Chapter

Advances in Biomaterials for Corneal Regeneration

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Abstract

The human cornea acts as a protective covering for the eye and plays an important role in light transmission into the eye for vision. Corneal defects due to trauma, infection, or disease can have detrimental effects on the vision, and severe cases lead to vision loss. Twenty-three million people are estimated to be affected by corneal blindness worldwide. Treatment involves corneal transplantation surgery, but there is a severe shortage of donor corneas worldwide. Furthermore, patients with severe pathologies risk rejecting conventional corneal transplantation, thus leaving them untreated. Therefore, there is an urgent need to develop new therapies to replace traditional corneal transplant surgery. This review focuses on recent potential biomaterials development for corneal regeneration and repair. It includes cell-based therapies, cell-free regeneration-inducing biomaterials, and injectable or in-situ gelation-based biomaterials for patients with a high risk of graft failure. It also consists of the emerging role of exosomes and extracellular vesicles in corneal infections and regeneration.

Keywords: corneal regeneration, biomaterials, corneal repair, transplantation, hydrogel, cell-free regeneration

1. Introduction

The cornea is an avascular, transparent, and multilayered tissue whose primary function is to act as a lens to direct light entering the eye toward the retina. It consists of three cellular layers, an epithelium, stroma, and endothelium, and two acellular layers, the Bowman's and Descemet's membrane [1]. A third acellular layer, the Dua membrane, has also been described. The epithelium forms the cornea's outermost layer and comprises stratified epithelial cells. These cells act as a barrier to protect the eye from pathogens and allow the diffusion of essential nutrients and oxygen. The stroma constitutes 90% of the cornea's total thickness and contains keratocyte cells interspersed in lamellae but forming a connected network. The cornea is responsible for focusing approximately 75% of light into the eye for vision. It also protects the eye while preserving its transparency by self-renewal of its epithelium in particular [2]. However, injury, aging, or disease could lead to irreversible loss of clarity and corneal blindness.

Corneal blindness is one of the significant causes of vision loss leading to 23 million patients who have unilateral blindness and 4.9 million patients who have bilateral blindness globally [3]. Although corneal transplant is the most common

transplanted tissue worldwide, corneal blindness is still rising, with 2 million cases yearly [4]. Due to the increasing demand, eye banks cannot provide the cornea to all the patients, leaving an estimated 12.7 million people on the waiting list for corneal transplantation [5]. Corneal transplant with a human donor cornea has been the go-to treatment for corneal blindness since the twentieth century. In penetrating keratoplasty or full-thickness grafts, a full-thickness button of healthy donor corneal tissue replaces excised pathologic corneal tissue. Only the damaged layers are replaced in lamellar keratoplasty or partial thickness grafts. This helps maintain the integrity of the cornea and surrounding tissue, leading to faster healing and vision improvement. Complications of corneal transplantation include graft failure, of which graft rejection is a leading cause. Disease transmission from graft to host is rare but has been reported, e.g., endophthalmitis, due to donor-to-host microbial transmission [6]. Other issues include complications in immune-compromised patients, infectious diseases, and astigmatism associated with corneal transplants. However, proper donor screening and processing may reduce the risk of infection and related post-surgical complications. However, the donor cornea shortage is a major issue, especially in developing nations. It is even difficult to carry out complex surgical procedures in some developing countries due to insufficient resources/facilities. Thus, there is an urgent need to develop new therapies or biomaterials as alternatives to donor transplantation.

In this chapter, we focus on the biomaterials rather than fabrication techniques such as electrospinning or 3D bioprinting. We refer the reader to Patel et al. 2021 for a review of electrospinning in corneal constructs [7] and to Bin et al. 2019 and Fuest et al. 2020 for reviews on bioprinting of corneal implants [8, 9].

2. Cell-based therapies

Several cell-based therapies are already approved for treatment in clinical settings [10–13]. These include resurfacing the cornea with limbal epithelial cells. Cellular therapy of corneal stroma has gained attention in recent years. Studies revealed that mesenchymal stromal (or stem) cells (MSCs) could survive and differentiate into adult human keratocytes in animal models without eliciting an inflammatory response. In addition, they produced new collagen in the host stroma and were able to remodel scars and improve transparency in animal models for corneal dystrophies [14, 15]. Cell-based therapies can be broadly divided into using stem cells to stimulate regeneration with and without scaffolds. This section focuses on stem-cell-based theories with scaffolds or templates.

Bio-fabrication of stem cell supports from silk fibroin/gelatin (SF/G) film and scaffold to make stem-cell-incorporating corneal epithelial and stromal equivalents for canine corneal regeneration. Canine limbal epithelial stem cells (cLESCs) were seeded in SF/G film, and canine corneal stromal stem cells (cCSSCs) were seeded in SF/G scaffold, respectively, which supported cell adhesion, viability, and proliferation along with differentiation of cLESCs and cCSSCs into keratocytes. Studies revealed endogenous ECM production after 14 days, thus mimicking native cornea. [16]. Pitarresi and coworkers were the first ones to use hydrogels based on hyaluronic acid (HA) cross-linked with polyaspartamide derivative (PHEA-EDA) as an alternative to amniotic membrane for the delivery of limbal stem cells (LSCs). HA/PHEA-EDA hydrogel showed biocompatibility with immortalized human corneal epithelial cells or primary cells but only moderate to poor cell adhesion capabilities. These features

allow the potential for clinical application of HA/PHEA-EDA as a delivery substrate with easy release of transplanted limbal stem cells to treat damaged corneas [17]. HCECs and human adipose stem cells (HASCs) were co-cultured in 3D hyaluronic acid hydrogel with and without collagen. HASCs could proliferate and differentiate within the proper cell density, and the survival of cells was better in the absence of collagen, showing potential for use in ocular surface reconstruction [18]. Hyaluronan hydrogel scaffold was used to expand human corneal epithelial stem cell ex-vivo in the xeno-free environment. The developed hydrogel behaved as native cornea equivalent, which reduced the risk of xeno-contamination and allowed the expansion of limbal stem cells [19].

3. Decellularized corneas repopulated with stem cells

Decellularized corneas comprising intact ECM composition and integrity hold the potential to use as an implant in the same or cross-species. They maintain the native mechanical strength and stromal structure but lack the cellular components, preventing graft rejection, or activating undesirable immune responses. Recently, Polisetti and coworkers explored the use of decellularized human corneas as scaffolds. The decellularized human corneal scaffold preserves the native extracellular matrix proteins, glycosaminoglycans, and tissue structure to allow regeneration of the epithelium and stroma of the host tissue ex-vivo [20]. Decellularized human limbus (DHL) was used as a biomimetic scaffold for transplanting limbal epithelial progenitor cells (LEPCs) in ex-vivo transplantation. The scaffold provided a limbal niche-specific microenvironment where native ECM composition was preserved and showed excellent biocompatibility for LEPCs and limbal melanocytes (LMS). Furthermore, the scaffold allowed complete epithelization with interspersed melanocytes and stromal repopulation from host tissue, thereby showing potential for regeneration of damaged cornea in patients suffering from limbal stem cell deficiency (LSCD) [21].

