens and toxins.

The integrity of these ocular barriers is crucial for maintaining eye health and clear vision. Damage to these barriers, through injury, infection, or degenerative diseases, can lead to various ocular pathologies. Understanding these barriers and their protective mechanisms is key to developing treatments for ocular diseases and injuries [5].

Globally around 285 million people are with visual impairment, and 39 million are blind. The foremost cause of visual impairment is the corneal disease. In clinical practice diseased cornea replacement is the major practice. The demand for donar cornea is highly increasing in the recent scenario. For every 70 persons only one donor cornea is the current scenario. In case of autoimmune disorders corneal allotransplantation is the major contraindication. The significant complication is the Donor-derived infection. Corneal regeneration may be achieved through Tissue engineering technology based construction of corneal substitutes.

2. Selection criteria

The inclusion /exclusion criteria mentioned in Table 1. Original research/review articles are considered for this review whereas excluded thesis, and short communications are excluded. Preliminary selection of few options were done independently. Association between functional biomaterials has been excluded in this review paper.

Table 1. Inclusion/exclusion criteria and order of application.

Order	Inclusion criteria	Exclusion criteria
1	English language articles	Non-English language articles
2	Research and Review articles	Thesis and short communications/mini- review
3	Biomaterials for corneal tissue regeneration	Biomaterials for dental/ocular/similar applications
4	Tissue regeneration for ocular corneal diseases	Tissue regeneration for non- ocular diseases

The articles obtained from the bases include 102 articles from web of science, 62 articles from scopus, 32 articles from science direct, 37 articles from PubMed. Herein the duplicate articles were excluded from the study. At the screening stage the articles were selected based on the abstract, keywords and title. At the eligibility stage the articles included for full reading

include 82 articles. The full text articles excluded for the study were 7 articles. Overall in this review 95 articles were included and remaining were of the case reports.

3. Factors influencing Corneal repair

The corneal epithelium may get easily disrupted. Integrity of cornea may gets affected due to the abnormalities. The persistent effect may be achieved based on the epithelial defects. The repeated defects at the ocular tissue level may causes the corneal healing [6,7]. The trauma or corneal dystrophies may cause Recurrent corneal erosions. The defective epithelial cell anchoring may cause the corneal epithelium to wear. The epithelial basement membrane dystrophy may be achieved due to the abnormalities in composition and formation of the basement. The redundant layers at the basement membrane also occurred at the corneal epithelium. Line based basement membrane as fingerprints are also observed. The cellular debris cause the cells to trap within the basement membrane to form cysts. Disruptions in corneal clarity was caused due to the abnormal cell layering and inadequate adhesion of cells to the underlying stroma [8–10].

The cellular/acellular components constitute the cornea along with the corneal layers present in the eye. Around 5-7 layers of cells with 50 μ thickness are present in the corneal epithelium. The smooth regular surface with non- keratinized stratified squamous epithelium is associated with the corneal epithelium which is composed of mucin. The epithelial cells of cornea exhibits a life span of 7 to 10 days. The optical transparency was associated with the high concentrations of intracytoplasmic enzyme crystalline [11]. The stroma makes up a significant amount of the cornea and 95% of the thickness of the cornea in humans. The extracellular matrix is made up of parallel-running lamellae seen in the stroma. Collagen fibrils with uniform sizes are evenly dispersed inside these lamellae. The cornea's transparency is caused by this homogeneous dispersion of collagen fibrils. Proteoglycans are macromolecular glycoconjugates that control the homogeneous distribution of collagen fibrils. Collagen-binding small leucine-rich repeat proteoglycans are a type of small interstitial PGs found in the extracellular matrix of the cornea. Either chondroitin sulfate⁄dermatan sulfate chains or kearatan sulfate chains are carried by these proteoglycans. Keratan sulfate chains are carried by lumiican, keratocan, and mimican; chondroitin sulfate⁄dermatan sulfate chains are carried by decorin, biglycan, and versican.

Around two thirds of the eye's refractive power comes from the transparent cornea, which makes up the anterior part of the outer layer of the eye. Its dual purposes are to shield the insides of the eye and to provide this power. The human cornea consists of five layers. Majorly the stroma tissue gets occupied. Desmosomes supports the epithelial cells. Within the basement membrane the basal cells gets attached. From the underlying layers the attachment prevents the epithelium. Any changes may leads to corneal erosions and nonhealing epithelial defects.

