

Biomaterials for reconstructing the corneal stroma

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9.1 Introduction—The requirement for stromal reconstruction

The stromal layer makes up the bulk of the cornea, constituting over 90% of its thickness. It imparts to the cornea its transparency, curvature, and strength, and protects the inner structures from the external environment. Its function relies heavily on the composition and organisation of the extracellular matrix (ECM), necessitating precise arrangement and orientation of collagen fibrils, lamellae and keratocytes, and relative deturgescence, which are conditions that are difficult to recreate in an in vitro model. Damage and aberrant wound healing of the stroma disrupts transparency and refractive function of the cornea, often leading to permanent vision loss and opacification (Fullwood, 2004).

The corneal stroma proper rests below a compact layer of ECM adjacent to the epithelial basement membrane known as Bowman's layer (Fig. 9.1). The stroma proper consists of roughly 300 distinct lamellae, which each consist of parallel-oriented bundles of collagen fibers, predominantly type I collagen (Bron, 2001). The cells of the stroma, called keratocytes are located between the lamellae where they form an interconnected network. The keratocytes are responsible for ECM synthesis and degradation. When the corneal stroma is wounded, the keratocytes are activated and transform into wound repair fibroblasts and myofibroblasts (West-Mays and Dwivedi, 2006). These activated cells are considered transient and necessary for wound healing, but excessive or prolonged inflammation leads to an altered composition and quantity of ECM components associated with fibrosis (Kamil and Mohan, 2021).

Physiologic stromal regeneration generally occurs over several years, likely allowing for the adoption of proper lamellar structure and orientation (Paik et al., 2018); when there is overactivation of myofibroblasts and aberrant collagen production, turnover times are shorter and stromal haze and scar tissue deposition are frequent (Wilson et al., 2012).

Whereas corneal blindness affects millions (Gain et al., 2016; Holland et al., 2021), current therapeutic practices for stromal replacement are limited. The mainstay

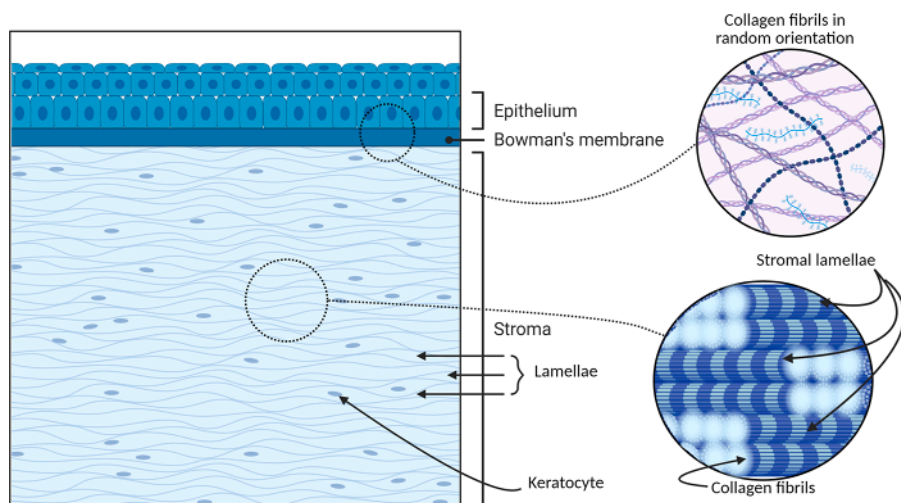


Figure 9.1 Structure of the corneal stroma located under the epithelium. The anterior-most Bowman's layer consists of randomly oriented collagen fibrils. The stroma proper consists of organized stromal lamellae, ECM molecules, and keratocytes.

Created in BioRender; <https://BioRender.com/ux8fg8k>.

treatment is donor cornea transplantation, by full-thickness grafts or by partial thickness stromal replacement. Transplantation of donor corneal grafts faces global shortages, with approximately 12.7 million people waiting for transplantation, with just over 1% being treated (Gain et al., 2016). The need for screening, transportation, and storage of donor tissue is also a considerable limiting factor, even in developed countries. Apart from the severe donor cornea shortage, the clinical results also have shortcomings, such as induction of scarring at the donor-recipient interface, immune rejection (Khodadoust and Silverstein, 1969), incomplete nerve regeneration (Al-Aqaba et al., 2012; Niederer et al., 2007; Tervo et al., 1985), and incomplete cellular repopulation (Niederer et al., 2007). Surgically induced astigmatism and other aberrations may lead to poor visual outcome despite an optically clear graft (Bhayani and Walkden, 2024). More importantly, where corneas are inflamed, the donor tissue is at high risk of graft failure due to immune rejection (Anderson et al., 2025).

In more severe cases where human donor tissue has failed or is contraindicated, keratoprostheses (KPro) have been used in some centers. The Boston KPro or the Osteo-Odonto KPro, two of the most commonly used models replace the entire thickness of the cornea including the stroma but they come with their own profile of complications including retroprosthetic membranes, corneal melting, severe glaucoma, endophthalmitis (Chew et al., 2009; Tan et al., 2012; Park et al., 2020). These devices are therefore reserved for end-stage corneas with severe corneal disease or trauma, typically following repeated failed transplantations (Avadhanam et al., 2015). KPro's are reviewed elsewhere in this book (Chapter 20) and will not be discussed further.

As the stroma represents the bulk of the cornea, it is important as a substrate for the epithelium and endothelium on either side of it. The cornea is the major refractive

element of the eye so bioengineered stromas need to be optically clear or transparent with minimal light scattering, for transmitting light into the eye for vision. Bioengineering technologies now range from delivery of stem cells directly into compromised stroma to restore function and optic clarity to tissue-engineered hydrogels and complex tissue reconstruction that allow for regeneration of host tissue. Since numerous materials and techniques have been used for stromal reconstruction, here, we discuss mainly examples of stromal replacements that have been evaluated in vivo preclinically and clinically.

9.2 Natural approaches to stromal repair

This section reviews current advancements in natural approaches such as self-assembled corneal constructs, use of decellularized corneal stromas and their clinical progress.

9.2.1 *Decellularized corneal implants*

During decellularization procedures, tissues and organs are stripped of their cells, leaving behind only the ECM to be pre-seeded with exogenous therapeutic cells (Fernández-Pérez et al., 2021; Gonzalez-Andrades et al., 2011) or re-colonized by healthy cells (Hashimoto et al., 2015; Lin et al., 2017). Decellularized corneas can then be used as scaffolds for replacing damaged corneas in therapeutic applications. A notable advantage of decellularized corneal implants is the lack of major histocompatibility complexes (MHC), thereby removing graft rejection as a potential complication (Fernández-Pérez and Ahearne, 2020). When tissues are insufficiently decellularized, macrophages have been shown to adopt a pro-inflammatory M1 phenotype in vivo and in vitro, and cellular components can be bound to complement proteins and immunoglobulins, eliciting macrophage and lymphocyte B activation (Keane et al., 2012). The decellularization, disinfection, and sterilization processes are therefore critical in determining the results of these implants, which have varied considerably with some success (Ayyildiz et al., 2008; Zalavras et al., 2006) and some failures (Doede et al., 2009; Zheng et al., 2005) in preclinical and clinical studies involving other organs.

