Rational Design of Peptide Based Implants for Corneal bioengineering

*Bapan Pramanik [a], Mohammad Mirazul Islam [b], Hirak K Patra [c]**

[a] Department of Chemistry, Ben Gurion University of the Negev, Be'er Sheva 84105, Israel

[b] Department of Ophthalmology, Massachusetts Eye and Ear and Schepens Eye Research Institute, Harvard Medical School, Boston, MA 02114, USA

[c] Department of Surgical Biotechnology, Division of Surgery and Interventional Science, University College London, NW3 2PF, United Kingdom

*Correspondence: Dr Hirak K Patra (hirak.patra@ucl.ac.uk)

Abstract

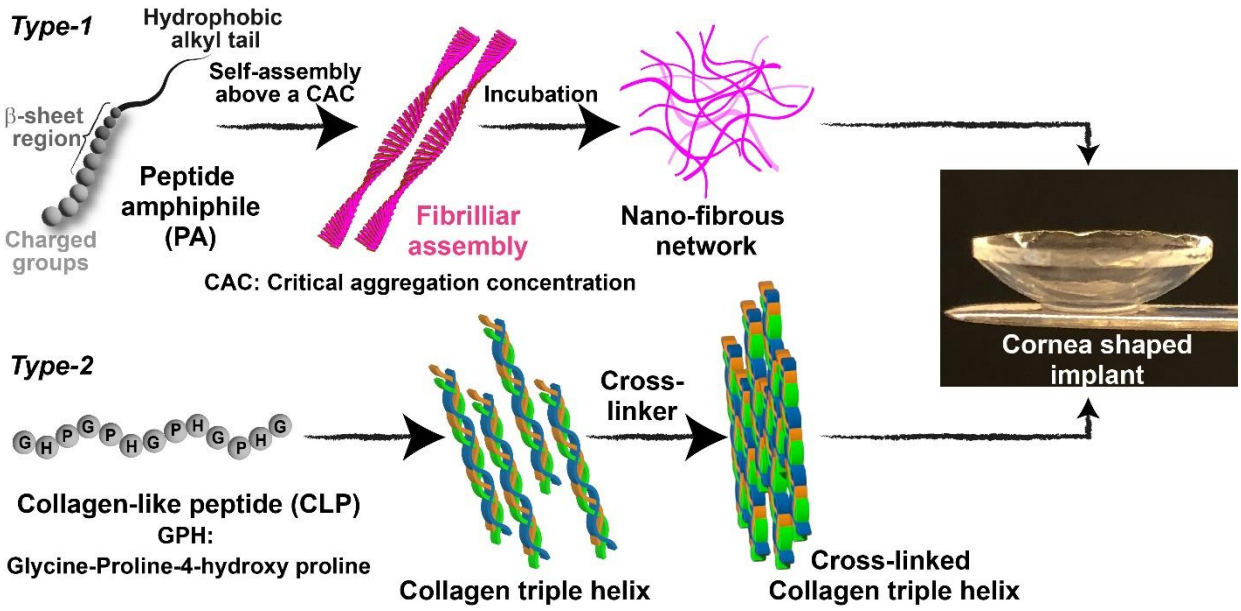
Regeneration of damaged cornea can save vision for millions of patients as globally 4 million people suffering from bilateral corneal blindness, and more than 20 million people affected by moderate to severe corneal visual impairment. Most of these patients are waiting for a donor cornea or its suitable substitute to transplant for restoring vision. Although donor cornea transplantation is the most accomplished treatment to replace the damaged one, shortage of the donor cornea leaves almost 69 out of the 70 patients untreated and the waiting list for the transplantation gradually increasing every year according to a pre-pandemic estimation. Therefore, corneal regeneration therapy and the use of artificial cornea are coming up as a cutting-edge alternative strategy. Developing appropriate corneal substitutes with biomaterial to mimic the native unique corneal structure and functions are challenging. In view of the peptides, especially collagen like peptides and peptide amphiphiles with bioactive functional motifs demonstrate promising avenue for the corneal tissue engineering and promoting regeneration, by their hierarchical self-assembling propensity to acquire desired nano to macro scale 3D architecture. In this report, we analyze rational designing, self-assembly process, and strategies of peptide/ peptide-based nanoscale building blocks to create the extracellular matrix mimetic implants for functional regeneration of the cornea. The critical balance and optimality on bio-integration vs biodegradability is considered in detail as the regenerative response. The pre-clinical prominence of these implants is being critically evaluated. The current challenges associated with and conceivable prospects for the clinical use of the peptides-based implants are argued focusing on their potential as artificial cornea for transplantation.

Introduction

Cornea, the transparent window of the eye, is an avascular, immune-privileged organ that covers the ocular surface and shields us from external environment and transmit light to the retina to provide vision [1,2]. Corneal disorders and injuries are the origins of irreversible loss of corneal functions [2]. Around 12 million people worldwide suffer from eternal vision impairment or blindness due to corneal complications [3]. In developing countries, corneal blindness has become one of the World Health Organization's (WHO) priority diseases. [4]. According to the WHO, corneal diseases have received 4th rank among the other corneal blindness diseases worldwide [5].