A rabbit corneal model was used to study the in-vivo biocompatibility of decellularized human corneal stroma with and without recellularization of human adipose-derived adult stem cells (h-ADASC). The decellularized corneal sheets had intact extracellular matrices and an excellent recellularization capacity with h-ADASC. In addition, it revealed good transparency with no clinical sign of rejection. The post-mortem analysis revealed the survival of transplanted stem cells within the graft and the differentiation of stem cells into functional keratocytes [22].

In a human clinical trial, decellularized porcine corneal stromas were transplanted into 47 patients with corneal fungal infection. There was no recurrent disease observed within 3 years of the study. Seventy-two percent of patients showed visual improvement. However, neovascularization was observed in 53% of patients but was suppressed eventually. Thus, the decellularized cornea has demonstrated promising potential in regenerating damaged corneas while preserving the native ECM composition. However, care must be taken to prevent zoonotic pathogenic transmission, as seen in recent years of global transmission of coronavirus from animals to humans.

4. Cell-free regeneration-inducing biomaterials

Recently biomaterials have shown enormous potential in tissue engineering due to their desirable physical, chemical, and mechanical properties, which can guide cells to

grow and promote functionality. Biomaterials developed as corneal substitutes should replicate the structure and functionality of the native cornea. They should mimic the in vivo microenvironment, e.g., extracellular matrix, to support resident cell growth, proliferation, and integration within the host tissue for bifunctional regeneration of damaged cornea. Biomaterials should also possess desirable characteristics such as biodegradability, biocompatibility, non-immunogenicity, and seamless integration with host tissues. In addition, biomaterials should possess sufficient mechanical (tensile) strength to support suturing or gluing and to withstand the intraocular pressure to support the fluctuations. Besides this, it is important to have optical transparency and a refractive index like that of a healthy cornea, plus allow the diffusion of nutrients into the scaffold and waste out of it [23]. Thus, it is important to have these considerations in mind for designing corneal construct mimicking native cornea. Griffith and coworkers have provided an extensive guide to consider beforehand while designing biosynthetic alternatives for clinical applications [24].

Natural, synthetic, and composite polymers are the main biomaterials used for corneal tissue engineering. Natural polymers tend to have inherent biocompatibility and bio-integration compatibilities, whereas synthetic polymers allow for customization of desired chemical and mechanical properties. Composite polymers have the advantage of both natural and synthetic polymers, i.e., they are both biocompatible and tunable to allow for desirable mechanical and chemical properties for corneal tissue engineering.

4.1 Naturally derived and synthetic hydrogels

The main advantage of using naturally derived polymers for corneal tissue engineering is their biocompatibility. Commonly used natural polymers for corneal regeneration include collagen, silk fibroin, gelatin, chitosan, cellulose, hyaluronic acid (HA), and decellularized cornea. They are formed by chemical cross-linking and formation of physical bonds and networks.

4.1.1 Collagen and collagen-based hydrogels

One of the most attractive natural polymers is collagen, the most abundant extracellular matrix component in several tissues including the human cornea. Collagen is the main component of corneal tissue. It possesses unique properties such as biocompatibility, bio-adhesiveness, suitable biodegradability, and low immunogenicity, which is to support corneal regeneration. In addition, collagen supports the corneal epithelial growth and cell adhesion without eliciting toxicity. The source of collagen plays important role in its physiochemical properties. Type I and type II collagen possess adequate tensile strength, whereas type III collagen is superior in mechanical strength and optical clarity [25]. Collagen from different sources such as porcine, bovine, rat, recombinant human collagen type I and III are commercially available. On their own, collagen hydrogels are soft and unstable. Attempts to improve the physicochemical properties of collagen-based hydrogels include chemical cross-linking and development of composite hydrogels with synthetic polymers to add mechanical strength. Examples include cross-linking by glutaraldehyde, 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC), and multifunctional dendrimers to improve the elastic and mechanical strength of collagen scaffolds [26]. One limitation of using chemical linkers is that they can be cytotoxic and not cell-friendly, limiting their use to fabricating cell-free scaffolds. A naturally derived cross-linker such as genipin was tested to reduce the cytotoxic effect of cross-linker, but the concentration

of genipin required to enhance the mechanical strength resulted in dark-blue-colored collagen scaffold [27].

Further, to reduce the heterogenicity of collagen derived from animal source, Fagerholm and team [28] performed the first successful clinical trials where they used the cell-free implant made from recombinant human collagen type III (RHCIII). Trial includes the treatment of 10 patients with keratoconus or central scarring with RHCIII implant, which showed stable regeneration of epithelium, stroma, and nerves after 2 years of implantation. The long-term observation also revealed the immune compatibility of implants. Cell-free implant containing carbodiimide cross-linked recombinant human collagen (RHC) was grafted into patients and revealed stable integration of regenerated neo-corneas without rejection. In addition, nerve and stromal cell regeneration observed over 4 years mimic microarchitecture of healthy corneas without recruiting inflammatory dendritic cells into the implant area [29]. Next, Griffith and coworkers conducted a clinical study in cornea patients with a high risk of implant rejection [30]. 2-methacryloyloxyethyl phosphorylcholine (MPC), a synthetic phosphorylcholine with reported inflammation suppressing properties, was incorporated into RHCIII to form interpenetrating networks. The resulting RHCIII-MPC hydrogels were implanted into the corneas of unilaterally blind seven patients (one dropout) by anterior lamellar keratoplasty after excision of the pathologic tissue. The patients were followed up for an average of 24 months (**Figure 1**). The implants supported the stable regeneration of the epithelium, stroma, and nerves over the observation period [30]. Short collagen-like peptides (CLPs) conjugated to polyethylene glycol (PEG) showed promising functionality equivalent to recombinant human collagen. CLP-PEG hydrogel supported stable regeneration of corneal tissue and nerve in preclinical animal testing [31].

Recently, a cross-linker-free supramolecular gel strategy has been explored to make collagen gel where collagen molecules were intertwined inside a pyrene conjugated dipeptide amphiphile (PyKC) without any functional group modification. The newly developed collagen implant was optically transparent, mechanically stable, and supported the growth of all corneal cells. It also triggered anti-inflammatory differentiation while suppressing the pro-inflammatory differentiation of human monocytes [32]. Plant-derived recombinant human collagen I was used to make hydrogel-based corneal graft to support mechanical stability, biocompatibility, and transparency in mini-pigs. Thus, showed the potential of plant-based RHC1 as an alternative to animal-derived collagen or allografts [33].