Fernández-Pérez and Ahearne (2020) have reviewed in detail the preparation methods and advancements in decellularized corneal implants (Fernández-Pérez and Ahearne, 2020). The process is usually a combination of physical, chemical, and biological methods. Some studies advocate for the superiority of the high hydrostatic pressure (HHP) method over the detergent method for corneal ultrastructure preservation (Hashimoto et al., 2016) and the basement membrane-eroding effects of detergent-based methods (Shafiq et al., 2012). Table 9.1 summarizes recent clinical trials involving decellularized corneal implants.

9.2.2 *Self-assembled corneal equivalents*

The self-assembly approach constitutes induction of corneal stromal cells to produce sheets of tissue in vitro, which can then be air-lifted and stacked together to promote

Table 9.1 Summary of clinical trials with decellularized corneal implants, from both allogenic and xenogeneic sources.

Study description	Source of ECM	Findings	Source
Anterior lamellar keratoplasty grafts in 47 patients with fungal corneal ulcers	Acellular porcine corneal stroma (APCS)	Successful epithelialization in all grafts but four (dissolution of graft) Transparency and no rejection in 87% (n = 41). Improvement in best corrected visual acuity (BCVA) by 2 lines in 72%.	Zhang et al. (2015)
Deep anterior lamellar keratoplasty and double lamellar keratoplasty in 9 and 5 patients with herpes simplex keratitis, respectively	APCS	Transparency or minimal opacities in 7 patients (53.8%). Neovascularization present in 11 patients (84.6%), though stabilized 3 patients (23.1%) had graft dissolution.	Zheng et al. (2019)
Deep anterior lamellar keratoplasty in 39 patients with infectious keratitis	APCS	12 patients experienced graft failure (reasons being non-infectious graft melting, extensive neovascularization, and recurring infection). Significant and higher relative risk of graft rejection in herpes keratitis and in large grafts (>8 mm) 40% increase in patients with visual acuity of 20/40 or better.	Li et al. (2019)

Prospective trial with 14 keratoconus patients	Adipose-derived adult stem cells (ADASC) in n = 5; decellularized human corneal laminae (dCL) of 120 µm (n = 5); dCL recellularized with human ADASC (n = 4)	Higher immune cell infiltration in the group receiving dCL-ADASC, compared to ADASC alone.	El Zarif et al. (2024)
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further integration (Le-Bel et al., 2020; Proulx et al., 2010). The corneal construct is completed by seeding corneal epithelial cells atop the stack, and endothelial cells to the other side (Proulx et al., 2010). These reproducible in vitro corneal models were originally developed for fundamental and preclinical studies including toxicology and pharmacology and served as precursors to organ-on-a-chip models. While corneal stromal fibroblasts (Le-Bel et al., 2020) are typically used to generate the self-assembled stromal tissue, several studies have reported equivalent results when using dermal fibroblasts (Carrier et al., 2009) and stem cells derived from umbilical cord (Karamichos et al., 2011) or Wharton's jelly (Garzón et al., 2014). These cells are then stimulated in vitro with ascorbic acid (Vitamin C) and its derivative, 2-O- α -D-glucopyranosyl-L-ascorbic acid, to secrete collagen and other ECM macromolecules. While studies of self-assembled cornea constructs are largely in vitro, in 2014, a pre-clinical in vivo study evaluated the transplantation of self-assembled cornea constructs in eight healthy feline eyes. The grafts remained healthy, transparent, and avascular after 4 months, and corneal innervation returned to baseline levels (Boulze Pankert et al., 2014).

Macromolecular crowding (MMC) is a special form of self-assembly. To recreate human corneal stromal-like tissue, MMC works by adding inert, high-molecular-weight polymers to the cell culture media where human corneal fibroblasts are grown to simulate the dense environment found within living tissue. This environment then drives the corneal cells to accelerate the natural production and deposition of their own extracellular matrix components that include several collagens and fibronectin (Gurdal and Zeugolis, 2025). MMC using Ficoll as the crowding agent allows rapid formation of corneal stromal-like assemblies where the cells have been shown to produce stromal markers such as vimentin and keratocan. The ultimate goal is to develop implantable corneal stromal constructs for clinical application.

9.3 Bioengineered corneal stromal equivalents that promote regeneration

9.3.1 Stromal implants

In addition to the natural approach, bioengineered corneal constructs that specifically target the regenerative capacity of corneal tissues and nerves have also been examined. Bio-friendly and bio-responsive materials that elicit migration, proliferation, and differentiation of the patient's endogenous progenitor cells have been developed. Biomaterials that facilitate delivery of nucleic acids, biomaterial-directed epigenetic transformation, and transcription factors for in situ and in vivo gene editing, resulting in the desired phenotypic changes have also been developed (Gaharwar et al., 2020). Here, we discuss biomaterials that can promote regeneration on their own.

A popular candidate for a biomaterial in corneal stromal reconstruction is collagen. Its high biocompatibility is attributable to its predominance in the stroma, amongst other ECM molecules, such as proteoglycans, glycosaminoglycans, and other macromolecules. It was first successfully used to regenerate corneal epithelium, stroma, and

nerves in mini-pigs by the Griffith led-team with a network of type 1 collagen from bovine and poly(N-isopropylacrylamide) (PNIPAAm) and laminin-derived cell-adhesive peptide YIGSR (Li et al., 2003). The animal extracted collagens from bovine and later porcine sources used for research were replaced with recombinant human collagen in preparation for clinical transplantation (Merrett et al., 2008). Table 9.2 details advancements in current bioengineering literature for pro-regenerative stromal implants, in preclinical in vivo studies made from proteins, with a preponderance of collagen and its derivatives (gelatin) and mimetics. Other biological materials that have been tested include silk fibroin, keratins, chitosan, and glycosaminoglycans such as hyaluronan. These include prefabricated implants that were molded or 3D-printed through advanced manufacturing techniques.

Severe corneal infections and injuries can also result in ulcers, thinning or perforations. While cyanoacrylate glue (or other commercial glues) is used to seal these defects, the treatment is sub-optimal as none of these promote regeneration, thereby requiring a follow-up corneal transplantation in over 50% of cases (Yin et al., 2019). Pro-regeneration liquid biomaterials formulations are evaluated and summarized in Table 9.3. The convenience and scalability of injectable hydrogels make them an attractive delivery method for stromal sealants and fillers (Reithofer et al., 2014; Suanno et al., 2024).