Transplantation with a cadaveric donor cornea is the current clinical practice to restore the vision for the corneal blind patients [2,6,7]. Despite the progress in organ donation, there is a massive shortage of the donor corneas worldwide for various reasons, including i) poor awareness about donations, ii) limited facilities to store the corneas, predominantly in the developing countries and iii) incompatible donated corneas [7-10]. A pre-pandemic estimation showed 1 cornea is available for 70 needed, and this condition became worse due to COVID-19 impact as eye banking becomes complex and non-life-threatening hospitalization becomes restricted [8,11-13]. In many developed countries, such as Japan and Canada, people are waiting for several years to get a cornea transplantation. The scarcity has become so severe that UK National Health Service (NHS) started sending specific reminder for ongoing need for cornea donors to mark on National Eye Health Week [13]. In the United States donated cornea recoveries (-20.4%) and transplant numbers (-22.8%) both reduced in 2020 for COVID-19 pandemic [14]. However, even with successful transplantation with donor cornea, transplants have associated with high risks of donor-derived infection, immune rejection, tissue unsuitability and allograft failure [15,16].

Figure 1. Simplified schematic self-assembled cornea implant for regeneration therapy.



Scientists have devoted innovating new strategies to develop functional artificial cornea or corneal substitutes. In an ideal scenario, corneal substitute should hold i) appropriate biocompatibility, ii) bio integrability, iii) high transparency, iv) appropriate refractive index, v) adhesiveness to the local microenvironment and coherence to the adjacent native ocular tissues, vi) adequate mechanical stiffness and finally v) low immunogenicity etc. [9]. These will help and support post-operative endogenous host tissue reconstruction, and finally will have clinical compliance for the ease of

application and use [10]. In light of this, various biopolymers such as collagen, gelatin, chitosan, fibrin, silk to name a few have been tested as the implant biomaterial for corneal bioengineering, however, complex isolation procedure, inferior mechanical strength, and low enzymatic degradation tolerance are the major challenges for the clinical translation [3,15,17]. Additionally, some of the biopolymers failed to support cell growth owing to the residual toxic chemicals used during cross-linking modifications etc. [3]. Alternatively, peptide-based bottom-up approaches, particularly, peptide amphiphiles (annotated here as PA)/ collagen-like peptides (CLPs) are paving the way for corneal tissue engineering due to their i) hierarchical self-assembling propensity, ii) controllable and tunable bio-physical properties, iii) easy manufacturing and implementation capabilities, iv) high biocompatibility and v) tunable structural diversity at the primary sequence (**Figure 1**). These self-assembled peptide (SAP) scaffolds further offer cellular adhesion, proliferation, and differentiation of corneal cells, and sometimes act as an immunosuppressants to accelerate corneal regeneration [6,18]. Such peptide-based scaffold is also advantageous for corneal tissue engineering due to its exclusive high surface to volume ratio with higher density of epitopes for functions. Several groups are therefore emerging with SAP and demonstrated such bioactive peptides for corneal tissue engineering applications [19-24]. In this opinion, we confer on how engineered peptide-based biomaterials designed at the molecular level to form a nanoscale to macroscale structure through their assembly process and enlighten recent advances in translating these biomaterials to corneal regeneration therapies by making artificial corneal tissue.

Peptide based approaches for corneal regenerations

Peptides, the versatile small building blocks of a protein, can be designed and crafted to either implants or scaffolds relatively easily than a gigantic protein and have been universally recognized as a prime elements to the field of tissue engineering for primarily their i) homogenious synthesis and less batch to batch variations, ii) easy customizable modification, iii) low immunogenicity risk, iv) low pathogenic transmission, v) cytocompatibility and finally vi) feasibility on an industrial scale production [5,25-27]. They further can be assembled into a hierarchically defined bottom-up architecture to serve both structural and functional aspects of proteins [28]. The PAs have been explored as appropriate bioactive implant biomaterial. For example, in corneal implants, which is largely depends on ultrastructure organization of the cornea for maintaining appropriate functions. Collagen is the main extra cellular matrix (ECM) in cornea, decorated with different glycosaminoglycan which bind to a vast number of cell-surface receptors. Lack of cellular attachment to the structural ECM can lead to the irreversible loss of transparency of the cornea. PAs can form nanofiber networks to provide biological signals to the cells to achieve controlled cell–ECM interactions [1]. PAs are generally composed of hydrophobic alkyl tails, β-sheet forming sequences and charged groups and can self-assemble into nanofibrous networks via hydrophobic, electrostatic and van der Waals interactions [29] (**Figure 2A**). In general, cell-adhesive peptide motifs/bioactive epitopes such as RGD, RGDS, IKVAV, YIGSR, KTTKS, PHSRN, DGEA etc. are employed in designing PAs for corneal biomaterials [30]. These bioactive epitopes/signals are integrated into PA sequences in directing the lineage cells' commitment and to amplify the cell adhesion, proliferation, and alignment of human corneal stromal fibroblasts (HCSF) [1]. Interestingly, these self-assembled nanofibrous scaffolds are biocompatible and biodegradable in nature. Additionally, it mimics the structural and functional properties of the native ECM environment of the cornea [31]. Classically, a β-sheet forming peptide sequence is most suitable and studied for secondary structures compared to the other secondary structures, including β-turn, and coiled-coil structures, mainly due to their short sequences, and high self-assembling propensity. The other advantages apart from their high surface area of the resultant nanofibrous network, porosity, and ease of positional modification of the epitopes, etc. Mechanistically, the oppositely charged peptide molecules undergo self-assembly at