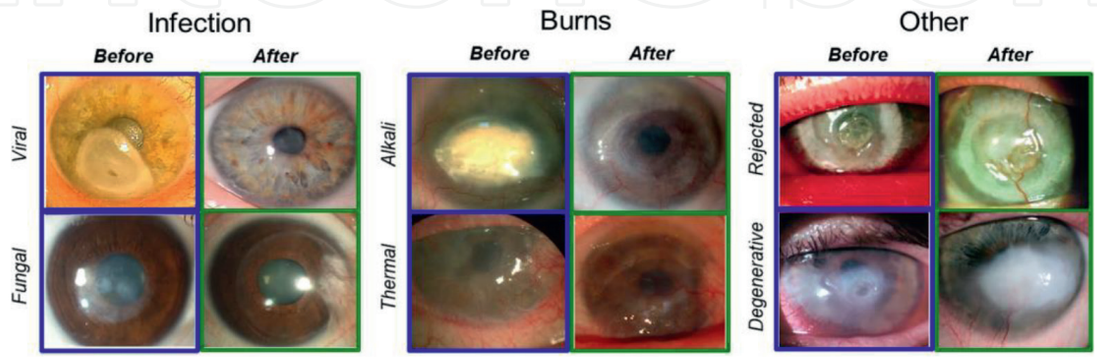

Figure 1.
Clinical trial of RHCIII MPC corneal implant in high-risk patient at the last follow-up showing three groups of patients as per the preoperative diagnosis: Infection (herpes and fungal keratitis), burn (alkali or thermal), and other (failed graft and post-stroke neurotrophic keratitis). Patient with infection showed best recovery with mostly clear regenerative cornea followed by cornea with burns. Reproduced from [30].

4.1.2 Gelatin-based hydrogel

Recently, methacrylated gelatin (GelMA)-based hydrogels have gained attention for corneal tissue engineering because of their appropriate mechanical strength and optical properties. GelMA hydrogels showed good biocompatibility with 98% survival rate of keratocyte after 21 days of culture. In vivo studies of Gel MA in rabbit model showed no signs of ulcer, edema, or infection when hydrogel was implanted in mid-stromal pocket and observed for 8 weeks. Hematoxylin and Eosin staining showed hydrogel integration within the host tissue with negligible foreign body reaction [34]. The efficacy of GelMA in repairing rat corneas compared with lamellar keratoplasty (LKP) showed significant differences between the two groups in terms of inflammatory cell infiltrates, corneal cell thickness, and expression of α-SMA and TGF-β after 3 months of observation. Thus, supporting the ability of Gel MA in alleviating the corneal stroma fibrosis and reducing the loss of corneal refractive power due to fibrosis [35]. Electrospinning was used to make Gel MA fibers that were then complexed with poly (2-hydroxymethyl methacrylate) (p(HEMA)) through UV photo-polymerization to form GelMA-p(HEMA) composite hydrogels. The composite hydrogel showed optical transmittance equivalent to that of the human cornea, highly promising mechanical strength, improved structural integrity, and supported proliferation of BCE C/D¬1b corneal endothelial cells in 3D culture [36].

Gelatin-based hydrogels have also been explored for the drug delivery applications to overcome the poor bioavailability of traditional eye drop method. Dual cross-linking reactions prepared gelatin hydrogel/contact lens composite via in situ free radical polymerization and carboxymethyl cellulose/N-hydroxysulfosuccinimide to encapsulate rutin for corneal wound healing. The composite hydrogel showed sustained release of rutin for 14 days and without eliciting toxicity. In vivo studies in rabbit cornea injury model showed that the rutin-encapsulating composite hydrogel supported faster healing (98.3% ± 0.7%) at 48 h post-operation compared with the composite alone (healing rate, 87.0% ± 4.5%). Results also revealed the role of ERK/MAPK and PI3K/AKT signal pathways in corneal wound healing [37]. Injectable photo-cross-linked gelatin hydrogels were formed using acrylated gelatin and thiolated gelatin with tunable mechanical properties, biocompatibility, and biological properties that support the repair of corneal wound. The hydrogel showed promising cell viability in cell seeding and cell encapsulation studies and supported the regeneration of new tissues under focal corneal wounds in rabbit corneas [38]. Collagen/gelatin/alginate (CGA) hydrogels were used for the sustained delivery of moxifloxacin (MFX) and dexamethasone (DEX) loaded into nanoparticles, for the treatment of infectious ocular keratitis. Lipid nanocarrier loaded with MFX/DEX (Lipo-MFX/DEX) and encapsulated within the hydrogel showed promising cell proliferation ability when co-cultured with ocular epithelial cells. CGA-Lipo-MFX/DEX composite hydrogel showed the sustained release of drug for up to 12 h and exhibit the ability to inhibit bacterial growth to improve corneal wound healing [39]. Thus, gelatin-based hydrogels are not only promising alternatives for stimulating corneal regeneration but could also be a viable option for ocular drug delivery applications.

4.1.3 Silk-fibrion-based hydrogels

Silk fibroin is a structural protein extracted from the silkworm's cocoon, Bombyx mori. Silk represents a unique choice to use as a biomaterial for tissue engineering due to its tunable and robust mechanical properties, controlled degradation, and

non-immunogenic properties [40]. Photoactivated, in-situ forming antimicrobial silk-based hydrogels were developed for treating corneal injuries. Gentamicin-loaded methacrylated-silk (SilkMA) hydrogels adopted the corneal injury shape and gelled within a few minutes upon exposure to low-intensity UV. The mechanical strength, transparency, and water content approached those of the human cornea, and hydrogel inhibited the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* for up to 72 h [41]. Silk-fibroin-based films (SF) were also complexed with lysophosphatidic acid (LPA) to form a functionalized graft for corneal endothelial cell (CEnCs) regeneration. The functionalized graft supported cell adhesion provided good biocompatibility and expressed CEnCs-specific genes and protein when tested in rabbit corneas [42]. A hybrid transparent fibrillar film made from SF/GelMA could be tuned as needed to form matrices with different properties by modifying the volume ratio of SNF to GelMA. The SNF/GelMA ratio of 30/70 was optimal and showed mechanical strength, transparency, and stability (reduction in degradation rate) that approximated the properties of the human cornea. The SNF/GelMA film supported cell attachment, spreading, and proliferation of stromal cells, making it an ideal candidate for corneal regeneration [43].

Recently, a study reported the upregulation of cytokine IL-1β, which induces undesirable phenotypic changes in keratocytes by downregulating the gene and protein expression of keratocyte corneal stromal markers: Keratocan, Lumican, Aldh3a1, and CD34. These markers play important role in maintaining corneal epithelial homeostasis and corneal transparency during normal conditions [44, 45]. The selective IKKβ inhibitor, TPCA-1, can reverse the phenotypic change and preserve the keratocyte phenotype in diseased corneas. Zhang and coworkers developed silk fibroin hydrogels with sustained release of TPCA-1, which accelerated in vivo wound healing, and increased keratocyte expression marker while supporting the regeneration of epithelium and stroma [46]. Thus, showing the promising potential of silk-fibroin-based hydrogel for corneal regeneration and drug delivery applications in vivo (**Table 1**).