The basal cells releases Type IV collagen and laminin, make up the 40–60 nm thick basement membrane of epithelial cells. The basement membrane contains the lucida and densa.. Fibronectin levels rise in the event of basement membrane disruption, and the repair

process may take up to six weeks. The majority of the cornea's structural framework, or about 80%–85% of its thickness, is made up of the corneal stroma. This causes major abnormalities in the eye. The extracellular matrix (ECM) and stromal fibers are precisely arranged to give the stroma its distinctive transparency. Type I collagen makes up the majority of the collagen in corneal fibrils. The stroma also contains Type XII and Type VI collagen [12].

4. Corneal repair

Hemostasis, inflammation, cell proliferation, and remodeling phases are the four phases involved in corneal wounds. In case of wound healing the missing tissue or cellular structures paves a major role. During wound healing cornea has to be overlooked [13–16]. Blinding corneal infections and inflammations causes the corneal repair. The low bioavailability high therapeutic concentration supports the promotion in the corneal wound healing in case of the eye drops. Regeneration approaches creates a great challenge in eye research. Disease, infection, and trauma, causes risk of vision loss which may cause Corneal transparency loss [2]. Majority of the patients suffer from corneal disorders each year. The low and middle-income regions causes over 90% of corneal blindness [17] and transplantation surgery with 53% of patients with corneal blindness. The responsible factors for corneal repair are shown in Figure 1.

The naturally derived biopolymer with characteristics similar to the native cornea is the Gelatin plus light GelCore with chemically modified porcine gelatin mixed with light-activated compounds [18]. GelCore solidifies and firmly adheres to the corneal tissue upon application to the wound with a cross-linking system which seals the defect without sutures. The light intensity used in the cross-linking system is well below the maximum exposure limit based on the biosafety concerns [19–21].

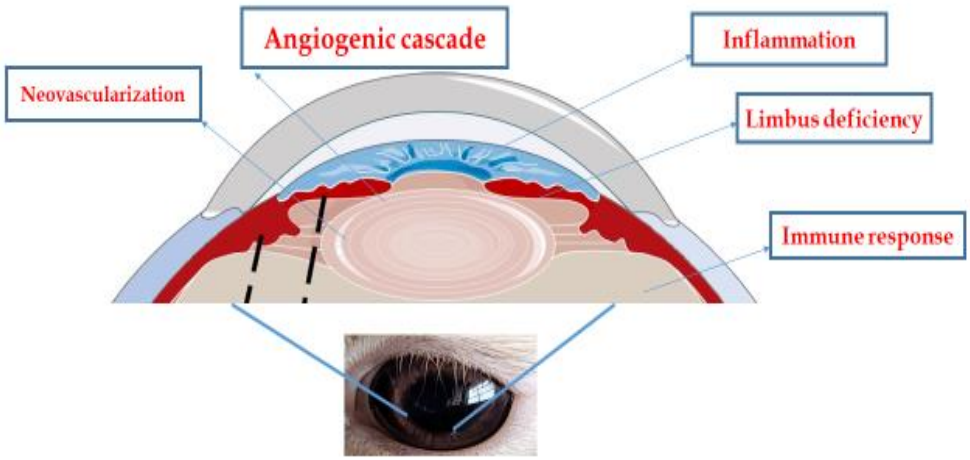

Figure 1. Factors responsible for corneal repair.

5. Corneal regeneration

Corneal tissue engineering has become a viable therapeutic option due to the deficiency of corneas and problems with traditional corneal transplantation. A World Health Organization

(WHO) research states that the lack of corneal donors causes 15%–20% of individuals in need of corneal transplantation to go untreated. In addition to concerns about infectious illnesses, the scarcity of donor tissues over the past few decades has sped up research on non-transplantation treatments. One such therapy option is the keratoprosthesis, or artificial cornea [22]. In order to facilitate the regeneration of new tissue from the host, regenerative medicine should supply the proper milieu, components, cells, and/or signals. Various cell delivery strategies have been applied for corneal regeneration. Rebuilding the deteriorated tissue is the goal of extracellular matrix (ECM) factors released by healthy host cells that are either supplied or stimulated by paracrine signalling [23–25].