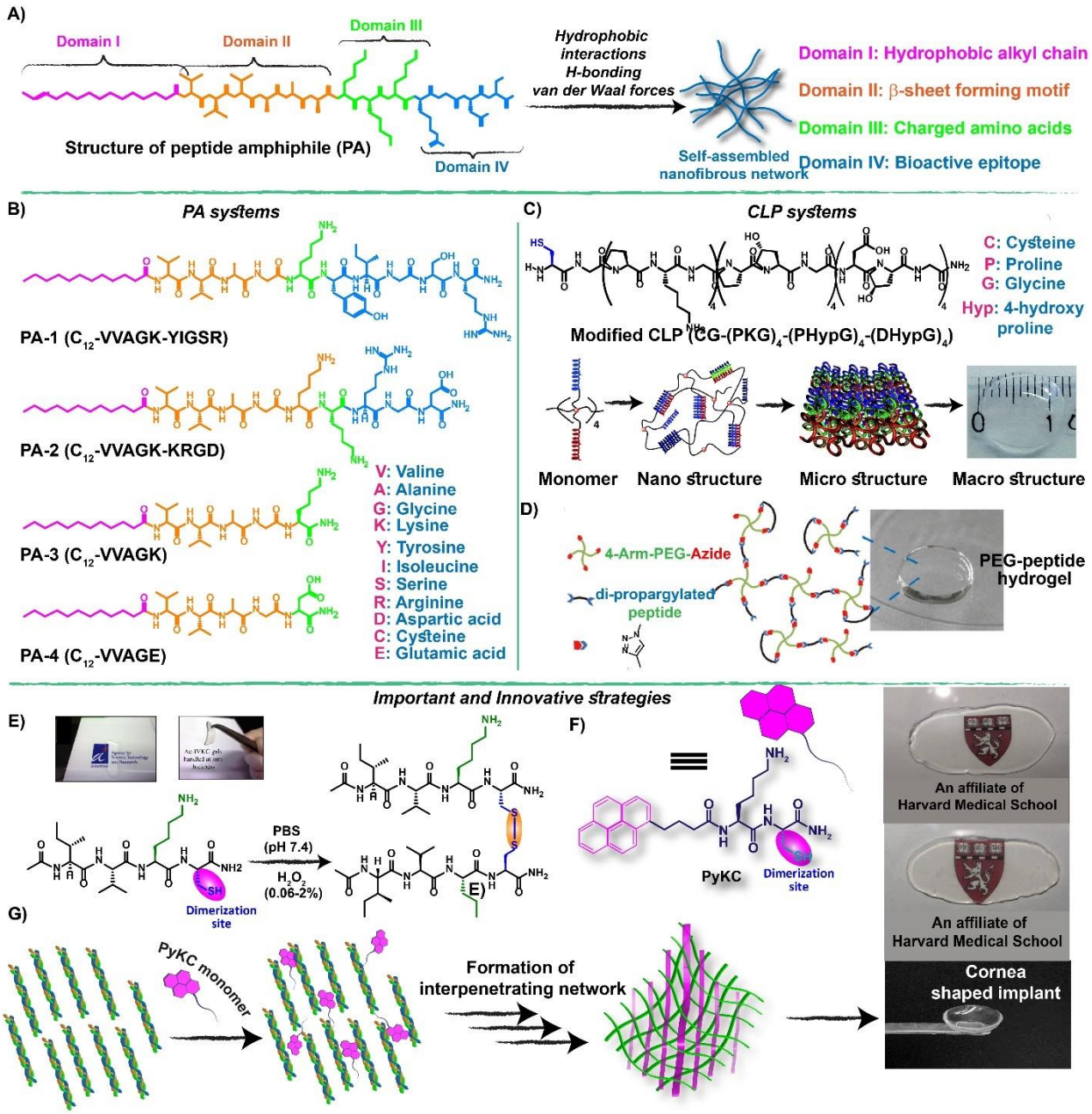
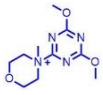
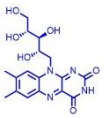


Figure 2: (A) Simplified schematic representation of a peptide amphiphile (PA) and the self-assembly process through non-covalent interactions; (B) Chemical structure of PAs used to prepare implants for corneal regeneration; (C) Molecular structure of collagen-like peptide (CLP) and schematic illustration of self-assembled CLP-PEG hydrogel to form different structure from nano to macro level; reproduced with permission from ref. 11 Copyright 2016, Royal Chemical Society (D) Schematic of PEG-peptide hydrogel through the triazole ring formation; reproduced with permission from ref. 45 Copyright 2022, Royal Chemical Society (E) Chemical structure of the peptide hydrogelator and representative membrane formation; reproduced with permission from ref. 27 Copyright 2019, Elsevier publisher (F) Chemical structure of PyKC. (G) Schematic illustration for the Cross-linker free corneal implant preparation. reproduced with permission from ref. 46 Copyright 2022, Nature publisher.