Biomaterial	**Target tissue**	**Novelty**	**Developmental status**	**Refs.**
Natural and Biosynthetic hydrogels				
Collagen	Stroma	Collagen-based porous hydrogel with good transparency and mechanical strength; showed sustained release drug delivery in-vitro, developed new Flap-ALK surgical implantation model inspired from LASIK surgery	Animal model: rabbit	[47]
Gelatin	Corneal surface: Epithelium	Glycidyl methacrylate grafted on gelatin to form elastic protein-based, adhesive hydrogel that showed 4 times better stretchability, biocompatibility, and higher tensile strength	In-vitro studies	[48]
Gelatin	Stroma	Visible-light-based stereolithography (SLA) 3D bioprinting methodology developed to make gelatin methacrylate (GelMA) hydrogels that can be molded into dome shaped corneal implants	In-vitro studies	[49]

Biomaterial	Target tissue	Novelty	Developmental status	Refs.
Recombinant human collagen type III	Epithelium and stroma	Biosynthetic recombinant human collagen implant can be fabricated using chemical or photochemical cross-linking. The former cross-linking methodology results in hydrogels promoting in situ tissue regeneration; of sufficient toughness to allow handling but requires overlying sutures for retention	Animal model: mini pigs, rabbits	[25]
o-nitroso benzaldehyde group (NB)-modified gelatin (GelNB)	Corneal surface: Epithelium	Developed molecular coating to attach covalently to corneal surface, enhanced corneal epithelial cell migration in-vitro, promoted self-healing, and inhibit disorder of regeneration	Animal model: rabbit	[50]
Recombinant human collagen	Epithelium and stroma	RHC hydrogel tested in 10 patients with significant vision loss and results showed stable integration, supported corneal reepithelization, nerve regeneration and restore touch sensitivity:	Pilot clinical trial	[28]
Composite hydrogels				
Collagen (Col) and polycaprolactone (PCL)	Epithelium	Corneal graft made from Col-PCL membrane, comprising transparent central part made from Col and mechanically robust edge from Col-PCL with high tensile strength (1.1 ± 0.3 MPa)	In-vitro and ex-vivo studies	[51]
Gelatin methacrylate (GelMA) and long-chain poly (ethylene glycol) diacrylate (PEGDA)	Epithelium and stroma	3 D printing of bi-layer dome shaped cell laden scaffold made from GelMA and PEGDA, demonstrated high transmission, swelling, nutrient permeation and appropriate degradation rate	Animal model: rabbit	[52]
methacrylated gelatin (GelMA) and poly(2-hydroxyethyl methacrylate) (pHEMA)	Stroma	Developed novel cell loaded IPN hydrogel made from GelMA-HEMA for corneal stroma model, appropriate mechanical strength, and high transparency	In-vitro	[53]
Collagen and glycol polymer	Full thickness cornea	Interpenetrating polymer network (IPN) hydrogel made from type I collagen and 6-methacryloyl-a-D-galactopyranose (MG) slow biodegradation	In-vitro	[54]
Collagen and phosphorylcholine	Full thickness cornea	Improved mechanical strength and enzymatic stability from collagenase, promising optical clarity	Animal model: mini pigs	[55]

Biomaterial	Target tissue	Novelty	Developmental status	Refs.
Collagen (Col) and pyrene conjugated dipeptide amphiphile (PyKC)	Epithelial, stroma and endothelium	Developed cross-linker-free collagen hydrogel, optically transparent, mechanically, and enzymatically stable, restrict human adenovirus propagation	In-vitro	[32]
Gelatin and poly (ε-caprolactone)-polyethylene glycol)	Stroma	Poly (ε-caprolactone)-poly (ethylene glycol) micro-fibrous scaffold was infused with gelatin methacrylate (GelMA) to make 3D fiber hydrogel, supported differentiation of limbal stromal stem cells to keratocytes or fibroblasts	Animal model: rat	[56]
Recombinant human collagen type III (RHC III) and 2 methacryloyl oxyethylphosphoryl-choline (MPC)	Full thickness cornea	Optimized RHC-III MPC hydrogel to enhance mechanical strength and make it suitable for post-fabrication modification that included micro-contact printing and laser re-shaping	In-vitro studies	[57]
Recombinant human collagen type III (RHC III) and 2 methacryloyl oxyethylphosphoryl-choline (MPC)	Epithelium and stroma	Optimized RHCIII-MPC hydrogel, where the incorporation of the MPC polymer network with reported inflammation suppression properties allowed stable regeneration in corneas of patients with active ulcers and infectious keratitis scarring	Pilot clinical study	[30]
In-situ forming, injectable hydrogels				
Gelatin	Stroma	Visible light crosslinking was used in conjunction with the bioadhesive in-situ GelCORE hydrogel. This hydrogel showed higher tissue adhesiveness than commercially available adhesives	Animal model: rabbit	[58]
Hyaluronate and collagen	Stroma	In-situ forming bio-orthogonally linked hydrogel, highly transparent and good mechanical strength	Animal model: rabbit	[59]
Short collagen-like peptides (CLPs) and polyethylene glycol (PEG)	Epithelium and stroma	In-situ forming, self-assembled liquid hydrogel (LiQD cornea) which gelled spontaneously at body temperature, stimulated regeneration of corneal epithelium, stroma, and nerves for over 1 year, acts as an effective glue filler for patching perforated corneas	Animal model: rabbit and cat	[60]

Table 1.
Advances in the use of natural, biosynthetic, composite, and in-situ forming biomaterials for corneal reconstruction.

5. In-situ gelation-based biomaterials

Recently, in-situ gelation-based, injectable hydrogels have gained attention in corneal tissue engineering because of several advantages over conventional solid implants. Injectable hydrogels can be delivered in a doctor's or ophthalmologist's clinic instead of an operating theater, since there is no need for surgical intervention. The risk of parasurgical infection, patient discomfort, or scar formation is reduced. The in-situ forming hydrogel can adapt to complex tissue cavities, mold, or irregular wounds with better integration [61]. It is easy to encapsulate therapeutic molecules such as peptides, drugs, or exosomes into the hydrogel for synergistic effect. The clinical potential of in-situ forming hydrogels for corneal regeneration has been reviewed by Poudel et al. [61].

In-situ composite hydrogels made from hydroxypropyl chitosan (HPCTS) and sodium alginate dialdehyde via Schiff's base chemistry showed histocompatibility, nontoxicity, and biodegradability. The composite hydrogel incorporating corneal endothelial cells was tested in a rabbit cornea injury model and showed its ability to support the reconstruction of corneal endothelium [62]. An Avastin® containing in-situ forming PEG hydrogel was explored for sustained release of the drug to treat corneal neovascularization. The transparent PEG hydrogel was nontoxic to L-929 cells after 1 week of incubation. The in-situ forming hydrogel showed sustained release of Avastin for up to 14 days without any apparent hydrolysis of the drug molecule [63].

To improve the drug efficacy and increase ocular drug availability, another in-situ forming, thermoresponsive chitosan-gelatin-based hydrogel was developed. The fast-gelling hydrogel was fabricated using β-glycerophosphate disodium salt hydrate (β-GD) and genipin to encapsulate timolol maleate for sustained release. The chitosan gelatin solution was instilled into the lower conjunctival sac of rabbits' eyes to form a hydrogel that provided sustained release of timolol maleate for up to 24 h [64]. Chun et al. developed injectable in-situ forming hydrogel comprising collagen type I that was cross-linked using a multifunctional polyethylene glycol (PEG)-N-hydroxysuccinimide (NHS). When tested for its ability to heal corneal defects, the hydrogel allowed the migration of epithelial cells to form multilayer at the site of corneal stromal defects without inflammation [65].