The approach of regenerative medicine changes epithelium and endothelium layer regeneration from invasive to non-invasive procedure further focus towards 2D into 3D cell delivery methods for stromal tissue regeneration. The uniform distribution and size of stromal collagen fibers (48–113 nm) are responsible for corneal transparency. Therefore, in stromal tissue regeneration, appropriate surface structure and characteristics can promote cell proliferation and differentiation. The overall tissue thickness up to 10% is made up of the corneal epithelium, which is made up of at least six layers: two layers of wing or superficial cells. In contrast to the stroma and endothelium, the epithelium heals by means of stem cell proliferation, specialized epithelial cell development, and migration to the site of injury. Otherwise, minor wounds can be self-healed by core epithelial cells. On the other hand, endothelium regenerates mostly through cell migration, while stromal cells change. The process of regeneration involves a number of substances and mechanisms of action that precisely control various cellular processes. The complicated network explains the intricacy and its management. Growth factors are the important growth factors involved in the injury-induced intercellular crosstalk [26,27]. The different formulation approaches reported for corneal tissue regeneration was shown in Figure 2. Angiogenic factors play a crucial role in the process of corneal neovascularization and regeneration. These factors include both proangiogenic and antiangiogenic molecules. The balance between these two types of factors is essential in maintaining corneal clarity and function. Vascular endothelial growth factor (VEGF), particularly VEGF-A, is a key proangiogenic factor, stimulating various steps of hemangiogenesis such as endothelial cell proliferation and migration. On the other hand, antiangiogenic agents like endostatin and angiostatin help maintain the avascularity of the cornea. In conditions where there is an imbalance, leading to a surplus of proangiogenic factors, corneal neovascularization may occur, potentially compromising corneal clarity and visual acuity. This understanding of the molecular mechanisms involved in corneal vascularization has been instrumental in developing treatments for corneal diseases and in corneal tissue engineering.

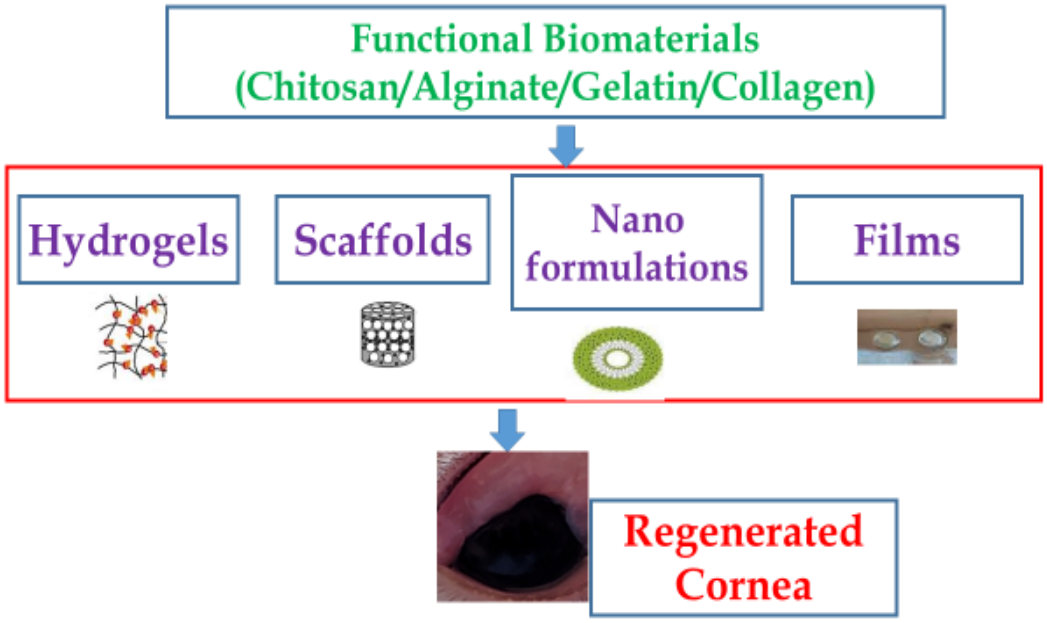

Figure 2. Formulation approaches for corneal tissue regeneration.

6. Functional biomaterials for corneal regeneration

6.1. Collagen

In the human and mammalian body the most abundant protein is the collagen. As a scaffold in tissue engineering Collagen gets widely utilized with occurrence in wide range of organisms. The fibrillar collagen are considered to be biocompatible. Collagen properties include tensile strength improvement, biodegradable, biocompatible *etc.* In case of burns/wounds, Collagen based materials holds its superior properties [28]. Three polypeptide chains are observed in the helical form of collagen. Proline and hydroxyproline are found at the positions X and Y. The triple helix gets supported by the electrostatic interactions and hydrogen bonding support. The various forms of collagen shown better responsive properties at different environment [29]. The typical characteristics of functional biomaterials focussed for corneal tissue regeneration is shown in Figure 3.

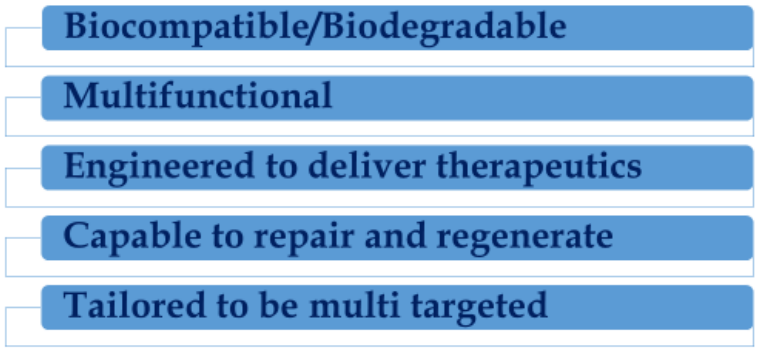

Figure 3. Typical characteristics of functional biomaterials for Corneal tissue regeneration.