9.3.2 3D-printed corneal stromal constructs

The first 3d printed corneal tissue mimics with integrated cells were reported in 2018 (Sorkio et al., 2018). Since then, the amount of literature surrounding 3D-printed corneal constructs has grown rapidly, making it clear that additive manufacturing paradigms are set to rapidly change this field. Even within the years 2023–2025, numerous review papers have covered and compared the wide range of 3D-printed corneal materials and the current state of the growing field (Balters and Reichl, 2023; Orash Mahmoudsalehi et al., 2025; Yuan et al., 2025). In 2025, Yuan et al., surveyed all 33 original research papers about 3D-printed corneas described on PubMed, and found that the majority of studies (~80%) focused on stromal repair. Regarding printing methods, the majority of papers used material extrusion (73.5%), with (in decreasing popularity) digital light processing (14.7%), inkjet printing (8.8%), and stereolithography (2.9%) also being used (Yuan et al., 2025). The most popular primary materials being printed for corneal materials were gelatin (42.4%) and collagen (39.4%), followed by hyaluronic acid (15.2%) and cellulose (6%).

Given the next section's focus on the use of collagen mimetic materials, we briefly discuss a few 3d printed applications of collagen materials in stromal repair. Collagen I remains by far the most popular material in printing. Campos et al., printed droplets of collagen-I alginate bioinks loaded with viable keratocytes and successfully fabricated transparent dome shaped tissue mimics (Duarte Campos et al., 2019). Isaacson et al., also extrusion printed a transparent material using a collagen-I alginate bioink with encapsulated keratocytes, though the resulting materials did not yield proper keratocyte morphology (Isaacson et al., 2018). Kim et al., extrusion printed a proof-of-concept collagen containing bioink based on decellularized and lyophilized extracts

Table 9.2 Stromal implants for corneal regeneration: sampling of recent advancements in animal studies to repair wounds or corneal perforations.

Implants	Components	Description	Exogenous cells	Results	Reference
CLP-PEG	Collagen-like peptides (CLP) and polyethylene glycol (PEG)	CLP is conjugated with PEG and crosslinked with carbodiimide chemistry. In vivo rabbit and guinea pig herpes simplex keratitis (HSK) model with 3 strains of herpes simplex virus (HSV) and anterior lamellar keratectomy.	None	Successful tissue regeneration at 3 months post-operative, with haze and neovascularization. Did not prevent HSV-1 reactivation nor reinfection.	Buznyk et al. (2025)
Biomimetic convex implant	Gelatin methacrylate (GelMA), collagen (Bioink)	Pneumatic, dual extruder 3D printing 2- and 6-month study of flat versus convex implants in New Zealand white rabbits by anterior lamellar keratoplasty.	None	Stronger epithelium adhesion Greater collagen induction and nerve fiber regeneration Highlights importance of corneal implant shape.	Xu et al. (2023)
RHCI	Plant-derived recombinant human collagen type I (RHCI)	Chemical crosslinking with EDC/NHS. 12 month study by anterior lamellar keratoplasty in Gottingen minipigs.	None	Regeneration of epithelium, stroma, nerves.	Haagdorens et al. (2022)

GelMA/ PEGDA	GelMA and long-chain poly(ethylene glycol) diacrylate, photoinitiator lithium phenyl-2,4,6 trimethylbenzoyl phosphinate	Digital light processing printing technology Photocrosslinking of ink with LAP with blue light (405 nm, 10 mW/cm ²) 28-day study in New Zealand white rabbits Comparison of cell-laden with cell-free implants.	Rabbit corneal epithelial cells (rCEC) and rabbit adipose-derived mesenchymal stem cells (rASC) pre-seeded as epithelium and stromal layers.	3D printed biomimetic epithelial and stromal bilayer hydrogel implant facilitates corneal regeneration with efficient epithelial and stromal regeneration. Comparable healing and regeneration in cell-laden and cell-free groups but higher expression of lumican in cell-laden group No observable inflammation or neovascularization. Comparable biocompatibility to control group treated with horse amniotic membrane.	He et al. (2022)
3D keratin/ PVA nanofibers (NF)	Keratin/polyvinyl alcohol (PVA) nanofiber membrane	Electrospun nanofiber mat immersed into 0.1M sodium borohydride aqueous solution for optical transparency 8-week study in female New Zealand white rabbits.	None		Jung et al. (2021)

Continued

Table 9.2 Continued

Implants	Components	Description	Exogenous cells	Results	Reference
CLP-PEG-MPC	Collagen-like peptides (CLP), polyethylene glycol (PEG), 2-methacryloyloxyethyl phosphorylcholine (MPC), 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM)	CLP-PEG, as used in previous study by Jangamreddy et al. (2018) . MPC crosslinked with DMTMM and molded to form hydrogels in shape and size of cornea 12-month study in alkali-burn cornea model in Gottingen minipigs.	None	CLP-PEG-MPC implants had reduced corneal swelling, haze, and neovascularization, when compared to CLP-PEG implants Overall more rapid healing and nerve regeneration.	Simpson et al. (2021)
PCL-GelMA	Poly(ε-caprolactone)-poly (ethylene glycol) (PCL), gelatin methacrylate (GelMA)	Direct writing electrospun PCL scaffold infused with GelMA, with and without chemical factors (β-FGF, ascorbic acid, insulin) 3-month intrastromal implantation study in male SD rats.	None	Successful regeneration of stroma reported, aided by factors.	Kong et al. (2020)

CLP-PEG	Collagen-like peptides (CLP) and polyethylene glycol (PEG)	CLP conjugated to PEG-maleimide Chemical crosslinking with EDC/NHS and molded to form hydrogels of shape and size of cornea 12-month study by anterior lamellar keratoplasty in Gottingen minipigs.	None	Epithelial, stromal, and nerve regeneration comparable to results from control group (recombinant human collagen type III-MPC, tested clinically).	Jangamreddy et al. (2018)
Multilayered silk film	Silk fibroin with RGD sequences	Silk fibroin films cast in a PDMS mold with leachable polyethylene oxide to create pores. RGD peptides conjugated onto surface of film using EDC/NHS chemistry.	Human corneal stromal stem cells (hCSCs) and human corneal fibroblasts	Successful genetic expression of keratocyte-specific ECM markers detected (keratan sulfate, lumican, keratocan), with better upregulation compared to 2d cell cultured systems.	Ghezzi et al. (2017)
SF-PA	Silk fibroin (SF) and polyacrylamide (PA)	3d porous (25–66 μ m pores) hydrogel made of different ratios of SF to PA, with N, N'-methylene bisacrylamide as a crosslinker.	Human corneal stromal cells	Cells saw improved cellular adhesion, enhanced proliferation, and elevated GAG deposition. High porosity ~84% facilitated keratocyte migration.	Bhattacharjee and Ahearne (2022)

Table 9.3 Liquid stromal filler-sealants for corneal regeneration: Sampling of recent advancements in animal studies developed to fill wounds or corneal perforations.