The GelCORE bioadhesive hydrogel was developed for the sutureless repair of corneal injuries. The highly biocompatible, transparent hydrogel is cured in-situ by visible light cross-linking. In-vivo studies in rabbit corneas showed regeneration of the stroma and epithelium after excision of rabbit corneal tissues [58]. Bio-orthogonally cross-linked hydrogels comprising hyaluronan and collagen showed improved mechanical strength for corneal repair. The in-situ forming hydrogel possesses low refractive index than the native cornea, showed excellent biocompatibility and epithelization in in-vitro and in-vivo studies of partial thickness defects [59].

Griffith and coworkers have recently explored the clinical potential of a fully synthetic hydrogel made from CLP-PEG that incorporated fibrinogen. The "LiQD Cornea" gelled spontaneously in situ at body temperature without the need for light exposure. The hydrogel stimulated the stable (over 1 year) regeneration of corneal epithelium, stroma, and nerves, serving as an alternative to a partial thickness anterior lamellar allografts (**Figure 2C**) [56]. For patients who have corneal perforations (**Figure 2A**), these are emergencies that require patching. The LiQD Cornea was tested and found to also be effective glue-fillers for patching perforated corneas in rabbits, mini-pigs (**Figure 2B** and **C**) [60], and in a cat model [66].

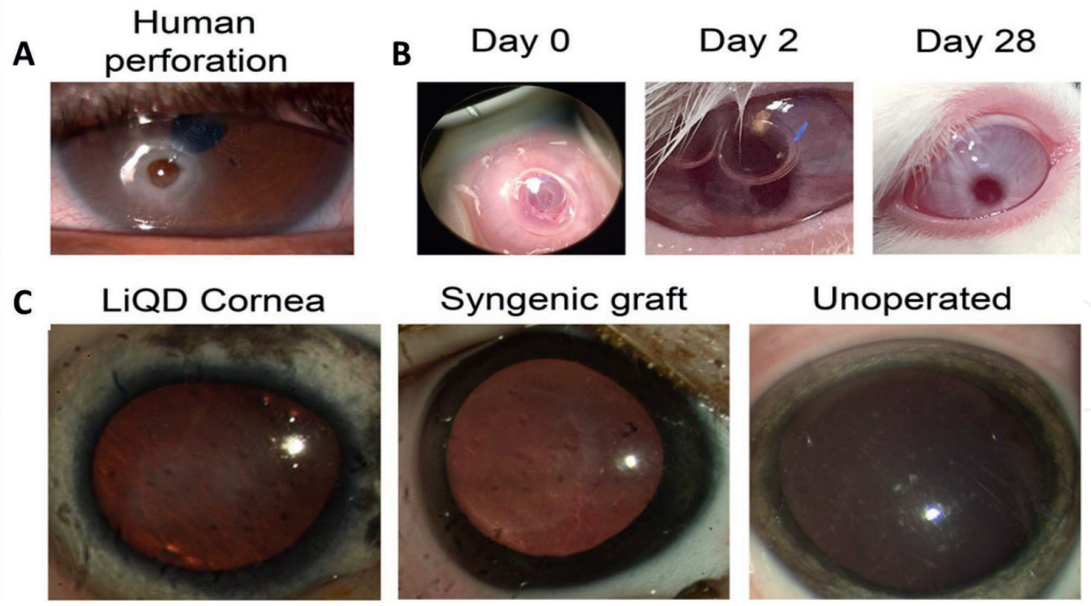

Figure 2.
LiQD hydrogel evaluation in rabbit and mini-pigs (A) Human cornea perforation. (B) Postsurgical perforated rabbit cornea containing LiQD cornea hydrogel at Day 0, 2, and 28. Perforation can be easily seen at Day 0. At Day 2, the air bubble introduced during surgery was prominent, indicating the perforation was sealed. Perforation was healed after 28 days of injecting LiQD cornea hydrogel. (C) Mini pig corneas showing presence of LiQD cornea, syngeneic graft, and unoperated cornea after 12 months of surgery. Reproduced from [60].

6. Role of exosomes in corneal infection and regeneration

Exosomes are membrane-bound extracellular vesicles (EVs) secreted from most cell types into the extracellular space, ranging from 30 to 150 nm in size. The role of exosomes in corneal diseases and their biogenesis during pathological or physiological immune responses has been documented in several studies [67–69]. Secreted exosomes could regulate immune responses as they have the potential for transferring and presenting antigenic peptides, regulating gene expression, and inducing various immune signaling pathways. Furthermore, their detection in a biological fluid can offer a window to the altered cellular or tissue states, and cell-to-cell communication in healthy and disease patients could lead to early diagnosis and potential treatment [70].

Exosomes are natural nanovesicles and are superior to synthetic nanovesicles like liposomes in terms of their stability, biocompatibility, plasma half-life, ability to enter non-accessible tissue regions, and their role in immunomodulation. Exosomes are potentially effective drug delivery vehicles, but their nonspecific targeting feature limits their use in drug delivery applications [71]. However, engineered exosomes secreted by specific cell types could overcome this problem.

Exosomes have recently shown potential in regulating therapeutic functions via cell-to-cell communications. Induced pluripotent stem cells derived exosomes (iPSCs-Exos) are being examined as a natural therapeutic nanoparticle for treating corneal epithelial defects. iPSCs-Exos have induced cell proliferation, migration, cell cycling, and apoptosis inhibition of human corneal epithelial cells (HCECs) in-vitro. Studies also showed the upregulation of cyclin A and CDK2 that induce the HCECs to enter the S phase of the cell cycle for potential regeneration. In vivo studies also revealed the accelerated healing potential of iPSCs-Exos in treating corneal epithelial defects [72]. Zhong and coworkers showed that when exosomes

derived from human umbilical cord mesenchymal stem cells (HucMSC-Exos) were combined with an autophagy activator, they positively affected the repair of a corneal injury (CI). The repair process was regulated by accelerated cell proliferation and migration while inhibiting apoptosis and inflammation by activating the AMPKmTOR-ULK1 autophagy pathway [73]. Han et al. [74] examined the possible role of exosomes in corneal wound healing and neovascularization. They showed exosomes between epithelium and stroma after epithelium debridement during the wound healing process. Exosome-like vesicles were also observed in the stroma after anterior stromal keratectomy. They were fused with keratocytes to induce myofibroblast transformation in vitro. These epithelial-derived exosomes also helped induce endothelial cell proliferation, indicating the ability of exosomes to communicate among corneal epithelial cells, keratocytes, and vascular endothelial cells [74].

As these naturally derived nanotherapeutics show emerging potential in treating corneal infection and regeneration, it would be interesting to encapsulate these particles into scaffold/hydrogel for sustained release in clinical applications. In this context, chitosan-based thermoresponsive hydrogels encapsulating exosomes derived from induced pluripotent stem cell–derived mesenchymal stem cells (iPSC-MSCs) were reported to reduce corneal scar formation and accelerated wound healing [75]. In addition, the sustained release of miRNA-containing exosomes helped regenerate the corneal epithelium and stromal layer. The exosomes prevented ECM deposition by downregulating the translocation-associated membrane protein 2 (TRAM2) gene, providing a new strategy for the clinical treatment of fibrotic corneal diseases [75]. Exosomes are paving a new path with their potential utility for early diagnosis and treatment of corneal diseases. Their incorporation into biomaterial-based hydrogels could facilitate their use as therapeutic particles for sustained release in clinical applications.