physiological pH to form supramolecular nanostructures through electrostatic interactions without any external trigger [32,33]. Several groups including Tekinay, Guler, Aydin and co-workers rationally designed a series of PAs composed of aliphatic hydrophobic chain along with oppositely charged amino acids. Two of them are bio-active, PA-1 (C_{12}-VVAGK-YIGSR) and PA-2 (C_{12}-VVAGK-KRGD), incorporating laminin and fibronectin derived most ubiquitous bioactive motifs,

YIGSR, and RGD, respectively, for cellular adhesion and proliferation during corneal stroma regeneration. Other two are non-bioactive PAs: PA-3 (C_{12}-VVAGK), PA-4 (C_{12}-VVAGE) as their respective controls to check the functions of bioactive unit of a PA [1] (**Figure 2B**). The presence of PA-3 and PA-4 enhances the epitopes' spacing in PA-1 and PA-2 for optimal recognition by receptors or proteins of interest by displaying hang-out-like patterns from the fiber surface [34]. At the physiological pH, the oppositely charged PAs (PA-1/PA-3; PA-2/PA-3 and PA-3/PA-4) can self-assemble to form nanoscale fiber structures like a natural ECM. The self-assembly initiation majorly expected to be driven by hydrophobic interactions from hydrophobic C_{12} alkyl tail, followed by the hydrogen bonding between the peptide backbone of -CO and -NH and electrostatic interactions from charged amino acids like lysine and glutamic acid and $\pi-\pi$ stacking from an aromatic amino acids like tyrosine. The electrostatic interactions are important to promote the hierarchical 3D network to form the hydrogel through β-sheet structure [1].

Bioengineered corneas using ECM protein such as collagen and CLPs showed great promise to the future for alternative to human donor cornea through the clinical trials. Collagen, the most abundant ECM component attracted enormous attention in corneal tissue engineering because of their inherent biocompatibility and pro-regenerative properties [12,35,36]. However, the collagen hydrogel based implants are generally with low mechanical properties and translucent/opaque in high concentrations limit its applicability in corneal transplantation [37]. For such implants collagens are cross-linked through a range of cross-linkers (carbodiimide, glutaraldehyde, genipin etc.) to surmount the issues [37]. Alternating strategies to make a composite materials with other double or interpenetrating network is tested to improve biomechanical properties [12]. Fagerholm *et al.* [38] reported a bioengineered acellular corneal implant for the first time using recombinant human collagen type III (RHC III), cross-linked through a zero-length cross-linker, N-(3-dimethyl aminopropyl)-N'-ethyl carbodiimide /N-hydroxy succinimide (EDC/NHS) to conduct Phase I clinical study for endogenous corneal tissue regeneration in human [39]. The corneal substitutes were found to be very well integrated into the host without complications like inflammation, neovascularization, rejection, or adverse effects after the surgery. The group successfully regenerated human corneal epithelial, stroma and nerves, supported by the clinical data. Four years postoperative follow up data showed that patients with artificial corneas had an average corrected visual acuity of 20/54 and gained more than 5 Snellen lines of vision on an eye chart [40].

Cross-linker	Abbreviation	Cross-linker type	Chemical structure	Bond type
N-(3-dimethyl aminopropyl)-N'-ethyl carbodiimide /N-hydroxy succinimide	EDC/NHS	Zero-length		Amide 
4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium	DMTMM	Zero-length		Amide 
Riboflavin	RF	Zero-length		Imine 
Glutaraldehyde	GTA	Non-zero-length		Imine 
Hexamethylene diisocyanate	HMDIC	Non-zero-length		Urethane 

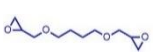
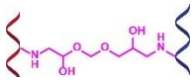
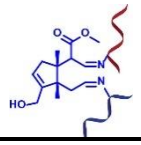
1, 4-butanediol diglycidyl ether	BDDGE	Non-zero-length		C-N 
Genipin	GP	Non-zero-length (C-N bond)		Imine 

Table 1:Driven factors behind the cross-linking

The success of this pioneer clinical evaluation ignites the idea of exploring shorter analogues of the full-length collagen, CLPs or collagen-mimetic peptides (CMPs) for corneal bioengineering. These are synthetic peptides capable of harnessing the triple helical structure of native collagen and their associated benefits in fostering corneal regeneration [41]. The approach is emerging as an alternative strategy to develop a collagen mimetic as the full length collagen is extremely challenging to synthesize and handle due to their triple-helical special structure, gigantic protein size, limited solubility in most of the working buffers, thermal instability and possibility of contamination with pathogenic substances [41,42]. Following a pioneering work by O'Leary *et al.* [43], Griffith *et al.* demonstrated a modified CLP, composed of $(PKG)_4(PHypG)_4(DHypG)_4$ (P: Proline, Hyp: 4-hydroxy proline) in addition to a Glycine (G) spacer and a cysteine (C) amino acid at the N-terminal with an intention of thiol-Michael addition through the free sulfhydryl group (-SH) of the Cys's side chain [11] (**Figure 2C**). The peptide undergoes covalent bond formation in the presence of an 8-armed polyethylene glycol (PEG) maleimide. The existence of a multi-arm template maintains a tolerable balance between rigidity and flexibility, whereas PEG promoted the triple helices of CLP into a higher ordered hierarchical supramolecular self-assembly which crosslink in presence of EDC/NHS to form CLP-PEG hydrogels, leading to a stabilized cornea shaped implant with optical transparency and robustness (**Figure 2C**). A detailed comparative optical, physical, and mechanical properties of such implants are described in **Table 1**. The developed implant was found to be relatively stable even at a higher concentration of collagenase solution (5 U/mL), reflecting greater resistant to biodegradation. The CLP–PEG implants exhibited minimal degradation compared to well-established recombinant human collagen-2-methacryloyloxyethyl phosphorylcholine (RHC-MPC) implants, which were found to be stable in severely pathologic eyes [12]. The RHC-MPC implants supported the proliferation of Human corneal epithelial cells (HCECs) and indicated strong cornea compatibility.