Implants	Components	Description	Exogenous cells	Results	Reference
Liquid collagen analog hydrogel (CAH)	Collagen-like peptidess (CLP), polyethylene glycol (PEG), 2-methacryloyloxyethyl phosphorylcholine (MPC), 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM)	Conjugation of CLP to poly MPC with a PEG template, injected with DMTMM crosslinker and allowed to gel in situ. In vivo anterior lamellar keratectomy in white New Zealand rabbits.	None	At 16-week, transparency in corneas receiving CAH was comparable to allografts. CAH supported complete corneal tissue and nerve regeneration.	Timmerman et al. (2025)
Kuragel	Thiolated gelatin (Gel-SH), methacrylated hyaluronic acid (HA-MA), photoinitiator Eosin Y-TEOA	HA-MA (50 mg/mL) and Gel-SH (120 mg/mL) were mixed with the photoinitiator solution and exposed to white light (10 mW/cm ²) 3-month study in physical corneal injury model in New Zealand white rabbits, treated Kuragel group versus untreated damaged group.	None	Kuragel-treated eyes demonstrated reversal of diseased state and return to near-healthy levels of corneal thickness, transparency, epithelial cells, keratocytes, and sub-basal nerve density.	Agrawal et al. (2024)
Collagen/PEG hydrogel	Thiolated collagen–polyethylene glycol (PEG) hydrogel	Mixing thiolated collagen with PEG-maleimide (bioorthogonal reactions).	Cell-free corneal epithelial extracellular vesicles (CE-EVs)	CE-EVs significantly accelerated corneal epithelial wound closure in vitro in scratch assays.	Lybecker et al. (2024)

Cornea-in-a-Syringe (CIS)	Linear carboxylated polymers 2-methacryloyloxyethyl phosphorylcholine, collagen-like peptides (CLP), adhesive collagen-citrate glue, GF19	One pot aqueous polymerization of LCPP polymer, purified with dialysis and lyophilization, combined with collagen components and DMTMM crosslinker under controlled temperature. In vivo surgical perforation and HSV-1 model in 24 white New Zealand rabbits. Some rabbits received GF19 cationic peptides to block HSV-1 activity.	None	At 6 months, all CIS-treated groups had relatively clear and non-vascularized corneas. CIS- treatment limited inflammation while the delivered antiviral GF19 blocked viral activity allowing regeneration.	Simoliunas et al. (2024)
GelMA/HA-NB	Methacrylated gelatin (GelMA), N-(2-aminoethyl)-4-(4-(hydroxymethyl)-2-methoxy-5-nitrosophenoxy) butanamide (NB)-modified glycosaminoglycan hyaluronic acid	Photocrosslinking with lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) (405 nm). 6-month study in New Zealand white rabbit large diameter (6 mm) partial thickness perforation model.	None	Excellent transparency at 6 months post-operation in hydrogel group, comparable to native cornea and superior to control group (treatment undefined), despite slight central haze at 1–2 months Confirmation of regenerative properties through proteomic analysis and upregulation of proteins.	Wang et al. (2023)

Continued

Table 9.3 Continued

Implants	Components	Description	Exogenous cells	Results	Reference
Collagen/PEG hydrogel	Thiolated collagen–polyethylene glycol (PEG) hydrogel	Mixing thiolated collagen with PEG-maleimide (bioorthogonal reactions).	None	Hydrogel remained shape-retaining and non-swelling in PBS for ≥ 1 year. Injectable up to 72 hours post-mixing, offering practical surgical handling. Supported viability and proliferation of human corneal epithelial cells.	Rosenquist et al. (2023)
CLP-PEG-fibrinogen LiQD Cornea	Collagen-like peptidess (CLP), polyethylene glycol (PEG), fibrinogen	Conjugation of eight-arm PEG-maleimide to 38-amino-acid CLP via formation of thioether linkage and reconstitution with fibrinogen to a final dilution of 10% and 1% (w/w), DMTMM crosslinker. In vivo studies in three white New Zealand rabbits and Gottingen mini-pigs.	None	LiQD Cornea formula withstood 170 mm Hg on bursting pressure testing. Successful sealing of corneal perforations and supported regeneration in animal studies, restoring corneal structure and nerve function.	McTiernan et al. (2020)

GelCORE	Gelatin derived from porcine collagen, chemically modified with methacryloyl groups, photoinitiator Eosin Y, Triethanolamine (TEA), N-vinylcaprolactam (VC)	Crosslinking in situ with visible light (450–550 nm) for 4 minutes 14-day study on New Zealand white rabbits with lamellar keratectomy.	None	Implant remained optically clear with smooth surface Complete homogenous reepithelialization in GelCORE group by day 14, whereas untreated controls exhibited heterogenous epithelialization CD45 ⁺ leukocyte infiltration in GelCORE group, indicating normal inflammatory response.	Shirzaei Sani et al. (2019)
Alginate-Chitosan hydrogel	Sodium alginate dialdehyde (SAD), Carboxymethyl chitosan (CMCTS)	Hydrogel formed covalent bonding between CMCTS amino groups and SAD aldehyde groups (Schiff base chemistry).	Rabbit limbal stem cells (LSCs)	Highly transparent, fast-gelling, biocompatible, low cytotoxicity. Significant improvement in epithelial reconstruction, but less expression of differentiated epithelial markers (cytokeratin 3 and 12).	Xu et al. (2019)

from bovine stromal tissue, that exhibited acceptable flexibility and good transparency (Kim et al., 2019). Song et al., focused on solving the issue of reduced transparency when extruding bioinks with high concentrations of collagen (for improved mechanical strength) by adding calcium salts into the buffer solution (Song et al., 2022). To date, there are no reported 3D-printed cornea structures for implantation or regeneration that have used collagen mimicking peptides directly in the printing process, though contact lenses using collagen mimicking peptides have been recently printed for drug delivery (Ch et al., 2024).

The main challenges facing the field of 3D-printed corneal materials are (1) achieving transparency and mechanical strength simultaneously, (2) fabrication of curved, defect free structures, and (3) a lack of standardization for material properties reported and measurement techniques for comparison between materials (Balters and Reichl, 2023; Orash Mahmoudsalehi et al., 2025; Yuan et al., 2025).

As shown previously in Fig. 9.1, the hierarchical structure of the corneal stroma uses careful alignment and structuring of nanometer scale collagen fibrils into micron scale lamellae to achieve the correct optical and strength properties simultaneously needed for the eye to function (Meek and Knupp, 2015). The cornea is quite anisotropic, being much stiffer in directions parallel to the collagen layers, which helps it maintain its curved shape and ability to handle intraocular pressure (Elsheikh and Alhasso, 2009). There is a consensus among current reviews of the field that current printing techniques are unable replicate this fiber alignment as the scope of additive material during printing is limited to much larger micron to millimeter size scales (Balters and Reichl, 2023; Orash Mahmoudsalehi et al., 2025; Yuan et al., 2025). In contrast to the randomly distributed fibers resulting from typical materials extrusion printing, Kim et al., demonstrated that alignment of collagen fibrils in 3D-printed materials improved its transparency by reducing light scattering (Kim et al., 2019). Composite corneal materials that blend another stronger material can better match the bulk mechanical properties of the cornea, but do not recapture the mechanical anisotropy (Zhang et al., 2019; Vijayaraghavan et al., 2024).