ECM fibril-forming collagens in the cornea are the Types I and V collagens are the main characteristics. The extracellular matrix (ECM) and other corneal structures including the basement membrane (BM) remodelling was influenced by the Collagen types. Corneal visual impairments in humans involving corneal opacification are the severe physiological and clinical abnormalities associated with the collagen. Adhesion of the epithelium was found with the Collagen type VII [30]. Around 70% of the cornea was occupied with collagen, collagen has been well utilized in corneal bioengineering. Collagen has been also utilized for *in vivo* animal and human modeling.

Collagen prepared using electro-compaction techniques is widely used in corneal bioengineering applications. Due to the well-organized and biomimetic fibril structure the mechanical characteristics may get significantly improved. The tensile and compressive modulus creates a ten-fold increase in its strength. Compared to normal human cornea higher glucose permeability was afforded with the electro-compacted collagen (ECC) membrane. Human corneal epithelial cells was associated with adhesion and multiplication. The required mechanical, optical, glucose-permeable, topographic, structural, and cell support qualities was emphasized by the ECC [31]. In order to create an artificial cornea with restoring specific layers the cellular regeneration paves the therapeutic focus. Anterior and posterior lamellar keratoplasty occurs due to the advent of novel, less invasive surgical methods and they replace injured corneal tissue while sparing the surrounding healthy tissue, has made this transition feasible. When compared to penetrating keratoplasty, which replaces the entire cornea, these methods can lead to better results in terms of graft survival and the quantity of postoperative problems.

Ocular tissue shortage is the restorance of ocular corneal tissue. Biocompatibility, strong, and transparency are the characteristic features of the biomaterials. Restoring the mechanical and optical properties the Viscoll collagen membrane has been reported to replace the stroma tissue. The implantation of Viscoll collagen membrane shown better effect. The nerve regeneration was promoted by the integration approaches. In case of the corneal stroma regeneration the safety and efficacy of the Viscoll collagen membrane was maintained. They conclude that this regenerated material is as efficient as stromal tissue for up to 10 months [32].

Bioengineered corneal tissue from unprocessed starting materials can be offered as an alternative for donor tissue. During phase I human clinical trial 10 patients associated with keratoconus or stromal scarring received anterior lamellar grafts. The removal of cornea stroma/epithelium was associated donar cells. The overlying sutures was used in addition with the anterior grafts because their strength was insufficient for direct suturing through the material. They observed that the implanted materials steadily integrated without rejection four years after implantation, moreover the vision recovery was limited due to irregularities on the ocular surface and surgical sutures with delay in re-epithelialization. The wound healing and maintenance of corneal homeostasis was accomplished by corneal nerve regeneration—which is essential for over the long term—but not to normal levels [33,34].

The artificial corneas made of crosslinked collagen are a unique regenerative option to offer promising clinical results. The Crosslinkers removed from implants avoid cytotoxicity. Enzymatic degradation was associated with the crosslinked product. Using pyrene

conjugated dipeptide amphiphile (PyKC), which is made up of cysteine and lysine, it has been reported that a crosslinker-free supramolecular gelation technique in which collagen molecules are entangled within the PyKC network without the collagen's functional groups being altered. The ability to block UV light was associated with the recently created collagen implants, known as Coll-PyKC, which possess the characteristics of sutureable, afforded by the Coll-PyKC implants which may stimulate anti-inflammatory differentiation in human monocytes while inhibiting pro-inflammatory differentiation, thereby the Implants containing Coll-PyKC can limit the spread of human adenoviruses [35].

6.2. Gelatin

Gelatin is derived from collagen. Due to the tertiary structure the aqueous solubility of gelatin was found to be superior compared to Collagen [36–38]. Gelatin is obtained from animal collagens those derived from the tissues of pigs, fish, and cows. Gelatin quality is decided by the extraction time, temperature, and pH used in the collagen processing process. Based on the acidic environment he molecular weight and isoelectric point varies based on the gelatin quality [39–41]. Bloom strength and proline-hydroxyproline content decides the Gelatin triple-helix content [42–44]. Gelatin based polymers are highly biocompatible with Methacrylate (GelMA) hydrogel as mechanical properties. The differentiation of rBM-MSCs into keratocyte-like cells, supports the tissue-engineered corneal stromal transplantation was afforded by the GelMA promotion [45,46].