7. Conclusion and perspective

Corneal transplantation is the only widely accepted treatment for corneal blindness. However, the shortfall of high-quality donor cornea worldwide has made it very challenging. Several approaches have been explored to replace donor corneas, ranging from incorporation of stem cells and decellularized extracellular matrix scaffold to cell-free approaches. Cell-free biomaterial approaches have shown promising potential in promoting in situ corneal regeneration without aggravating the risk of rejection. In-situ gelation of biomaterials opens a new path in corneal regeneration as it does not require extensive surgical intervention, and could reduce the risk of infection, scar formation, and patient discomfort. However, this technique is ineffective in patients with depleted stem or progenitor cells.

More research focusing on developing of biomaterials for high-risk patients is needed. Exosomes derived from cells have recently shown potential in early diagnosis and capabilities to modulate pathological disease state. Future studies should focus on understanding the role of exosomes in corneal pathologies and combining biomaterials with exosomes to explore the additive effect of both strategies.

Finally, it is essential to consider regulatory pathway beforehand while designing and developing corneal implants for clinical applications for effective translation from bench-to-bench side.

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Conflict of interest

MG's previous university has filed patents that cover RHCIII and RCIII-MPC implants. She has IP disclosures filed for the LiQD Cornea and fully synthetic pro-regeneration corneal implants.

Nomenclature

ALDH3A1	aldehyde dehydrogenase 3A1 (ALDH3A1)
β-GD	β-glycerophosphate disodium salt hydrate
CD34	Cluster of differentiation 34
CEnCs	Corneal endothelial cells
CI	Corneal injury
cLESCs	Canine limbal epithelial stem cells
CLPs	Collagen-like peptides
Col	Collagen
DEX	Dexamethasone
DHL	Decellularized human limbus
ECM	Extra cellular matrix
EDC	1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride
EVs	Extracellular vesicles
Gel MA	Methacrylated gelatin
HA	Hyaluronic acid
HASCs	Human adipose stem cells
HPCTS	Hydroxypropyl chitosan
HucMSC	Human umbilical cord mesenchymal stem cells
iPSCs-Exos	Induced pluripotent stem cells derived exosomes
iPSC-MSCs	Induced pluripotent stem cell-derived mesenchymal stem cells
LEPCs	Limbal epithelial progenitor cells
LKP	Lamellar keratoplasty
LMs	Limbal melanocytes
LSCs	Limbal stem cells
LSCD	Limbal stem cell deficiency
MFX	Moxifloxacin
MPC	2-methacryloyloxyethyl phosphorylcholine
MSCs	Mesenchymal stem cells
NHS	N-hydroxysuccinimide
PCL	Polycaprolactone
PEG	Polyethylene glycol

PEGDA	Poly (ethylene glycol) diacrylate
p(HEMA)	Poly (2-hydroxymethyl methacrylate)
RHC	Recombinant human collagen
RHC III	Recombinant human collagen type III
SF/G	Silk fibroin/gelatin
Silk MA	Methacrylated-silk

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References

[1] Mobaraki M, Abbasi R, Omidian Vandchali S, Ghaffari M, Moztarzadeh F, Mozafari M. Corneal repair and regeneration: Current concepts and future directions. Frontiers in Bioengineering and Biotechnology. 2019;**7**:135

[2] Mahdavi SS, Abdekhodaie MJ, Mashayekhan S, Baradaran-Rafii A, Djalilian AR. Bioengineering approaches for corneal regenerative medicine. Tissue Engineering and Regeneration Medicine. 2020;**17**(5):567-593

[3] Oliva M, Schottman T, Gulati M. Turning the tide of corneal blindness. Indian Journal of Ophthalmology. 2012;**60**(5):423

[4] Ong JA, Auvinet E, Forget KJ, Lagali N, Fagerholm P, Griffith M, et al. 3D corneal shape after implantation of a biosynthetic corneal stromal substitute. Investigative Ophthalmology & Visual Science. 2016;**57**(6):2355

[5] Gain P, Jullienne R, He Z, Aldossary M, Acquart S, Cognasse F, et al. Global survey of corneal transplantation and eye banking. JAMA Ophthalmology. 2016;**134**(2):167

[6] Hassan SS, Wilhelmus KR, Dahl P, Davis GC, Roberts RT, Ross KW, et al. Infectious disease risk factors of corneal graft donors. Archieves of Ophthamology. 2008;**126**(2):235-239

[7] Patel G, Na KS, Lee HJ, et al. Electrospun fibers for corneal regeneration. Current Ophthalmological Report. 2021;**9**:146-157

[8] Zhang B, Xue Q, Li J, Ma L, Yao Y, Ye H, et al. 3D bioprinting for artificial cornea: Challenges and perspectives. Medical Engineering & Physics. 2019;**71**:68-78

[9] Fuest M, Yam GHF, Mehta JS, Duarte Campos DF. Prospects and challenges of translational corneal bioprinting. Bioengineering. 2020;**7**(3):71

[10] Hsu CC, Peng CH, Hung KH, Lee YY, Lin TC, Jang SF, et al. Stem cell therapy for corneal regeneration medicine and contemporary nanomedicine for corneal disorders. Cell Transplantation. 2015;**24**(10):1915-1930

[11] Sacchetti M, Rama P, Bruscolini A, Lambiase A. Limbal stem cell transplantation: Clinical results, limits, and perspectives. Stem Cells International. 2018;**2018**:8086269

[12] Calonge M, Nieto-Miguel T, de la Mata A, Galindo S, Herreras JM, López-Paniagua M. Goals and challenges of stem cell-based therapy for corneal blindness due to limbal deficiency. Pharmaceutics. 2021;**13**(9):1483

[13] Baylis O, Figueiredo F, Henein C, Lako M, Ahmad S. 13 years of cultured limbal epithelial cell therapy: A review of the outcomes. Journal of Cellular Biochemistry. 2011;**112**(4):993-1002

[14] Demirayak B, Yüksel N, Çelik OS, Subaşı C, Duruksu G, Unal ZS, et al. Effect of bone marrow and adipose tissue-derived mesenchymal stem cells on the natural course of corneal scarring after penetrating injury. Experimental Eye Research. 2016;**151**:227-235

[15] Yan D, Yan C, Yu F, Zhang S, Chen L, Wu N, et al. Exploitation of human mesenchymal stromal cell derived matrix towards the structural and functional restoration of the

ocular surface. Biomaterials Science. 2020;**8**(17):4712-4727

[16] Torsahakul C et al. Bio-fabrication of stem cell incorporated corneal epithelial and stromal equivalents from silk fibroin and gelatin-based biomaterial for canine corneal regeneration. PLoS One. 2022;**17**(2):e0263141