Table 2: Optical, mechanical, and thermal properties of the implants**.**

Implant	Cross-linker	Transmission (%)	Refractive index	Water content (%)	Tensile strength (MPa)	Young's modulus (MPa)	Storage modulus (G') (kPa)	Loss modulus (G") (kPa)	Denaturation temperature (°C)	Elongation
Human cornea		87.1 ± 2.0	1.373–1.380	78	3.81 ± 0.40	3-13	-	-	65.1 ± 0.0	N/A
RHCIII	EDC/NHS	95.1 ± 0.05		91.5 ± 0.9	0.286 ± 0.062	1.749 ± 0.782	-	-	54.21 ± 0.91	20.149 ± 7.614
CLP-PEG	EDC/NHS	32-92(UV) 92-99(Vis)		92.67 ± 0.85	0.56 ± 0.21	0.150 ± 0.015	22.36 ± 1.489	0.0433 ± 0.006		49.96 ± 8.10
RHCIII-MPC	EDC/NHS	92.1 ± 0.1	1.334 ±0.0	85.5 ± 0.2	0.26 ± 0.06	3.63 ± 0.84	-	-	56.96 ± 1.05	12.15 ± 0.84
CLP-PEG	EDC/NHS	92.4 ± 0.95	1.34 ±0.0	91.65 ± 1.10	0.07 ± 0.02	0.18 ± 0.06	-	-	151.30 ± 9.91	58.30 ± 4.49
LiQD	DMTMM	19–93% (UV) 93–99% (Visible)	1.354 ± 0.037	91.2 ± 2.3	0.02	-	0.16	-	64 ± 8.5	
CLP-PEG-MPC	DMTMM	29-80(UV) 80-97(Vis)	1.340 ± 0.005	90.94 ± 0.78	0.022 ± 0.004	0.044 ± 0.010	15.15 ± 1.086	0.1522 ± 0.0569	-	59.50 ± 7.70
Coll-PyKC	NA	~80 (400-600 nm)	-	~90%	-	-	-	-	-	-

	~ 90 (600-800 nm)

Both the implants exhibited appropriate light transparency (~92%) comparable to human cornea (~87%) and similar refractive index values (~1.37-1.38), perquisite for artificial cornea (**Table 1**). The denaturation temperature of CLP-PEG hydrogel was found to be around 152 °C, much higher than control hydrogel (~57 °C) and human cornea (~ 65 °C). Mechanically, the implant also showed enhanced (~4-folds elongation) elasticity compared to control implant. Notably, the optical and physical properties like water content, optical transparency, collagenase degradability of the CLP-PEG implants was retained even after storing it for more than 12 months at 4 °C. *N*either CLP-PEG nor RHCIII-MPC were cytotoxic and showed similar proliferation in vitro. This has further been supported by Haagdorens and co-workers with immortalized human corneal epithelial cell (iHCEC) and primary Limbal Epithelial Stem Cells (LESCs) on EDC and DMTMM ((4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium)) cross-linked CLP-PEG hydrogels [44]. Simpson *et al.* further improved the CLP-PEG implant by replacing the crosslinker EDC by DMTMM to reduce probable residual inflammatory response due to EDC, followed by linked to MPC (to reduce further inflammation) to form CLP-PEG-MPC [7] implants. The CLP-PEG-MPC hydrogels blocked up to 60% transmission in the wavelength range of 300–400 nm (UV A) and the other properties summarized in **Table 1**. The field is progressing and recently, Lei *et al.* developed a newer PEG-peptide-based hydrogel for corneal tissue engineering to prepare Pep-PEG hydrogel [45] (**Figure 2D**). At first, di-propargylated peptides were synthesized, which undergoes intermolecular cross-linking (click reaction) with 4-arm-PEG-N_3 via 1,2,3-triazole ring formation to form hydrogels.