A gelatin based hydrogel was reported using the 3 D fiber hydrogel construct sililar to the natural cornea. This hypothesis was supported strongly. The only effective treatment is the organ rejection or infection, corneal transplantation. The main goal of tissue-engineering-based approaches is the finding safe and innovative strategies. For corneal stromal applications the potential of gelatin based system showed its effect. With a methacrylation degree of 75% the Marine GelMA was synthesized. The formulations of ascorbic acid shown enhanced collagen production [47].

Chondroitin sulfate (CS) shown better effect with cross-linked porous nano based gelatin materials shown for designed corneal keratocyte scaffolds. These indicate that there is a positive correlation between the CS content in the biopolymer matrices and the NHS to EDC molar ratio [48].

With the influence of dual crosslinking reactions Rutin based hydrogel system has been reported with the aid of *in situ* based systems. Rutin composites shown a 14 days release and its biocompatibility assay confirms the composites' nontoxicity. Rutin-encapsulated composites cured the cornea within 48 hours with a healing rate of 98.3% ± 0.7%. ERK/MAPK pathway was observed for the corneal wound healing [49]. The effect of gelatin methacrylate (GelMA) hydrogel repairing process was reported in rat cornea after surgery of lamellar keratoplasty (LKP). Whereas the GelMA group's rats also had a GelMA hydrogel embedded during corneal transplantation, the LKP group's rats received enhanced green fluorescent protein lamellar stroma matrix transplants from Sprague-Dawley rats. Days 7, 30, and 90 were dedicated to harvesting grafted eyes. The process of corneal regeneration and

restoration was investigated using slit-lamp microscopy, immunofluorescence staining, optical coherence tomography, scanning electron microscopy, and hematoxylin and eosin staining.

Around 42 rats in all, comprising 6 rats in the control group and 18 rats in each of the experimental groups, were examined. The degree of inflammatory cell infiltration between the GelMA group and the LKP group was observed after three months with a difference ($P < 0.001$). Furthermore, a noteworthy distinction in corneal thickness was noted between the GelMA group and the LKP group after several comparisons. Additionally, there was a difference ($P < 0.001$, $P < 0.001$) in the outcomes between the LKP group and the control group [50].

6.3. Silk Fibroin

Silk fibroin is a insoluble protein obtained from the mulberry silkworm *Bombyx mori* (Bm) [51–55]. Transparent thin silk films supports the generation of a corneal epithelial cell sheet *in vitro* [56]. For damaged corneal surface the silk fibroin film obtained from non-mulberry Antheraea mylitta (Am) offers to be a promising which supports cell growth and differentiation [57–59]. Rigidity associated with other cellular events has been offered by the scaffold. Ocular abnormalities does not shown any markable changes. These indicates as a corneal scaffold the silk fibroin film may be utilized [60,61].

Silk fibroin, is a hydrophilic protein. The two types of proteins that forms *Bombyx mori* worm cocoons are the sericin and silk fibroin [62–64]. Sericin was observed for silk fibroin. Neatly arranged sheets and are embedded in the amorphous matrix are found in the shimmering circuits [65]. Silk fibroin reported to be promising role in the ocular drug delivery too. The movement of epithelial cells that substitute injured tissue in ocular wound healing was observed. The injured region was supported by the silk fibroin [66]. For CEC regeneration Curcumin (CC) and silk fibroin (SF) were used as biocompatible materials to build transparent film scaffolds. The film scaffolds underwent hydrophilicity assessment, stiffness, transparency, thermal characterization, and surface analysis. This promotes tissue integration and cellular contact was observed with the CC/SF film scaffold's. All films displayed stable thermal characteristics and an enhanced ability for cell growth when the appropriate quantity of CC was added to the SF film scaffolds [67].

The reported work aimed to produce insulin (INS) films based on silk fibroin (SF), a naturally occurring polymer possessing anti-inflammatory properties. The films were designed to treat corneal wounds by gradually releasing a bioactive INS into the eye. SF films with 100 IU/cm^2 INS and glycerine as a plasticizer were created through casting. SF was found to be in the β-sheet/Silk II conformation and INS and SF had non-covalent interactions, according to analysis performed using DSC, FT-IR and SEM revealed that the original globular form of the INS was embedded in the film. The released insulin from the film actually maintained its native conformation and biological activity *in vivo*, as shown by circular dichroism, which also caused the blood glucose levels of Wistar rats to drop. The film shows burst release, suggesting that it could be helpful in repairing damaged corneal epithelium without needing to be administered several times a day. Consequently, SF films

demonstrated potential in the sustained delivery and liberation of INS, rendering them a feasible treatment choice for corneal injuries [68].