[17] Fiorica C, Senior RA, Pitarresi G, Palumbo FS, Giammona G, Deshpande P, et al. Biocompatible hydrogels based on hyaluronic acid cross-linked with a polyaspartamide derivative as delivery systems for epithelial limbal cells. International Journal of Pharmaceutics. 2011;**414**(1-2):104-111

[18] Kiiskinen J. Co-culture of corneal epithelial cells and adipose stem cells – toward the use of hydrogels in ocular surface reconstruction. University of Tampere: Tampere, Finland. 2016:58

[19] Chen D, Qu Y, Hua X, Zhang L, Liu Z, Pflugfelder S, et al. A hyaluronan hydrogel scaffold-based xeno-free culture system for ex vivo expansion of human corneal epithelial stem cells. Eye. 2017;**31**:962

[20] Polisetti N, Schmid A, Schlötzer-Schrehardt U, Maier P, Lang SJ, Steinberg T, et al. A decellularized human corneal scaffold foranterior corneal surface reconstruction. Scientific Reports. 2021;**11**(1):2992

[21] Polisetti N, Roschinski B, Schlötzer-Schrehardt U, Maier P, Schlunck G, Reinhard T. A decellularized human limbal scaffold for limbal stem cell Niche reconstruction. IJMS. 2021;**22**(18):10067

[22] Alio del Barrio JL, Chiesa M, Garagorri N, Garcia-Urquia N, Fernandez Delgado J, Bataille L, et al. Acellular human corneal matrix sheets seeded with human adipose-derived mesenchymal stem cells integrate functionally in an experimental animal model. Experimental Eye Research. 2015;**132**:91-100

[23] Chen Z, You J, Liu X, Cooper S, Hodge C, Sutton G, et al. Biomaterials for corneal bioengineering. Biomedical Materials. 2018;**13**(3):032002

[24] Griffith M, Poudel BK, MalhotraK,AklaN,González-AndradesM, Courtman D, et al. Biosynthetic alternatives for corneal transplant surgery. Expert Review of Ophthalmology. 2020;**15**(3):129-143

[25] Liu W, Merrett K, Griffith M, Fagerholm P, Dravida S, Heyne B, et al. Recombinant human collagen for tissue engineered corneal substitutes. Biomaterials. 2008;**29**(9):1147

[26] Nong LM, Zhou D, Zheng D, Jiang YQ, Xu NW, Zhao GY, et al. The effect of different cross-linking conditions of EDC/NHS on type II collagen scaffolds: An in vitro evaluation. Cell and Tissue Banking. 2019;**20**(4):557-568

[27] Avila MY, Gerena VA, Navia JL. Corneal crosslinking with genipin, comparison with UV-Riboflavin in ex-vivo model. Molecular Vision. 2012;**18**:1068-1073

[28] Fagerholm P, Lagali NS, Merrett K, Jackson WB, Munger R, Liu Y, et al. Biosynthetic alternative to human donor tissue for inducing corneal regeneration: 24-month follow-up of a phase 1 clinical study. Science Translational Medicine. 2010;**2**(46):46ra61

[29] Fagerholm P, Lagali NS, Ong JA, Merrett K, Jackson WB, Polarek JW, et al. Stable corneal regeneration four years after implantation of a cell-free recombinant human collagen scaffold. Biomaterials. 2014;**35**(8):2420-2427

[30] Islam MM, Buznyk O, Reddy JC, Pasyechnikova N, Alarcon EI, Hayes S, et al. Biomaterials-enabled cornea regeneration in patients at high risk for rejection of donor tissue transplantation. NPJ Regeneration Medicine. 2018;**3**(1):2

[31] Jangamreddy JR, Haagdorens MKC, Mirazul Islam M, Lewis P, Samanta A, Fagerholm P, et al. Short peptide analogs as alternatives to collagen in pro-regenerative corneal implants. Acta Biomaterialia. 2018;**69**:120-130

[32] Islam MM, Chivu A, AbuSamra DB, et al. Crosslinker-free collagen gelation for corneal regeneration. Scientific Reports. 2022;**12**:9108

[33] Haagdorens M, Edin E, Fagerholm P, Groleau M, Shtein Z, Ulčinas A, et al. Plant recombinant human collagen Type I hydrogels for corneal regeneration. Regeneration and Engineering Translational Medicine. 2022;**8**(2):269-283

[34] Kilic Bektas C, Burcu A, Gedikoglu G, Telek HH, Ornek F, Hasirci V. Methacrylated gelatin hydrogels as corneal stroma substitutes: In vivo study. Journal of Biomaterials Science. 2019;**30**(18):1803-1821

[35] Chen Y, Dong L, Kong B, Huang Y, Zhong S, Connon C, et al. Effects of gelatin methacrylate hydrogel on corneal repair and regeneration in rats. Translational Vision Science & Technology. 2021;**10**(14):25

[36] Arica TA, Guzelgulgen M, Yildiz AA, Demir MM. Electrospun GelMA fibers and p(HEMA) matrix composite for corneal tissue engineering. Materials Science and Engineering: C. 2021;**120**:111720

[37] Zhao L, Qi X, Cai T, Fan Z, Wang H, Du X. Gelatin hydrogel/contact lens composites as rutin delivery systems for promoting corneal wound healing. Drug Delivery. 2021;**28**(1):1951-1961

[38] Li L, Lu C, Wang L, Chen M, White J, Hao X, et al. Gelatin-based photocurable hydrogels for corneal wound repair. ACS Applied Materials & Interfaces. 2018;**10**(16):13283-13292

[39] Chang MC, Kuo YJ, Hung KH, Peng CL, Chen KY, Yeh LK. Liposomal dexamethasone–moxifloxacin nanoparticle combinations with collagen/gelatin/alginate hydrogel for corneal infection treatment and wound healing. Biomedical Materials. 2020;**15**(5):055022

[40] Lawrence BD, Marchant JK, Pindrus MA, Omenetto FG, Kaplan DL. Silk film biomaterials for cornea tissue engineering. Biomaterials. 2009;**30**(7):1299-1308

[41] Barroso IA, Man K, Hall TJ, Robinson TE, Louth SET, Cox SC, et al. Photocurable antimicrobial silk-based hydrogels for corneal repair. Journal of Biomedical Materials Research. 2022;**110**(7):1401-1415

[42] Choi J, Jeon H, Song J, Oliveira J, Reis R, Khang G. Biofunctionalized lysophosphatidic acid/silk fibroin film for cornea endothelial cell regeneration. Nanomaterials. 2018;**8**(5):290

[43] Farasatkia A, Kharaziha M, Ashrafizadeh F, Salehi S. Transparent silk/gelatin methacrylate (GelMA) fibrillar film for corneal regeneration. Materials Science and Engineering: C. 2021;**120**:111744

[44] Kao R, Liu CY. Roles of lumican and keratocan on corneal transparency. Glycoconjugate Journal. 2002;**19**(4-5):275-285

[45] Koppaka V, Chen Y, Mehta G, Orlicky DJ, Thompson DC, Jester JV,

et al. ALDH3A1 plays a functional role in maintenance of corneal epithelial homeostasis. PLoS ONE. 2016;**11**(1):e014

