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Tissue Engineering Collagen-Based Implants for Corneal Regeneration

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Abstract

Comprising the refractive component of the eye, the cornea is a fundamental component of the human visual apparatus. The vitality of the structure is recognized in instances where it is functionally impaired, most commonly due to ulceration-induced scarring, infection, traumatic events, as well as autoimmune disorders. Consequently, such pathologies result in blindness. With the current global incidence of corneal blindness affecting over 10 million individuals, further research and development in this area of healthcare is crucial. Current approaches addressing corneal blindness include transplantation with cadaveric donor corneas, or, more recently, inserting the ologenTM Biocornea as a temporary patch over a corneal injury until a donor cornea is made available. Several attempts have been made to produce a viable synthetic cornea using collagen-based biomaterials, a few of which will be discussed in this review.

Keywords: Corneal structure; Mechanical strength; Keratoplasty

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Introduction

The intricacies associated with corneal tissue engineering can be understood by considering the multifunctionality of the corneal structure, which facilitates vision by means of lubrication, optical clarity, and mechanical strength [1]. These unique characteristics are imparted by a complex corneal architecture, which largely consists of an intricate alignment of collagen fibrils [1, 2]. Ultimately, synthesizing a biomimetic corneal scaffold becomes notably more daunting in comparison to other tissues. This review will discuss the materials and associated methodologies required to produce a collagen-based corneal implant. The viability of such materials will be assessed by comparing their performance in in-vitro and in-vivo models to native corneal tissue, analysing characteristics pertaining to transparency, mechanical toughness, and grafting potential.

Human Corneal Anatomy

Composed of several interspersed collagen fibrils that focus light onto the retina, as well as a 6-layer body including an air-facing epithelium, Bowman's membrane, stroma, pre-Descemet and Descemet's membrane, and most posteriorly, endothelium, the cornea is an exceptionally complex structure. Comprising 90% of the cornea, the especially complex stroma, a collection of collagens I and trace amounts of type V, VI, XII, XIII, XIV and XXIV collagens, adds to corneal sophistication [3]. This multi-layer-multi-component anatomy imparts great difficulty when attempting to engineer an anatomically identical cornea that would ultimately be suitable for clinical use [4, 5].

Current Approaches in Addressing Corneal Shortages

A current approach addressing corneal donor shortages includes transplantation with full-thickness human corneas via a penetrating keratoplasty, a procedure necessitating access to eye banks; a timely process that is an impediment to swift cornea retrieval in emergency situations [3]. Developments in corneal regenerative medicine are needed to provide solutions for patients with such high-risk grafts. The first level at which corneal regeneration was studied was at the corneal epithelium by Pelligrini et al. in 1997, after which it was concluded that unilateral corneal epithelial defects could be salvaged using limbal grafts derived from the uninjured eye [6].

Keratoprostheses (KPros) serve as alternatives to donor corneas for high-risk grafts, comprised of an optical core that gently

communicates with the host's eye [7]. Commonly used KPros include the Boston KPro and osteo-odonto-keratoprosthesis, however these are not ideal corneal substitutes due to challenges pertaining to the irreversible nature of their insertion, as well associated complications including infection, which can lead to indefinite antibiotic and immunosuppressant use [8]. As evidenced by Sweeney et al., advances in KPros development seek to preserve native corneal functionality, aiming to permit epithelial growth. This is necessary to maintain tear film, as well as prevent infection and implant extrusion [9]. Recently, such advances have become a reality due to improved lithographic and surface chemistry altering techniques [8, 10].

Collagen- Based Implants

The most abundant component of the cornea, utilizing collagen for corneal implants increases implant success due to its similarity to native tissue, which ultimately improves implant grafting potential [3, 11]. The collagen microstructure consists of a tripeptide arginine-glycine-aspartic acid (RGD) sequence, which permits recognition by adjacent integrin receptors and plays an essential role in guiding cell behavior [2, 12]. Small leucine-rich proteoglycans (SLRPs) regulate fibril thickness of corneal collagen fibrils during development to achieve distinctive optical transparency [13]. In order to preserve the functionality of the cornea when developing corneal implants to the greatest extent, it is fundamental to understand how such developmental processes contribute to complex corneal micro-anatomy.