The effects of biodegradable silk fibroin was studied and reported. Throughout the cultivation process, the scaffolds with 250 and 500 ng/mL GDNF exhibited the highest keratocyte proliferative activity. In an experimental model of epithelial-stromal damage mice on different group was injected with GDNF exhibited smaller corneal epithelial defects in comparison to the other groups. The best positive immunohistochemical responses were seen in groups 2 and 3, when using antibodies against certain pathways. Consequently, GDNF-based silk fibroin scaffolds accelerate the stromal nerve plexus's formation, the process of epithelialization, the growth of keratocytes and epithelial cells, and their anti-apoptotic characteristics [69].

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Silk fibroin films' permeability, transparency, strength, and thickness have been demonstrated to make them an effective carrier of corneal epithelial and limbal cells. Silk fibroin has been used synergistically to improve cell adhesion, with polyethylene glycol (PEG) and RGD peptides. Silk fibroin has a high Young's modulus (6–8 GPa129), is flexible, and can form thin films, which makes it an ideal material for corneal endothelial graft engineering.. In order to investigate nerve regeneration into a cornea, Wang *et al.* developed Corneal stromal cells seeded into multiple layers of silk fibroin and then joined with a collagen hydrogel. At that point, epithelial cells were applied to this section of the construct. Following the encapsulation of dorsal root ganglion neurons in a silk sponge along the outer rim of the construct, immunofluorescent staining was used to track the nerve growth into the central construct. In this work, transparent ultrathin film scaffolds for corneal endothelial cells (CECs) using silk fibroin (SF) and aloe vera (AV) gel was utilized. The scaffolds' physical and chemical characteristics were ascertained by analyzing their transparency and contact angle using FT-IR and FESEM.

The immunofluorescence, and other cell mediated assays for CECs were measured through the use of RT-PCR techniques. A tiny quantity of AV gel was added, which improved cell viability and preserved its functions. Small incisions were made to easily handle the scaffolds for transplantation. Without causing a noticeable inflammatory response, the scaffolds integrated with the surrounding corneal tissue and adhered to the corneal stroma's surface. These findings suggest that AV blended SF film scaffolds could be a good stand-in for other corneal grafts in transplant procedures [71].

Silk fibroin (SF), and GelMA/SF (GS) with high adhesion behaviors. The materials prepared using silk fibroin showed improved adhesion properties. The prepared GS tissue adhesives showed high physiological adhesion. The adhesive strength of GS can be modulated up to three times higher than that of G. Additionally, it was found that GS is biocompatible and has a high potential for healing. Moreover, the obtained GS transmission

value is similar to that of the human cornea. GS promoted cell adhesion and proliferation. It was found that the GS-60s sealants had a greater burst pressure strength for fresh cornea [72].

Using electrospinning and permeation techniques, silk-gelatin composite scaffold has been reported. To physically crosslink the silk nanofibers, ethanol vapor (T) was applied. Micrographs from scanning electron microscopy shown micro/nano-scale characteristics of the manufactured scaffolds. Characterizations were done by Fourier transform infrared spectroscopy. Using UV-visible spectra, the scaffolds' transparency was assessed. The highest level of transparency was displayed by measuring 77.75 ± 2.3%. This value is comparable to the native cornea's transparency when it comes to acute vision.

Physically and microscopically, treated samples showed significantly more stability than untreated ones. These scaffolds showed remarkable cellular compatibility with mouse fibroblast cells (L929 RFP) and rabbit corneal fibroblast cells (SIRC) when cultured on artificial scaffolds, as compared to SF (in aqueous; T). A manufactured new scaffold is most likely a good choice for corneal tissue reconstruction. Furthermore, the results demonstrate by its best stability, maximum transparency, and enhanced cytocompatibility of both silk and gelatin, is a better choice for a corneal stromal analog than the other manufactured scaffolds.

6.4. Chitosan

Among its advantageous qualities are low toxic/immunogenic/biodegradable/ biocompatible/absorbable. Chitosan is a polysaccharide derived from natural chitin. Chitosan hydrogels have found extensive application in medicinal field due to their exceptional water-absorption and water-retention capabilities [73]. For corneal epithelium regeneration, thermosensitive chitosan-gelatin hydrogel has been reported. It was observed that the proliferation, chemotaxis, and migration of stem cells could be enhanced by exogenous cells and mesenchymal stem cells (MSCs) exhibited significantly elevated expression levels of related genes *in vitro*. The corneal fate of the LESCs was suppressed by MSCs. Upregulation of the expression of genes was carried out by the histological analysis. Growth factors may be secreted via the SDF-1/CXCR4 chemokine axis and stem cell homing may be activated to accomplish this [74]. The molecular structure of Chitosan makes it insoluble at neutral and high pH whereas at low pH chitosan can be protonated, acetic acid (1%–3%, v/v) are often used to solubilize chitosan.