Printing corneal stromal implants requires precise fabrication of curved materials to best mimic the curvature of the eye. Most 3D-printing techniques today generate scaffold structures to ensure the integrity of the curved shape during the print process (Yuan et al., 2025). The problem is post-processing often requires careful removing of support structures which can damage the printed part or leave burrs and defects. Note that this is an issue with externally printed implants, not in-situ based printing strategies.

9.4 Extracellular mimetic peptides and collagen-like peptides: The next frontier in stromal repair

Extracellular matrix (ECM) proteins are large macromolecules that are complex to extract. While laminin, fibronectin, elastin peptides have been examined as alternatives to their full-length counterparts (Nishida et al., 2015), collagen-like peptides

(CLPs) or collagen mimetic peptides, CMPs) have been extensively examined because the corneal stroma comprises mainly collagen. However, self-assembling laminin and fibronectin nanofibers have successfully been tested in rabbit corneas with increased keratocyte migration and regeneration (Uzunalli et al., 2014).

CLPs mimic sequences from native collagens in order to recapture its desired properties (self-assembly, biocompatibility, mechanical properties, etc.), without needing to use full length collagens (Luo and Kiick, 2013). Capturing the self-assembling properties of peptide chains is necessary for replicating the mechanical properties of larger materials made from these peptides (Hennink and van Nostrum, 2002). Collagen contains trimer motifs $(\text{Gly-X-Y})_n$ that forms sinistral (left turning) parallel triple helices (Persikov et al., 2005). Fig. 9.2 provides a visual to understand this trimer structuring and the relative sizing of the collagen triple helix. This structure depends on closely packed polyprolines (as X or Y with substitutions possible) and side-chain free, flexible glycines spaced every three residues (Ramachandran, 1963). For example, CLPs will often contain >4 repeats of the trimers GPP or POG in order to mimic collagen's tertiary helical structure that helps provide the mechanical

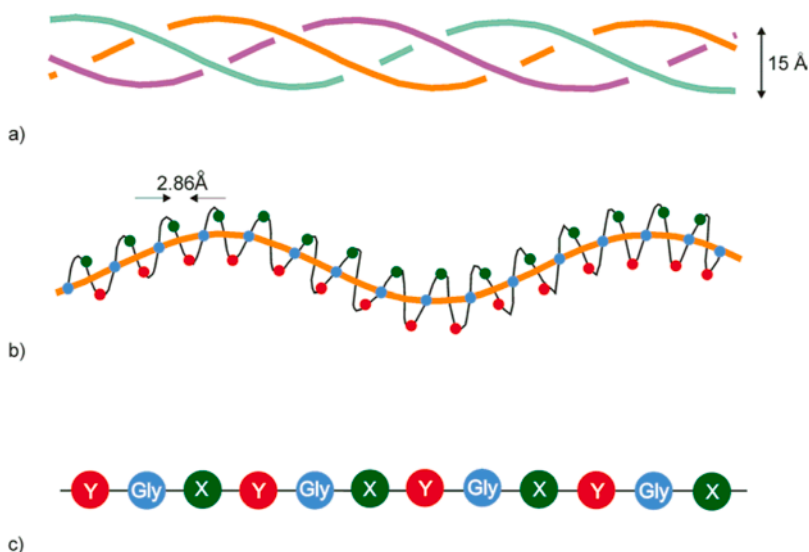


Figure 9.2 Collagen triple helix sizing, spacing and peptide sequence. (A) Three polypeptide alpha-chains twist together to form a helix. (B) Visualization of glycine placement (blue dots) in the left-handed alpha-chain helix, relative to other peptides (red and green dots). Each individual alpha-chain is a left-handed helix. Tight packing means only glycine can fit in the center of the triple helix. (C) Gly- X-Y trimer repeat, where X and Y are often proline and hydroxyproline. Glycine residues are at every third position in the trimer and help to form hydrogen bonds to link the three chains together. Figure is used under the Creative Commons Attribution License.

Knupp, C., Squire, J.M., 2003. Molecular packing in network-forming collagens. *Sci. World J.*, 3, 558–577. <https://doi.org/10.1100/tsw.2003.40>.

properties of collagen (Shoulders and Raines, 2009; Kubyshev, 2019). Note that substitutions of X and Y in the (Gly-X-Y)_n trimers are not uniform in collagens, and even small substitutions can yield notable changes in the twisting behavior and structure of the triple helix (Kramer et al., 2001).

Although full length recombinant human collagens have been successfully integrated into corneas for regeneration (Fagerholm et al., 2010; Islam et al., 2018), CLPs have the notable advantage of being more reactive and amenable to chemical manipulation (Elsawy et al., 2022; Yang et al., 2004). Being smaller, they are less complex and therefore cheaper to produce and more straightforward to scale into industrial production (Muttenthaler et al., 2021). They avoid the risk of zoonotic issues with collagen extracted from animals to, and avoid chemical alterations and batch variation that may arise from extraction and purification of protein from animal tissues (Lagali, 2020). Natural polymers (Ex: polylactic acid, cellulose) are often used as an alternative to CLPs for stromal repair (Xu et al., 2019), but have the added drawback of imprecise structural design, batch to batch variation, weaker mechanical properties, and are potentially less biocompatible (Liu et al., 2023).

9.4.1 Design strategies for CLP in stromal implants

A wide range of sequence customizations and chemical modifications have been employed to recapture the desired properties of collagen in the stroma. Short CLP sequences are often conjugated to larger polymers to achieve the mechanical stiffness required for stromal applications. The use of multi-arm polyethylene glycol (PEG) remains a prevalent strategy to reach the desired stromal material properties, as seen in Fig. 9.3 (Stahl et al., 2010; Jangamreddy et al., 2018; Ross et al., 2024). Peptide production for tissue engineering applications can be expensive as it demands optimizing and iterating on peptide designs to reach desired material properties, and the price and time costs roughly scale with peptide length. More recently, Ross et al., strategically reduced these drawbacks in a PEG-based system by using small sample volumes and reducing the length of peptides (only 18–36 amino acids long) in a discovery screening pipeline of 21 peptide candidates (Ross et al., 2024).

Given the stroma is the primary determinant of the cornea's stiffness and mechanical properties (Kling and Hafezi, 2017; Elsheikh and Alhasso, 2009), implant materials typically aim to match its elastic modulus. Elastic moduli of ~1.1 and ~3.8 kPa have been measured by atomic force microscopy (AFM) for the anterior and posterior stroma of rabbit eyes, respectively (Thomasy et al., 2014). These measurements are notably softer than moduli reported for humans and pigs, ranging between 1.3 and 5 MPa (Jue and Maurice, 1986; Kampmeier et al., 2000; Wang et al., 1996; Wollensak et al., 2003). Such stiffness in short peptide-based materials is challenging to match, but has been achieved by using toxic chemical crosslinkers (Bax et al., 2017; Shen et al., 2023). Alternatively, nontoxic polymers like PEG can be conjugated to CLPs to increase the stiffness of these materials (Pérez et al., 2014; Stahl et al., 2010). The peptide:PEG ratio can be further tuned to control this stiffness (Ross et al., 2024).