Production methods

Collagen derivation can be categorized based on source and cross-linking methodology. Collagen is derived from animal models via transfection and viral infection [12]. Recombinant human collagen, however, is derived via cross-linking type I and III collagen. Furthermore, cross-linking methods can be separated into those employing physical methods using ultraviolet light or dehydrothermal treatment, transglutaminase for enzymatic crosslinking, or aldehydes, carbodiimides, isocyanate, or genipin to chemically crosslink collagen molecules [12]. It is important to note that derivation and cross-linking methods must be chosen carefully, as toxic degradative products are occasionally generated in the final collagen biomaterial (**Table 1**) [14-24].

In-vitro outcomes

In-vitro collagen-based corneal implant studies have been of particular interest as they provide data on the ratios of biomaterials needed to most resemble native corneal tissue [25, 26]. Co-culture methods, culturing corneal epithelial and fibroblast cells on collagen gels, are used to assess cellular interaction, which directs cell behavior in the native cornea [27]. Likewise, synthetic polymers, such as polyester urethane,

Table 1. Corneal collagen-based implants: a history of advancements promoting corneal regeneration.

Author (s)	Description
Zieske et al., 1994 [14]	Corneal endothelial cells added to corneal 3D construct; enhanced quality of basement membrane
Griffith et al., 1999 [15]	Multilayer tissue of collagen hydrogel, chondroitin sulphate, immortalized corneal cells
	Modified gene expression to incite response to chemical stimuli
Li et al., 2005 [16]	Attenuated NIPAAm polymer using YIGSR peptide copolymerized with bovine collagen I; formed transparent hydrogel that moulded into cornea shape
Duan and Sheardown, 2005/ 2006 [17]	Collagen hydrogel- TERP5 composite grafted on pigs; first corneal tissue to be derived from stromal and epithelial cell growth; improved mechanical properties by substitution of TERP5 with multifunctional dendrimers
Mosser et al., 2006 [18]	Liquid-crystal characteristics of collagen permit control of organization and transparency in dense collagen scaffolds
Torbet et al., 2007 [19]	Development of collagen-based scaffold with orthogonal lamellae, improving corneal mechanical strength
Fagerholm et al.2010 [20]	Human study using recombinant collagen type III artificial cornea with EDC/ NHS; epithelial and partial nerve regeneration and stromal cell growth observed
McLaughlin et al., 2010 [21]	Collagen-MPC implant as full-thickness transplant in guineapig; stimulation of nervous regeneration over 8 months
Karamichos et al., 2014 [22]	HCF produces stromal construct
	HCF-secreting stroma-like ECM composed of collagen type I and V
Sorkio et al., 2018 [23]	Multi-layered structure mimicking stroma
	Bioinks based on recombinant human laminin and human-derived collagen I
	hESC-LESCs used to mimic corneal epithelium
	Human adipose tissue-derived stem cells propagated stroma formation
Shojaati et al., 2018 [24]	Administration of stromal stem cells via compressed collagen to stimulate corneal healing
Majumdar et al., 2018 [13]	Developmentally, corneal collagen fibrils guided by SLRPs: regulate fibril diameter imparting optimal optical transparency
	CDs regulate collagen assembly during vitrification process
	Addition of β CD to collagen vitrigels produces materials with aligned fibres and lamellae, increasing artificial cornea stability

NIPAAm: N-isopropylacrylamide; YIGSR: pentapeptide (Tyr-Ile-Gly-Ser-Arg); TERP5: poly N-isopropyl acrylamide-coacrylic acid-coacryloxysuccinimide; EDC/ NHS: N-ethyl-N'-[3-dimethylaminopropyl] carbodiimide/N-hydroxy succinimide; MPC: 2-methacryloyloxyethyl phosphorylcholine; HCF: Human corneal fibroblasts; hESC-LESCs: embryonic stem cell-derived limbal epithelial stem cells; SLRPs: small leucine-rich proteoglycans; CDs: Cyclodextrins; βCD: β- cyclodextrin.

combined with human stromal stem cells, have been shown to direct cell behavior, specifically, by promoting an orthogonal alignment of collagen fibrils that is necessary for optimal optical transparency [1, 10]. Builles et al. demonstrated that gelation of collagen under a horizontal magnetic field generates scaffolds comprised of orthogonal lamellae of aligned collagen fibrils, replicating the interior structure of the human corneal stroma [19]. This specific collagen orientation also serves to direct keratocyte alignment, which illustrates that mimicking the differentiation pathways observed in native corneal tissue are necessary to promote differentiation in tissue engineered corneas [2]. Synthetic polymers demonstrate valuable in-vitro results due to their tunable properties, which allow them to be manipulated to the standard of native corneal tissue [28] more feasibly. For example, a composite material consisting of a type I collagen hydrogel- chitosan- polyethylene glycol- poly-lactic acid (PLGA) showed increased mechanical and optical properties compared to using synthetic material alone [29]. Therefore, by combining synthetic and natural materials, implants are more likely to bear characteristics of the native human cornea, including improved optical clarity and mechanical properties.