The other approach using peptides are developing rapidly for corneal bioengineering therapy aimed at partially damaged and perforated cornea that can lead to corneal blindness. In general, corneal perforation in those cases are sealed using cyanoacrylate glue. However, due to the toxicity of cyanoacrylate glue and post-translational complications, McTernan *et al.* engineered a peptide based regeneration-stimulating liquid corneal replacement, LiQD Cornea [8]. LiQD cornea is an alternative to traditional full corneal transplantation and used as sealants/fillers. LiQD is composed of a short CLP linked to PEG and mixed with fibrinogen to endorse adhesion within tissue defects and it is injectable. The CLP-PEG-fibrinogen undergoes self-assembly to form a porous hydrogel in presence of thrombin and DMTMM crosslinker. The sealants' optical, physical, and mechanical characteristics are illustrated in **Table 1**. The LiQD exhibited epithelial growth of HCECs and immune compatibility. Also, *in vitro* data of the LiQD Cornea formulation indicates negligible activation of dendritic cells, and hence has less rejection possibilities.

Two other interesting approaches are reported by Hauser *et al and Islam et al* to make cell free corneal scaffold using peptides in a different context [27] and [46]. The first group reported an ultrashort tetra peptide, Ac-IVKC-$CONH_2$, with a protected N-terminus with acetyl group for hydrophobicity (**Figure 2E**). The peptide self-assembled in PBS (pH 7.2) through non-covalent and covalent interactions through cystine and formed a self-supporting stiffer hydrogel with an interwoven fibrous network with very high storage modulus. The fabricated thin-layered hydrogel membrane is transparent and showed >95% transparency in the visible light region (400–750 nm), illustrating potential for corneal bioengineering. The second approach is recently reported by our group for the first time where we show a cross-linker-free collagen implant for corneal regeneration (**Figure 2F**). We rationally choose a self-assembling short peptide hydrogelator, PyKC [47,48] as an assembling unit to stitch the collagen fibrils (type 1) to form collagen-based artificial corneas [46] without chemically crosslinking the collagen chains. The PyKC undergoes self-assembly through non-covalent interactions (hydrophobic, $\pi-\pi$ stacking interactions and H-bonding) and a disulphide covalent interaction (–S–S–) between free sulfhydryl group of cysteine. Collagen molecules get entrapped into it to form the hydrogel without modifying the native

collagen molecules. The resultant formulation ($Coll_x$-$PyKC_y$, where x and y represent wt% of respective component) showed similar optical properties to the human cornea and was stable and exhibited resistance to collagenase mediated degradation. the implants showed biocompatibility with human corneal epithelial cells, human corneal endothelial cells, and human corneal fibroblasts, implying the feasibility in corneal tissue regeneration.

One important parameter for any corneal biomaterial is transparency. For a peptide hydrogel, it is primarily dependent on the solubility of peptides in the fabrication medium and the strength of the involved physical and covalent interactions among nanofibers. The overall net charge in the peptide sequence plays a vital role in getting transparency. While hierarchically self-assembling, the resultant nanofibers undergo repulsive interaction among themselves because of higher net charge, tending to be organized to inhibit the aggregate formation and leading to increased transparency. It seems, while rationally designing a peptide for corneal biomaterial, at least one additional charged amino acid per peptide monomer is required to stabilize nanofibers against aggregation [49]. The hierarchical assembly also plays an essential role in obtaining the transparency [46,47,50-55]. Any attempt to intensify the hierarchical self-assembly process (addition of additives), can be detrimental to both transparency and mechanical strength as the peptide monomers might not get sufficient time for homogeneous self-assembly. Secondly, the locoregional resultant network concentration can be too high due to immediate self-assembly in-homogeneously, leading to collapse of fibrillar networks and loss of transparency.

Preclinical studies to evaluate the clinical aspect of peptide based corneal regeneration

Peptide-based corneal regenerative approach holds tremendous potential either to develop an artificial corneal implant or for adding functionalities to the corneal scaffolds, however the true potential needs to evaluate in *in vivo* studies. For a clinical application, PA can also be easily customized for personalized treatment as their biological activities can be tuned by changing functional motives in the peptides. Therefore, presumably, PA has the potential to revolutionize the next generation artificial cornea development for human transplantation. Griffith lab has played a pioneer role in transplanting peptide based artificial corneal implant in animal models. They transplanted YIGSR containing collagen based artificial cornea into the pig eyes by lamellar keratoplasty and showed successful regeneration of cornea by host corneal cells [56]. Later, they made the artificial corneal with CLP-PEG and transplanted into animal models for pre-clinical evaluations. (**Figure 3A**). Biocompatibility of the implant was studied by subcutaneous implantation of hydrogels into the dorsum of rats. The implants were biocompatible as they were relatively intact and remained free of immune cells or thick fibrotic encapsulation after 90 days of implantation. Finally, these corneal implants were transplanted into mini pigs by anterior lamellar keratoplasty (ALK). The CLP-PEG implants remained stably incorporated and optically clear, and acellular implants infiltrated with host corneal epithelial, stromal and nerve cells. However, no blood or lymphatic vessels were observed within the transplanted corneas [11]. Ultrastructural analysis of regenerated corneas showed significant amount of extracellular vesicles secretion from the corneal epithelium and peptide implanted corneas were expressing markers for exosomes (CD9), and endosome-exosome (Rab-7) [25]. To further evaluate the potential of CLP-PEG, implants were further studied with fibrinogen using different crosslinker with thrombin and used this implant to correct corneal perforation in a rabbit study. These implants were eventually transplanted into mini pigs by ALK. Rabbit study showed that the hydrogels sealed the perforation in the majority of the animals with visible transparent corneas. Pig study confirmed the regeneration of the corneal tissue by the host cells [8].