Different strategies have been employed to mimic the self-assembly of nanoscale architectures that resemble collagen fibrils in the stroma, beyond classic trimer repeats

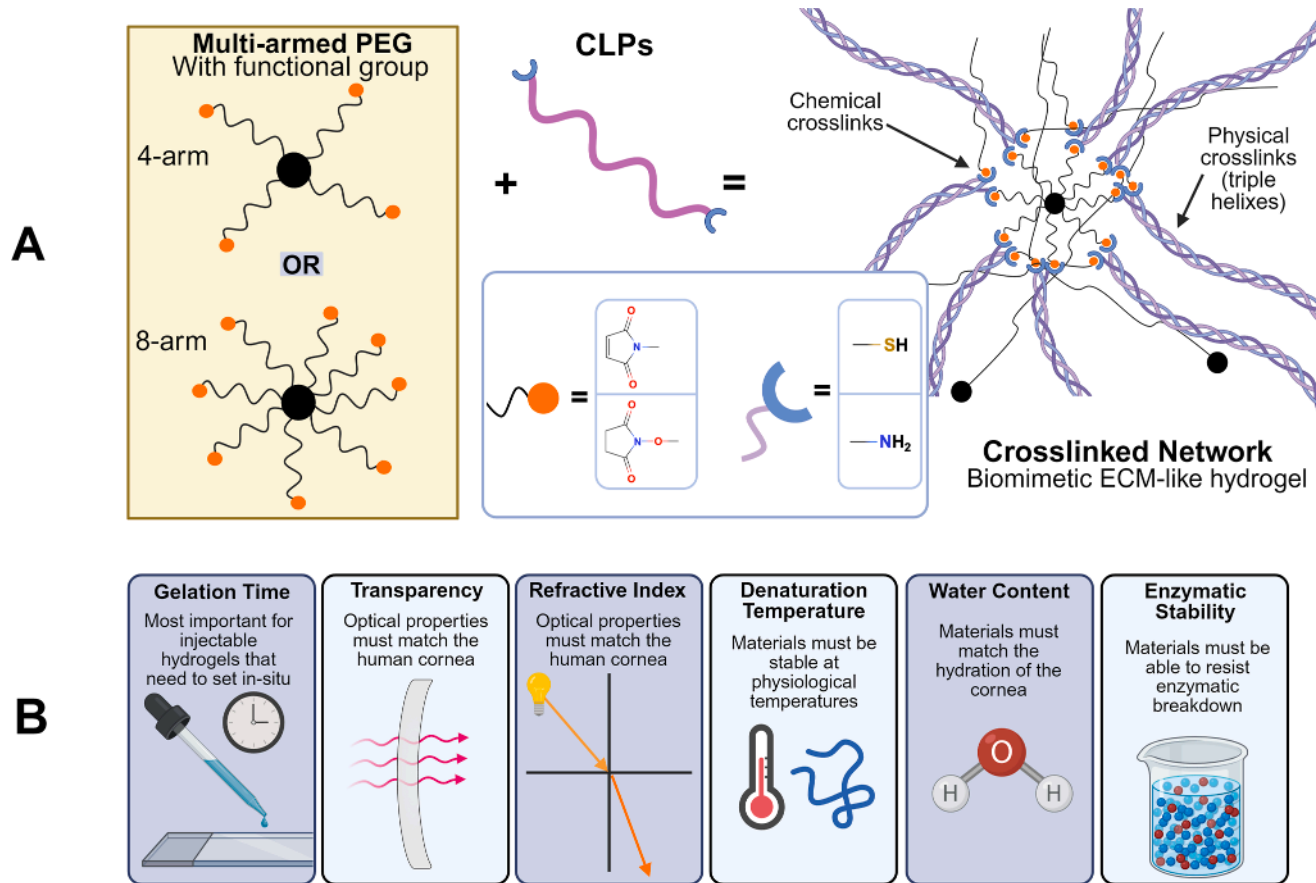


Figure 9.3 PEG-CLP conjugation strategies and design constraints for stromal materials. (A) Schematic of PEG-CLP conjugation chemistry utilized to engineer improved stiffness 3D networks, given the challenges of polymerizing truncated CLP sequences. This PEG-based approach is well established in stromal repair literature (Stahl et al., 2010; Jangamreddy et al., 2018; Ross et al., 2024). (B) Summary of material property performance targets for the selection of CLP sequences in stromal repair materials.

Created in BioRender. Griffith, M. (2025) <https://BioRender.com/lctjza3>.

(Gly-X-Y)_n (Kubyszhkin, 2019; Shoulders and Raines, 2009). Peptide amphiphiles contain polar and non-polar groups designed to enable self-assembly into a desired architecture (Karmakar et al., 2022), and such principles have been exploited for stromal regenerative materials (Uzunalli et al., 2014). CLPs have been designed into self-assembling amphiphiles (Miotto et al., 2015). Luo and Tong et al., were able to design CLPs that organized into nanofibers using integrin compatible GFOGER peptide motifs (Luo and Tong, 2011).

Crosslinking and gelation behavior are another design consideration when creating CLPs for stromal repair materials. Ross et al. (2024), relied on maleimide crosslinking to form PEG based gels, and carefully placed cysteines into candidate peptides by, (A) sandwiching cysteines between two glycines to maximize conformational freedom and reactivity of the thiol groups (Cheng et al., 2014), and (B) avoiding terminal cysteines for easier production (Tsuda et al., 2020). Other well reviewed amino acids such as lysines, arginines, or tyrosines could be used in future crosslinking chemistries (Jayachandran et al., 2022). Other stromal sealant materials that make use of methacrylated gelatin, chitosan, often use photocrosslinking mechanisms to gel the material once delivered (Suanno et al., 2024). Examples of CLP-based implants are shown in Tables 9.2 and 9.3.

9.4.2 Clinical evidence for *in situ* tissue regeneration

A team led by Griffith and Fagerholm conducted the world's first clinical study using cell-free biosynthetic hydrogels made from recombinant human collagen type III (RHCIII) to promote *in situ* corneal tissue and nerve regeneration (Fagerholm et al., 2010, 2014). The RHCIII was crosslinked with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and its co-reactant, N-hydroxysuccinimide (NHS). Crosslinking with EDC and NHS confers enhanced optical and mechanical characteristics, though the stability and durability depend on the crosslink density and formulation (Li and Wang, 2025). Fig. 9.4 displays the results 4 years post-operatively. There were no rejections nor adverse immune reactions at 4 years post-operation in the 10 patients (Fagerholm et al., 2014) receiving the hydrogel, whereas 1 of the 9 patients in the control group had a rejection episode. The patients in the control group, receiving human donor allografts by penetrating keratoplasty, demonstrated activation of stellate-shaped dendritic cells (Fig. 9.4) and had tortuous nerves. Those who received biosynthetic RHCIII hydrogels regenerated nerves in parallel arrangements and no mature dendritic cells, the lack of immune activation due to all cells contributing to the regeneration being endogenous. The tendency is for immune reactions to be directed against foreign cells (Niederkorn, 2011) or xenogeneic proteins (Sharifi et al., 2019).