Recently it has been reported that chitosan-N-acetylcysteine (C-NAC) shown wound healing effect. Herein 20 New Zealand White rabbits randomized, placebo-controlled, masked experiment were performed. Under general anaesthesia, manual scraping was used to cause a monocular epithelial debridement. The animals were randomly assigned to receive a placebo or C-NAC twice a day. OCT and fluorescein imagings were used to monitor the healing of corneal wounds. Following wound induction, measurements were taken both right away and up to 72 hours later. Following surgical debridement, there was no discernible variation in wound size between the treated/un treated groups. In comparison to the placebo group, the C-NAC group experienced noticeably faster wound healing (for both methods).

OCT is regarded as a non-invasive, dye-free substitute for traditional fluorescein staining in the evaluation of corneal wound healing in humans [75].

Chitosan is a conventional biomaterial for wound dressing because of its innate antibacterial and coagulation-promoting properties [76–78]. In the application, chitosan's solubility is another problem. Its application is largely restricted by its solubility in weak acid solutions and insoluble in water [79–81]. For example, quaternization can be used for its anti microbial activities, and acidification can be used to increase its anticoagulation properties. Furthermore, chitosan's structure and composition can be changed to provide them with appropriate performance and multifunction [82–84]. A thermosensitive hydrogel based on chitosan that releases murine nerve growth factor over an extended period of time was described as an ophthalmic solution (CTH-mNGF). Its efficacy was assessed in patients with neurotrophic keratopathy and corneal denervation (CD) mice. CTH-mNGF was evaluated in a murine corneal denervation model in the preclinical context. Transparent and thermosensitive, CTH-mNGF guaranteed continuous release of mNGF on the ocular surface for more than 20 hours, sustaining the local mNGF concentration at approximately 1300 pg/mL *in vivo*. This work reveals the transparency, thermosensitivity, and sustained-release characteristics of CTH-mNGF. Given that CTH-mNGF is convenient and affordable, its ability to repair corneal epithelial defects [85].

Photo-crosslinked tissue adhesive has been reported to use a photosensitizer methylene blue along with urocanic acid-modified chitosan (CS). Specifically, a 650 nm red diode was used to control the curing time. Photo-crosslinked, gel applied to a perforated cornea, which prevented aqueous humor from leaking. When applied to corneal wounds, the gel's blue appearance allowed for exceptional precision. Crucially, the dissipation of MB from tears caused the crosslinked gel to become transparent in less than 24 hours, and the gel sloughed off naturally without the need for artificial removal. The development of a novel photo-crosslinkable CS gel modified with urocanic acid was reported overall in the study, and it showed great promise for use in the healing of corneal perforations [86]. The various functional biomaterials for corneal tissue regeneration are shown in Table 2.

Table 2. Functional biomaterials for corneal tissue regeneration.

S No	Biomaterial	Mechanism	Therapeutic role	Reference
1	Collagen (Hydrogel)	Hydrogel offer sustained release of drug	Replacement of diseased corneal stroma.	[87]
2	Collagen–polycaprolactone (Col- PCL)	Facilitates cell mediated adhesion	Water adsorption property	[88]
3	Collagen (Viscoll collagen membrane)	Promotes nerve regeneration	Corneal stroma regeneration	[89]
4	PVA/Collagen (Composite Nanofibrous Electrospun Scaffold)	Proliferation and induction of Human keratocytes	Tissue-engineered cornea	[90]

Table 2. *Cont.*

S No	Biomaterial	Mechanism	Therapeutic role	Reference
5	Chitosan NPs	Promotes Human corneal endothelial cells proliferation	Corneal endothelial regeneration	[91]
6	Chitosan–poly(ethylene glycol) (hydrogel films)	Proliferation	Attractive candidates for the regeneration and transplantation of corneal endothelial cell	[92]
7	Gelatin (Hydrogel)	Regeneration of a new tissue	Treatment for Corneal Wound Repair	[93]
8	Gelatin (Hydrogel Filler)	Cellular adhesion and proliferation	Induce corneal stromal regeneration	[94]
9	Silk fibroin (film)	epithelial cells mediated sprouting	Utilized as a corneal scaffold.	[95]

7. Comparative analysis of synthetic and natural biomaterials in corneal tissue regeneration

In corneal tissue regeneration, a critical comparison between synthetic and natural functional biomaterials highlights their distinct characteristics and applications.