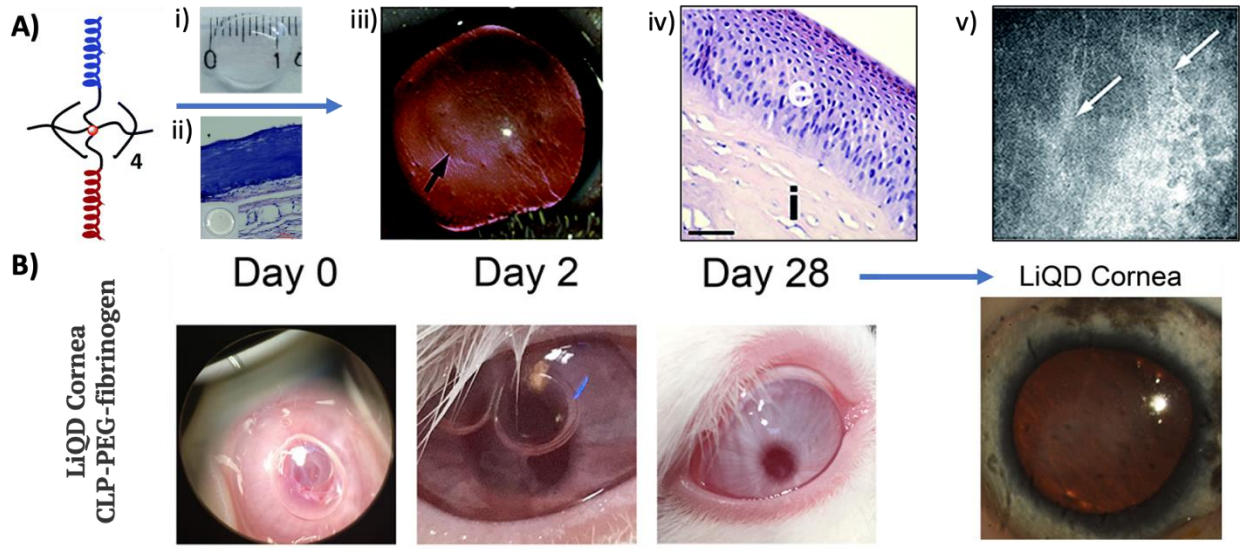


Figure 3: *In-vivo* biocompatibility of peptide based corneal therapies. (A) CLP-PEG implants transplanted subcutaneously into the rats and as artificial cornea in the mini-pig. (i) Transparent CLP-PEG cornea implant, (ii) H&E staining of a CLP–PEG hydrogel subcutaneously implanted for 90 days shows an even, unbroken material edge and there is no presence of fibrotic tissue or inflammatory cells. Inset: intact 10 mm diameter, 500 mm thick CLP-PEG hydrogel retrieved at the end of the study. (iii) Integration of CLP–PEG implant (arrowed) within the pig cornea, (iv) H&E staining of a regenerated CLP–PEG cornea shows stratified epithelium on the implant, (v) *in-vivo* confocal microscopy shows the regenerated nerve (arrows) in CLP–PEG transplanted cornea, reproduced with permission from ref. 11 Copyright 2016, Royal Chemical Society (B) Peptides based therapy as liquid cornea to correct the corneal perforation. (i) Images of rabbit corneas at different time points of sealing of the perforated cornea with LiQD. The perforated cornea was completed sealed by 28 days after operation. (ii) Mini-pig corneas at 12 months of transplantation with LiQD based artificial cornea. reproduced with permission from ref. 8 Copyright 2020, American Association for the Advancement of Science publisher.

In another approach, CLP-PEG was incorporated with MPC to make corneal implants and evaluated in a mini-pig cornea alkali burn model. This pig study data confirmed that implants reduced corneal swelling, haze, and neovascularization, at the same time promoted faster nerve regeneration and recovery of corneal sensation [7]. Noticeable other mention of animal study can be the works of Connon's lab for the reconstruction of corneal stroma with the assist of the PA. They have developed corneal stromal self-lifting analogous tissue equivalents by culturing corneal stromal cells on PA surface and implanted the cell generated tissue into the intrastromal pockets of rabbit's model. It was found that the implanted tissue got well integrated into the host stroma without any of toxicity and haziness of the cornea [57]. CMP $(Pro\text{-}Pro\text{-}Gly)_7$) without having the ability to form triple helices was investigated to promote epithelial healing in an acute corneal wound model in mouse. Topical application of a CMP formulation on the wound augmented the healing rate during 24 h period. It was also found that this CMP increased adhesion of the basal epithelial cells to the underlying substrate, enhanced epithelium thickness and promoted regeneration [58]. Finally, instead of making a full corneal implant, the CLP-PEG-fibrinogen being used as a sealant to develop LiQD cornea. The spontaneous gelation of LiQD peptide at body temperature makes it even more attractive. The in vivo rabbit experiment showing exciting observations with a perforated cornea model. The perforated injury was recovered by 28 days after surgery (**Figure 3B**).