The RHCIII base formulation was evaluated in a second clinical trial by anterior lamellar keratoplasty in seven unilaterally blind patients at high risk of conventional corneal allograft rejection. This group included patients suffering from scarring or ulcers due to infection, burns, and immune and degenerative disorders. The RHCIII base formulation was modified with the incorporation of 2-methacryloyloxyethyl phosphorylcholine (MPC), a synthetic lipid reported to suppress inflammation (Kakinoki

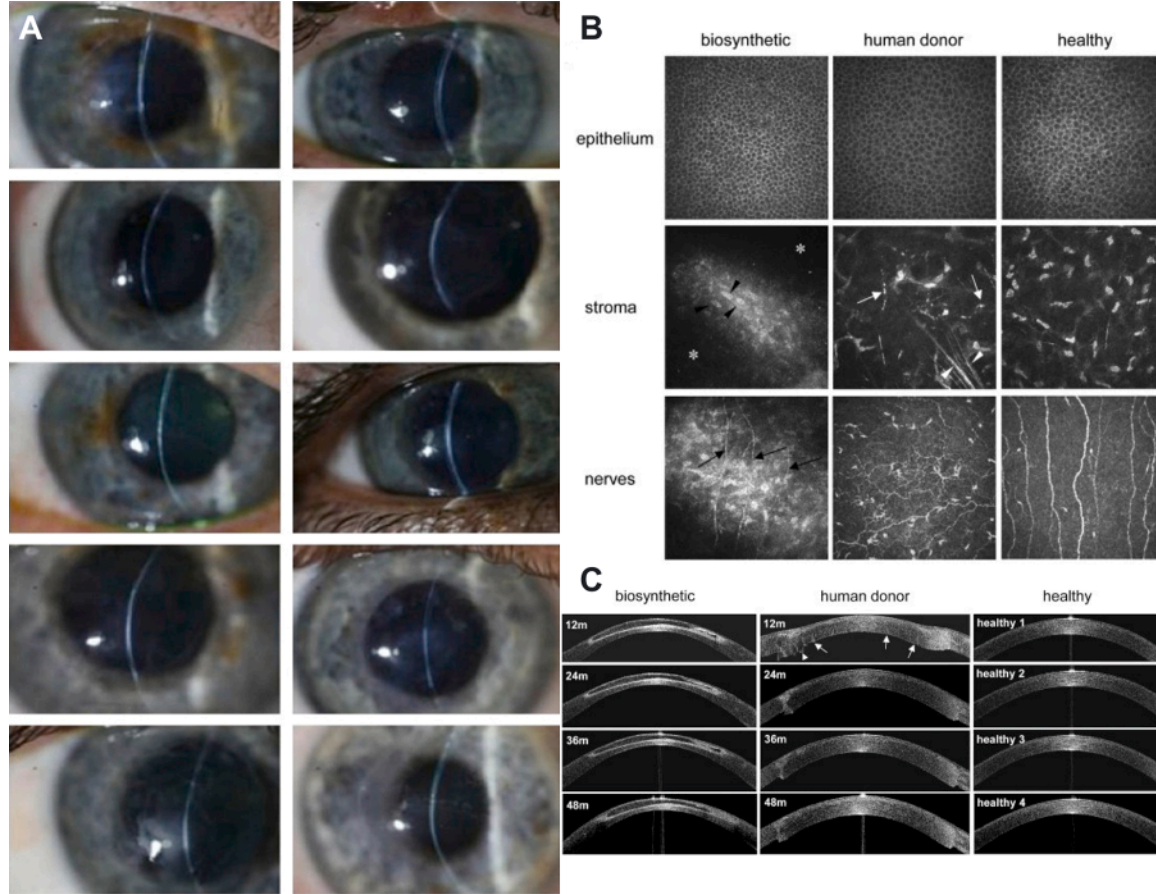


Figure 9.4 Results 4 years post-operative to RHCIII-EDC-NHS hydrogel implantation. (A) Slit lamp biomicroscopy of all 10 patients, demonstrating integration of corneal implants and transparency. (B) In vivo confocal microscopy images of biosynthetic grafts (hydrogel), human donor grafts, and healthy cornea. Epithelial regeneration is comparable across all groups. The biosynthetic group has keratocytes (black arrowheads), migrating into the cell-free scaffold area; the human donor cornea grafts have fewer keratocytes, linear reflective structures (white arrowheads), and particulate bodies (white arrows), suggestive of apoptosis. Nerves are parallel and linear in the biosynthetic grafts and resemble, to a lesser extent, the nerves in healthy corneas; nerves are tortuous with adjacent dendritic cell presence in human donor cornea grafts. (C) Anterior segment optical coherence tomography images: In the left column, the biosynthetic implant remains stable over time; in the middle column, the human donor corneal graft shows compression due to sutures, posterior donor-recipient edge mismatch (arrowhead), and stromal compression lines (arrows), which resolve following suture removal at 1 year post-operatively.

Reprinted from Fagerholm et al., 2014. Stable corneal regeneration four years after implantation of a cell-free recombinant human collagen scaffold. *Biomaterials* 34 (8), 2420–2427, Copyright (2014), with permission from Elsevier. License number: 6142650324867.

et al., 2014) and minimize neovascularization (Hackett et al., 2011). Patients with scarring or ulcers due to infection ($n = 3$) saw the greatest improvement, whereas those with immune and degenerative disorders (i.e., a previous graft rejection and neurotrophic keratitis) performed the worst. Corneal sensitivity in all patients was restored close to the levels of their healthy contralateral eyes (Islam et al., 2018).

In a similar clinical trial with cell-free collagen-based implants (Rafat et al., 2023), implanted cell-free lenticules made from double crosslinked (both chemically and photochemically) to treat patients with keratoconus. The lenticules were implanted into stromal pockets created using femtosecond lasers. The authors reported that 14 of 14 initially blind subjects achieved a best-corrected vision (spectacle or contact lens) of 20/36.

Other proteins that have been tested include fibrin. Campos, Alaminos and co-workers developed implants made from fibrin and agarose, but these contained human allogeneic stromal keratocytes as well as corneal epithelial cells (González-Gallardo et al., 2023). These stromal/epithelial tissue replacements, called NANOULCOR were clinically evaluated in a trial of five patients with severe trophic ulcers refractory to conventional treatment and reported improvement.