7.1. Synthetic biomaterials

These materials, including polymers like PMMA (Polymethylmethacrylate) and PHEMA (Polyhydroxyethylmethacrylate), are favored for their consistency, tailorability, and longer shelf life. They are engineered to precise requirements, such as specific mechanical strength, porosity, and degradation rate, making them highly versatile for corneal implants and contact lenses. Their predictability and lack of biological contaminants are major advantages. However, synthetic materials may lack intrinsic bioactivity, potentially requiring modification to enhance cell attachment and proliferation.

7.2. Natural biomaterials

Materials like collagen, fibrin, and hyaluronic acid, derived from biological sources, offer a more biologically relevant environment. They mimic the natural extracellular matrix, promoting better cell attachment, proliferation, and differentiation, essential for tissue integration and healing. Natural biomaterials can also provide critical signaling cues to cells, aiding in tissue formation. Yet, they often suffer from batch-to-batch variability and potential immunogenicity. Additionally, their other properties can be less predictable compared to synthetic counterparts.

7.3. Hybrid approaches

To leverage the advantages of both, hybrid biomaterials are being developed. These combine the mechanical robustness and tailorability of synthetic materials with the biological relevance of natural materials. For instance, bioengineered collagen-polymers or hydrogels infused with natural signaling molecules aim to create biomaterials that are not only mechanically suitable but also biologically instructive.

7.4. Biocompatibility and integration

A critical aspect in choosing biomaterials for corneal regeneration is biocompatibility. Synthetic materials, while less likely to carry biological contaminants, may invoke a foreign body response. In contrast, natural materials are generally more biocompatible but pose a risk of disease transmission. Advanced sterilization and bioengineering techniques are being explored to mitigate these risks.

7.5. Transparency and mechanical properties

For corneal applications, maintaining transparency is crucial. Synthetic materials often provide excellent clarity, but their integration with surrounding tissues can be challenging. Natural materials typically integrate well but may not always provide the necessary clarity or mechanical strength, especially in load-bearing scenarios.

7.6. Regeneration and healing

The ultimate goal of using these biomaterials is to support corneal healing and regeneration. Natural materials excel in promoting cellular activities that are conducive to healing due to their biological components. Synthetic materials, engineered with specific degradation profiles, can serve as scaffolds that gradually transfer load to regenerating tissues.

7.7. Future directions

Research is ongoing to develop biomaterials that combine the best features. Innovations in nanotechnology, surface modifications, targeted systems, stimuli responsive systems etc are areas of intense research. The goal is to develop biomaterials that not only support the physical structure of the cornea but also actively participate in the healing and regenerative processes.

In conclusion, both synthetic and natural biomaterials have distinct roles in corneal tissue regeneration, each with specific advantages and limitations. The choice between them depends on the specific requirements of the application and the desired outcome. As the field advances, hybrid and bioengineered materials are likely to play a significant role in addressing the challenges of corneal regeneration.

8. Recent insights on corneal tissue regeneration

Artificial corneal has been recently emerged using printing technology. The field of printing and regenerative medicine has seen significant advancements, offering new possibilities in personalized medicine and addressing donor shortages. This technology allows for the precise deposition of bio-inks, tailored to individual patient needs in terms of corneal curvature, thickness, and refractive power. Tissue engineered corneas, which can be custom-made with specific biomechanical, optical, and biological properties, overcomes the limitations of traditional donor corneas. These include the challenges of donor health screening, limited availability, and adverse effects. The emphasis in corneal tissue engineering is now on functional reconstruction, focusing on materials that provide clarity, such as hydrogels, and on incorporating cellular components and growth factors to replicate the natural structure of the cornea. In order to demonstrate the safety and tolerability of corneal implant and to offer better treatment to the eye an open-label and prospective clinical trial has been conducted who receive synthetic cornea (Id-NCT05667337). The biodegradable natural and synthetic bio-functional materials for corneal tissue regeneration have to be considered with relevance to its effect at the ocular cellular environment and its possible interactions has to be studied.

9. Conclusion

In conclusion, significant strides have been made in tissue engineering for corneal regeneration, leveraging both natural and synthetic functional biomaterials. These advancements have not only provided viable corneal substitutes but also revolutionized approaches through cell-based and gene-based therapies. This progress marks a significant turning point, offering hope for long-term success in restoring corneal function and addressing the global need for corneal transplants. The integration of these innovative strategies underscores a transformative era in ophthalmology, opening new avenues for treating and understanding corneal diseases and injuries.

Conflicts of interests

The authors do not declare any conflicts of interests.

Authors' Contribution

The author contribution for this manuscript are as follows, V.K.; writing—original draft preparation, M.K.; writing—review and editing, S.V.; visualization, S.A.J.R.; supervision. All authors have read and agreed to the published version of the manuscript.

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