In-vivo outcomes

The use of collagen for corneal implants, irrespective of its production method, has proved very promising in-vivo. A phase 1 clinical study demonstrated that implants remained stable and avascular after a 24-month follow-up on a cross-linked collagen gel scaffold [20]. Interestingly, stromal cell recruitment, tear film restoration, re-epithelialization, and neurogenesis were also observed [20].

Amongst other hybrid collagen-based gels, glutaraldehyde cross-linked collagen, presented with minimal immune rejection when implanted in dogs for 16 weeks and pigs for 12 months. Moreover, full-thickness collagen-based hydrogels promoted both corneal tissue and nerve regeneration when inserted in guinea pigs [29].

More recently, a Swedish-based, phase 1 human clinical study, which included 10 patients affected with keratoconus and stromal scarring, studied the grafting potential of recombinant human collagen-based implants. Although the grafts were stable for four years after the procedure, vision recovery was substandard due to delayed re-epithelialization [26]. For optimal integration and to minimize implant- associated inflammation, colonization by host stromal stem cells is critical. However, challenges pertaining to vascularization and opacification due to host immune response, must be further studied to improve grafting rates in human implant trials [5] (**Table 2**).

Discussion

In-vitro and *in-vivo* animal and human studies have demonstrated the potential and complexity with which collagen-based implants can be engineered to replace native corneal tissue. In-vitro studies have been pertinent to developing biomimetic implants, allowing molecular specifications of native corneal tissues to be identified through manipulation and combinations of various biomaterials. Moreover, the use of collagen in implant production has been substantiated by in-vivo animal studies. Although superior biocompatibility and low immunogenicity render collagen an ideal implantable device, it remains of questionable use due to its inability to adopt characteristics critical to normal corneal function, including optimal light transmittance, mechanical strength, and lubricative properties, longitudinally.

Future *in-vivo* trials should strive to refine implantation techniques to those that are minimally invasive, minimizing associated scarring and poor corneal re-epithelialization rates. Although human clinical trials for such transplants are lacking due to insufficient candidate number, they are likely to increase with the number of individuals affected by corneal blindness. Long-term follow up of human in-vivo studies would be necessary to assess implant success. However, assessing implant success according to patient description could have questionable reliability due to patient bias, degree of sight being a very subjective experience, ranging from mild visual impairment to complete blindness.

Tissue engineering corneal implants suitable for human use is a multi-step process requiring meticulous understanding of corneal anatomy and sophisticated production methods. Consequently, engineering implants is an arduous task that must overcome various challenges concerning biomaterial derivation and production, as well minimizing graft rejection, for the implant to

Table 2. Current corneal implant models and associated developments.

Cell-based, self-assembled corneal constructs [22]	**To increase tissue variety, ascorbic acid is used to stimulate fibroblastic cells to release ascorbic acid, stimulating ECM production by human umbilical cord MSCs; consequently, corneal stroma-like structure is produced**
Decellularized ECM [8]	Developing decellularized implants from cadaveric corneas avoids corneal shortages, but patients remain at risk for immunogenicity and disease transmission; cadaveric corneas are given to emergency patients; if unavailable, ologen Biocornea is used as a temporary seal until donor cornea is available
Implants made from carbodiimide-cross-linked RHCIII [4, 20]	Does not require immune suppression to engraft; in-vivo rabbit models demonstrated that RHCIII-based implants promote neovascularization in severely injured corneas; MPC was introduced in such models, blocking associated neovascularization
Peptide Analogs of ECM [25]	PAs containing RGD cell adhesions from fibronectin used to promote corneal regeneration
	Injected in rabbit models, demonstrated increased keratocyte migration, promoting corneal healing
	Lumican-based PA reported to stimulate collagen production in combination with corneal fibroblasts
Collagen- based implants incorporating antibiotics or NPs releasing acyclovir [7,10, 26]	Collagen-based implants genetically modified to release antibacterial and antiviral agents
ECM: Extracellular matrix; MSCs: mesenchymal stem cells; RHCIII: recombinant human collagen III; PA: peptide amphiphiles; RGD: Arg-Gly-Asp; NPs: nanoparticles.	