[46] Zhang W, Chen J, Qu M, Backman LJ, Zhang A, Liu H, et al. Sustained release of TPCA-1 from silk fibroin hydrogels preserves keratocyte phenotype and promotes corneal regeneration by inhibiting interleukin-1β signaling. Advanced Healthcare Materials. 2020;**9**(17):e2000591

[47] Xeroudaki M, Thangavelu M, Lennikov A, et al. A porous collagen-based hydrogel and implantation method for corneal stromal regeneration and sustained local drug delivery. Scientific Reports. 2020;**10**:16936

[48] Sharifi S, Islam MM, Sharifi H, Islam R, Koza D, Reyes-Ortega F, et al. Tuning gelatin-based hydrogel towards bioadhesive ocular tissue engineering applications. Bioactive Materials. 2021;**6**(11):3947-3961

[49] Mahdavi SS, Abdekhodaie MJ, Kumar H, Mashayekhan S, Baradaran-Rafii A, Kim K. Stereolithography 3D bioprinting method for fabrication of human corneal stroma equivalent. Annals of Biomedical Engineering. 2020;**48**(7):1955-1970

[50] Zhang Y, Li C, Zhu Q, Liang R, Xie C, Zhang S, et al. A long-term retaining molecular coating for corneal regeneration. Bioactive Materials. 2021;**6**(12):4447-4454

[51] Sun X, Yang X, Song W, Ren L. Construction and evaluation of collagen-based corneal grafts using polycaprolactone to improve tension stress. ACS Omega. 2020;**5**(1):674-682

[52] He B, Wang J, Xie M, Xu M, Zhang Y, Hao H, et al. 3D printed biomimetic epithelium/stroma bilayer hydrogel implant for corneal regeneration. Bioactive Materials. 2022;**17**:234-247

[53] Kilic Bektas C, Hasirci V. Cell loaded GelMA:HEMA IPN hydrogels for corneal stroma engineering. Journal of Materials Science: Materials in Medicine. 2020;**31**(1):2

[54] Deng C, Li F, Hackett JM, Chaudhry SH, Toll FN, Toye B, et al. Collagen and glycopolymer based hydrogel for potential corneal application. Acta Biomaterialia. 2010;**6**(1):187-194

[55] Liu W, Deng C, McLaughlin CR, Fagerholm P, Lagali NS, Heyne B, et al. Collagen–phosphorylcholine interpenetrating network hydrogels as corneal substitutes. Biomaterials. 2009;**30**(8):1551-1559

[56] Kong B, Chen Y, Liu R, Liu X, Liu C, Shao Z, et al. Fiber reinforced GelMA hydrogel to induce the regeneration of corneal stroma. Nature Communications. 2020;**11**(1):1435

[57] Mirazul Islam M, Cėpla V, He C, Edin J, Rakickas T, Kobuch K, et al. Functional fabrication of recombinant human collagen–phosphorylcholine hydrogels for regenerative medicine applications. Acta Biomaterialia. 2015;**12**:70-80

[58] Shirzaei Sani E, Kheirkhah A, Rana D, Sun Z, Foulsham W, Sheikhi A, et al. Sutureless repair of corneal injuries using naturally derived bioadhesive hydrogels. Science Advances. 2019;**5**:eaav1281

[59] Chen F, Le P, Fernandes-Cunha GM, Heilshorn SC, Myung D. Bio-orthogonally crosslinked hyaluronate-collagen hydrogel for suture-free corneal defect repair. Biomaterials. 2020;**255**:120176

[60] McTiernan CD, Simpson FC, Haagdorens M, Samarawickrama C,

Hunter D, Buznyk O, et al. LiQD Cornea: Pro-regeneration collagen mimetics as patches and alternatives to corneal transplantation. Science Advances. 2020;**6**:eaba2187

[61] Poudel B, Robert M, Simpson F, Malhotra K, Jacques L, Labarre P, et al. In situ tissue regeneration in the cornea from bench to bedside. Cells, Tissues, Organs. 2022;**211**:506-526

[62] Liang Y, Liu W, Han B, Yang C, Ma Q, Song F, et al. An in situ formed biodegradable hydrogel for reconstruction of the corneal endothelium. Colloids and Surfaces. B, Biointerfaces. 2011;**82**(1):1-7

[63] Yu J, Xu X, Yao F, Luo Z, Jin L, Xie B, et al. In situ covalently cross-linked PEG hydrogel for ocular drug delivery applications. International Journal of Pharmaceutics. 2014;**470**(1-2):151-157

[64] Song Y, Nagai N, Saijo S, Kaji H, Nishizawa M, Abe T. In situ formation of injectable chitosan-gelatin hydrogels through double crosslinking for sustained intraocular drug delivery. Materials Science and Engineering: C. 2018;**88**:1-12

[65] Chun YH, Park SK, Kim EJ, Lee HJ, Kim H, Koh WG, et al. In vivo biocompatibility evaluation of in situ-forming polyethylene glycol-collagen hydrogels in corneal defects. Scientific Reports. 2021;**11**(1):23913

[66] Juarez A, Djallali M, Piché M, Thériault M, Groleau M, Beroual S, et al. A liquid hydrogel to restore long term corneal integrity after perforating and non-perforating trauma in feline eyes. Frontiers in Bioengineering and Biotechnology. 2021;**9**:773294

[67] Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. Nature Reviews. Immunology. 2014;**14**(3):195-208

[68] Liu J, Jiang F, Jiang Y, Wang Y, Li Z, Shi X, et al. Roles of exosomes in ocular diseases. International Journal of Nanomedicine. 2020;**15**:10519-10538

[69] Li N, Zhao L, Wei Y, et al. Recent advances of exosomes in immune-mediated eye diseases. Stem Cell Research & Therapy. 2019;**10**:278

[70] Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science. 2020;**367**(6478): eaau 6977

[71] Li X, Corbett AL, Taatizadeh E, Tasnim N, Little JP, Garnis C, et al. Challenges and opportunities in exosome research-perspectives from biology, engineering, and cancer therapy. APL Bioengineering. 2019b;**3**(1)

[72] Wang S, Hou Y, Li X, Song Z, Sun B, Li X, et al. Comparison of exosomes derived from induced pluripotent stem cells and mesenchymal stem cells as therapeutic nanoparticles for treatment of corneal epithelial defects. Aging. 2020;**12**(19): 19546-19562

[73] Ma S, Yin J, Hao L, Liu X, Shi Q, Diao Y, et al. Exosomes from human umbilical cord mesenchymal stem cells treat corneal injury via autophagy activation. Frontiers in Bioengineering and Biotechnology. 2022;**10**:879192

[74] Han KY, Tran JA, Chang JH, Azar DT, Zieske JD. Potential role of corneal epithelial cell-derived exosomes in corneal wound healing and neovascularization. Scientific Reports. 2017;**7**(1):40548

[75] Tang Q, Lu B, He J, Chen X, Fu Q, Han H, et al. Exosomes-loaded thermosensitive hydrogels for corneal epithelium and stroma regeneration. Biomaterials. 2022;**280**:121320