9.5 Stromal implants incorporating delivery systems

9.5.1 Types of infections

9.5.1.1 Bacterial infections

In collagen hydrogels, antibiotics like vancomycin have been incorporated, and in one such 15% collagen hydrogel this demonstrated successful prophylactic activity against *S. aureus* in rabbits (Riau et al., 2015). Silver nanoparticles (AgNPs) serve as another alternative to antibiotics and have been integrated into collagen corneal constructs, demonstrating in vitro successful blocking of *P. aeruginosa* growth comparable to common antibiotics like gentamicin (Alarcon et al., 2016). Further developments like light-mediated grafting techniques have been developed to make it easier to integrate these nanoparticles into corneal implants (Khatoon et al., 2020).

9.5.1.2 Viral infections

Herpes simplex virus (HSV-1) infections are one of the most common infectious cause of blindness in developed world (McCormick et al., 2022). Several corneal implant materials have focused on preventing or treatment of these infections, as detailed in Table 9.4. One of these is a stromal filler-sealant with inflammation suppressing properties that also contained silica nanoparticles delivering an anti-HSV-1 peptide derived from the cathelicidin, LL37 (Simoliunas et al., 2024). The liquid filler gelled within the wound bed and was able to block viral activity when HSV-1 was introduced.

9.5.1.3 Fungal infections

Fungal infections from *Aspergillus* and *Fusarium* species are responsible for ~50% of keratitis cases in developing nations, being a major global cause of blindness (Sharma

Table 9.4 Mimetic biomaterials for stromal implants or filler-sealants with or without extra drug or therapeutic cell loading properties, and pre-clinical progress described.

Primary/ host component	Peptide or protein description	Delivery method	Drug/ loading	Crosslinking method	Stage of development	Reference
(PPG) ₇	Short CLP that combines into damaged collagen I	Topical injection	Not applicable	Not applicable	In-vivo accelerated wound closure and healing in mice within 24 hours.	Baratta et al. (2021)
Thiolated collagen	Collagen from human umbilical cords, thiolated.	Surgical implant	Levofloxacin, antibiotic	Disulfides	Ex-vivo rabbit eye adhesion and drug permeability, in- vitro reduces fibrotic episodes by reducing TGFβ1 and vimentin expression.	Gunasekaran et al. (2025)
Collagen nanosheet	Collagen I, functionalized with Tet213 peptides	Surgical implant	Tet213 antibacterial peptide	EDC/NHS carbodiimide chemistry	In vivo rabbit work showed enhanced antibacterial healing.	Huang et al. (2024)
RHCIII-MPC	Full length recombinant human collagen type III conjugated to 2-methacryloyloxyethyl phosphorylcholine	Surgical implant	Silica nanoparticles loaded with KR12 peptide from human cathelicidin LL37	EDC/NHS carbodiimide chemistry	In-vivo hydrogels partially blocked HSV-1 activity in infected rabbit corneas.	Malhotra et al. (2023)
Thiolated collagen— PEG	Thiolated porcine type I collagen crosslinked to 8-arm polyethylene glycol-maleimide	Injection	Mesenchymal stromal cells and endothelial cells	Maleimide click chemistry	In-vitro cell survival after encapsulation into hydrogel.	Pupkaite et al. (2019)

CLP-PEG	A library of 21 different CLPs 18–36 amino acids long, conjugated to 8 arm PEG	Injection	Not applicable	Maleimide click chemistry	9 CLPs identified, ex-vivo pig cornea implants integrated successfully. Custom injector was fabricated for delivery.	Ross et al. (2024)
CLP-LCPP (linear carboxylated phosphorylcholine polymer)	(DOG) ₄ (POG) ₄ (PKG) ₄	Injection	GF19 antiviral peptide in silica nanoparticles	Carboxylic activation chemistry (DMTMM)	In-vivo delivered GF19 blocked HSV-1 viral activity in infected rabbit corneas.	Simoliunas et al. (2024)
Silk fibroin/collagen film	Degummed natural silk and type I collagen	Surgical implant	GI-20 antiviral peptide in PLGA nanoparticles	EDC carbodiimide chemistry	In-vivo blocked HSV-1 viral activity in infected rabbit corneas, sustained 14 day release.	Sohrabi et al. (2025)
(PPG) ₇	short CLP that combines into damaged collagen I	Topical injection	Not applicable	Not applicable	In vivo mice with dry eyes - less nerve fragmentation, better sub-basal and epithelial neuronal coverage, better tear film production, and less corneal sensitivity.	Wareham et al. (2023)
Coll-PyKC	10% porcine type I collagen and pyrene dipeptide amphiphile (PyKC) monomer with lysine and cysteine	Injection	Not applicable	EDC/NHS carbodiimide chemistry	In-vitro cell work showed suppression of inflammatory markers.	Islam et al. (2022)

et al., 2022). Diagnosing and treating it is difficult due to delayed symptoms and slow culturing tests for confirming the infection, as well as limited availability of the common drug treatments natamycin and voriconazole. Arora et al. (2025) were able to target fungal keratitis using cell penetrating peptides that inhibited fungal growth in mice models (Arora et al., 2025). This along with other peptide strategies could be incorporated into bioengineered stromas in future designs for added functionality.

9.5.2 CLP stromal repair materials and drug delivery systems

Unlike donor corneas, biomaterials used for fabrication of stromal implants have the added advantage of being able to deliver drugs and therapeutic cells within their bulk. A key emerging trend is the increased use of drug loading, often with nanoparticles to allow controlled release to address specific problems like preventing implant infections, including targeted antiviral and antibacterial peptides. For example, Malhotra et al., incorporated the KR12 peptide (derived from human cathelicidin LL37) into RHCI-MPC hydrogels via silica nanoparticles (Malhotra et al., 2023). These composite hydrogels were shown to partially block HSV-1 activity in infected rabbit corneas (Malhotra et al., 2023). These and other examples hydrogels containing delivery systems for drugs or therapeutic cells are shown in Table 9.4.

9.6 Future outlook

9.6.1 Regulatory landscape for stromal implants

For clinical applications, researchers select biomaterials with patient safety and regulatory approval in mind. Approved biomaterials can enable faster regulatory approval. Scalability, cost, and ease of production are also crucial considerations. Briefly, the engineered constructs used as stromal replacements are classified as medical devices or as medicinal products (e.g., biologics in Canada or advanced therapy medicinal products (ATMPs) in Europe) depends on whether or not the materials contain cells, drugs, or bioactive components (Dieh et al., 2025; Pelletier et al., 2018). Bedford et al. provide a detailed description of the regulatory process approvals for bioengineered materials (Bedford et al., 2025).

9.6.2 Conclusion and future directions

A range of biomaterials from synthetic polymers to decellularized extracellular matrices, with or without cells, and fabrication techniques have been bioengineered as potential stromal equivalents and replacements. For regeneration, stable and seamless integration are critical. Much of their success is dependent on the material's ability to mimic the ECM collagen environment with sufficient equilibrium water content. Of particular importance for stromal constructs is optical clarity and light transmittance, and nutrient permeability (Holland et al., 2021). Advances in the stromal bioengineering include designing in of drug and therapeutic cell delivery systems, and the ability to control inflammation in compromised corneas.

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