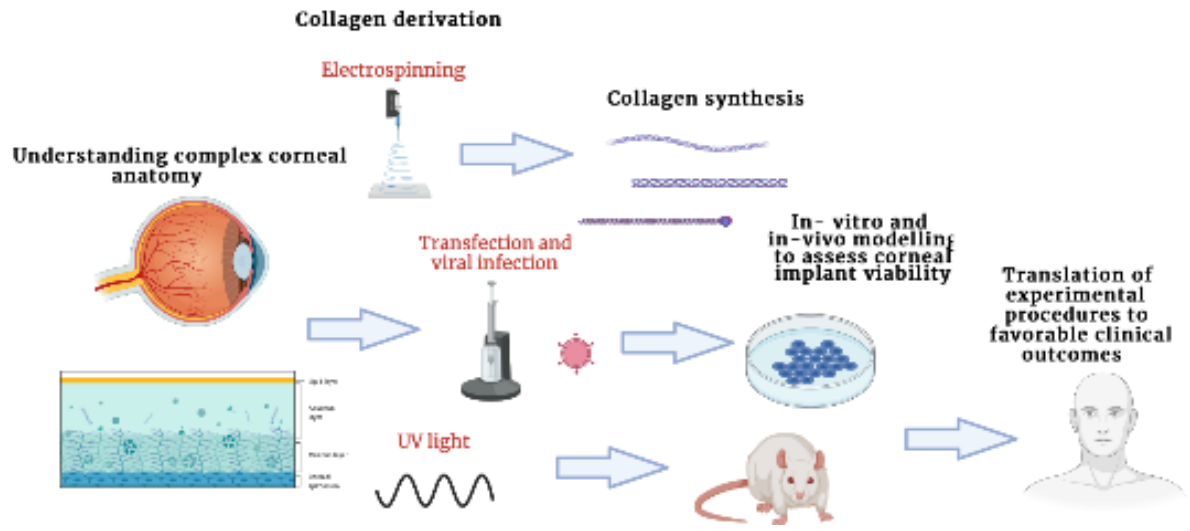

Figure 1 Pathways involved in tissue engineering viable corneal implants.

be able to functionally replace the native human cornea (**Figure 1**).

Clinical limitations and associated challenges

For bioengineered corneas to become acceptable forms of implants, several challenges must be overcome. Intrinsically, bioengineered collagen lacks the mechanical toughness and elasticity required for a longitudinally functional optical apparatus [2, 12]. Although these substandard properties have been improved through collagen cross-linking methods, determining optimal blend ratios of biomaterials to engineer a corneal implant similar to the native cornea remains a daunting process. For example, type I and type III collagen hydrogels have acceptable tensile strength and flexibility for handling, but using type III collagen hydrogels alone for implants better mimics the mechanical and optical properties of the native cornea [11, 20]. Similarly, light transmittance of corneal implants is increased by using natural biomaterials, although such materials are more difficult to electrospin, a commonly employed fiber production method used to improve biomaterial mechanical strength. Consequently, engineering corneal implants without the electrospinning technique decreases their mechanical strength [12]. Therefore, for corneal implants to be of optimal use in the clinical setting, producing implants with as many characteristics to human corneal tissue must be developed. The high costs associated with such complex production processes must eventually also be overcome [30].

Finally, better understanding the molecular complexity of corneal formation, exemplified mainly in the stromal architecture, is pertinent to discovering how to best replicate native corneal tissue.

Conclusion

Insufficient knowledge of corneal developmental processes and precise arrangement of corneal anatomy prevent tissue engineered collagen-based corneas from being routinely used as implants. However, advances in corneal regenerative potential have cascaded since they were studied by Pellegrini et al. As the complexity of corneal architecture is better understood, the possibility of engineering a multi-layered cornea to the standard of its native counterpart is nearly a reality. Producing viable collagen-based corneal implants would eliminate issues concerning global corneal shortages and most importantly, restore one's faculty of sight.

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