These studies tried to evaluate the safety and efficacy of the peptide-based approaches for human use. The knowledge gathered from these studies suggesting the feasibility of using

peptide analogs in clinical application for corneal bioengineering. We are close to seeing the successful transplantation of these peptide implants in a well-documented clinical trial.

Challenges and Opportunities of using peptides

The goal for any artificial organ development is to make the artificial substitute close to the native tissue by composition, characterization and by functional properties. The cornea is a unique tissue of the human body as it is transparent, full of nerve and absence of blood vessels. It has unique size, shape, and strength for having collagen fibers in specific orientation. To mimic the physicochemical and functional properties of the native cornea is the main challenge of peptide-based approach. Synthesis, isolation, purification, and quality control to generate clinical grade biomaterials can also be a big hurdle. Functional and structural reproducibility and batch-to-batch consistency are critical requirements for clinical grade biomaterial development [59]. Using crosslinkers to give mechanical strength and appropriate shape can be challenging as most of the crosslinkers are cytotoxic in nature. Additionally, some cross-linkers form inhomogeneous hydrogel owing to their fast gelation time, a major obstacle for clinical use. For human use, the full process of material development needs to be customized for GMP production, which can ultimately increase the production cost and the facilities can be restricted to only developed parts of the world. Sterilization of the biomaterials can be the next challenge to ensure the peptide components are sterile, and the process of the sterilization does not adversely affect the peptide and it self-assemble.

Recently many peptide sequences have been developed and over 150 peptides are in clinical development, and over 60 peptide-based drugs are approved in the United States and other major markets [60]. Most of the peptides are designed as functional adhesion motifs, growth factor and drug or combination of different sequences to get multiple properties. Many peptides have been developed for different intentions but can easily apply for corneal application. Injectable peptides used for local and sustained delivery of drug from the implant [61]. The same approach can be used by injectable hydrogel to seal corneal perforation and local drug delivery. Different groups also showed that peptides-based hydrogels can be used to release the therapeutic agent [62]and this approach can be adapted to release ocular drugs from artificial corneal hydrogel to enhance regeneration and prevent infection. We previously showed that hydrogels can be used for theranostic application with the help of MRI detectable nanoparticles [63]. Other groups have shown that peptide hydrogels can be developed with peptides conjugated with MRI contrast agents [64,65]. This contrasting agent conjugated peptide hydrogels can be evaluated for corneal application. Peptide based supramolecular hydrogels have the advantages for optical imaging, radionuclide imaging, computed tomography, ultrasound imaging and photoacoustic imaging [66]. Now it is well known that antimicrobial peptides play an important role in the ocular defense mechanism against microbes [67]. These peptides or their bioequivalent can be synthesized and can be used directly on the ocular surface as drugs, or they can be incorporated into the matrix of artificial corneal formulations. Nanoparticle based drug delivery from corneal implants showed potential in *in vitro* evaluation [63,68]. These same nanoparticles can be conjugated with peptides to develop a versatile tool for biomedical application [69]. Peptides conjugated to nanoparticles can help to modify the nanoparticle shape, dimension, and size for ocular application. These peptides can be crosslinked while developing the artificial cornea with ECM component, which can facilitate the local retention of nanoparticles for an extended period and get prolonged drug delivery from the conjugated nanoparticles.

Conclusion and perspectives

Peptides are natural biomolecules. Peptides are easy to synthesize quickly, with high purity and easily customizable without much difficulty. Therefore, there is enough space to create room for ready and scaled-up production. Additionally, peptides offer a broad window of mechanical strength just by tuning the amino acid sequence because mechanical strength is always a significant concern in corneal regeneration. The versatile nature of PA allows them to a broad clinically translatable biomimetic materials for tissue regeneration that not only provide healthy physiological well-being life, but also resolve existing problems connected with the corneal regeneration. The peptide analogs of ECM are emerging with advantages in comparison to full-length matrix materials because of their simplicity to amend at the seed level with variety of diverse functionalities. Those can then hierarchically assemble with desired and tunable properties such as enhanced solubility, stimuli responsiveness and even tethering with polymeric backbones. It is true that a lot of literature exists to exploit the PA in biological systems, but still scientific community are working on the PA to apply them in corneal tissue engineering and repair. This current opinion has enlightened this embryonic but potentially convenient focused field, and reveals the achievement from different aspects of physical, chemical, biological, and clinical sciences and it is the paradigm shift for tissue engineering and regenerative medicine. Despite the improvements in the progress of peptide assisted implants for cornea regeneration, there are still few bottleneck issues which should be taken under consideration for future research: (i) transparency, (ii) stiffness, (iii) stability, (iv) biodegradability etc. Therefore, extensive research should be performed to identify new peptide based non-cytotoxic transparent biocompatible implant for future generations to live happier life.

Author Contributions

HP formulated the idea of comprehensively summarizing the current updates on how peptides being useful in corneal bioengineering and regeneration therapy. BP drafted the MS with contributions from MMI and HP. The final manuscript was written through contributions from all authors. All authors have given approval to the final version of the manuscript.

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Conflict of interest statement

Nothing to declare.

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** Of outstanding interest

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