Advancements in Bioengineered Corneas for Vision Restoration -A Systematic Review

En Hui Ye[1], En Zhou Ye[2], Joseph Ye[3], and Run Zhou Ye[4,5,6]

[1]Blyth Academy
[2]Affiliation not available
[3]Académie Michèle-Provost
[4]Division of Endocrinology, Department of Medicine, Centre de recherche du Centre hospitalier universitaire de Sherbrooke, Université de Sherbrooke
[5]Department of Ophthalmology, Mayo Clinic
[6]Wilmer Eye Institute, Johns Hopkins University

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En Hui Ye[1], En Zhou Ye[2], Joseph Ye[3], and Run Zhou Ye[4,5,6*]

1 Blyth Academy, Toronto, Ontario, Canada; en.hui.ye@hotmail.com

2 Marianopolis College, Montreal, Quebec, Canada; enzhouye@outlook.com

3 Académie Michèle-Provost, Montreal, Quebec, Canada; joseph.en.ci.ye@outlook.com

4 Division of Endocrinology, Department of Medicine, Centre de recherche du Centre hospitalier universitaire de Sherbrooke, Université de Sherbrooke, Sherbrooke, Québec, Canada; Run.Zhou.Ye@usherbrooke.ca

5 Department of Ophthalmology, Mayo Clinic, Rochester, Minnesota, United-States

6 Wilmer Eye Institute, Johns Hopkins University, Baltimore, Maryland, United-States

* Correspondence: R.Z.Y.: Run.Zhou.Ye@usherbrooke.ca.

ABSTRACT

The quest for vision restoration has witnessed significant advancements in the field of bioengineering, particularly in the development of bioengineered corneas. The cornea, as a critical component of the visual system, plays an essential role in light refraction and focusing, directly impacting visual acuity. In recent years, bioengineered corneas have emerged as a promising solution for patients suffering from corneal blindness due to injury, disease, or congenital defects.

The review presented herein aims to comprehensively analyze and evaluate the latest advancements in bioengineered corneas for vision restoration. Through an extensive search of academic databases and scientific literature, relevant studies and research articles were identified and selected for inclusion in this review. The selected studies cover a diverse range of approaches and methodologies, including tissue engineering, cell-based therapies, 3D bioprinting, and regenerative medicine techniques.

Additionally, this review critically assesses the outcomes of preclinical and clinical studies involving bioengineered corneas, shedding light on the safety, efficacy, and long-term viability of these innovative approaches. Ethical considerations, regulatory hurdles, and potential challenges in large-scale implementation are also addressed.

The findings of this systematic review highlight the tremendous potential of bioengineered corneas in restoring vision for corneal blindness patients. While acknowledging the progress made thus far, it also identifies areas for further research and refinement. The pursuit of effective and accessible bioengineered corneal solutions stands to transform the field of ophthalmology, offering renewed hope for those afflicted with corneal blindness and paving the way for a brighter future in vision restoration.

INTRODUCTION

Corneal blindness is a significant visual impairment caused by various factors, including injuries, infections, degenerative diseases, and congenital defects. The limited availability of donor corneas for transplantation and the risk of graft rejection pose significant challenges in treating corneal blindness. As a result, there is a pressing need for innovative solutions to restore vision in affected individuals.

Bioengineered corneas have emerged as a potential solution to address the challenges of corneal transplantation. These bioengineered corneas are designed to mimic the structure and function of native corneas, offering the promise of stable and effective vision improvement. By using various tissue engineering techniques, cellular therapies, and biomaterials, these innovative approaches hold great potential in restoring visual acuity in individuals with corneal blindness.

The objectives of this systematic review are to comprehensively evaluate and analyze recent advancements in bioengineered corneas for vision restoration. The review aims to gather evidence from clinical studies, preclinical research, and reviews to assess the safety, efficacy, and potential of bioengineered corneas as an alternative to traditional corneal transplantation methods. The scope of this review covers various aspects of bioengineered corneas, including their development, in vivo testing, clinical outcomes, and their role as a potential solution for vision restoration.

METHODS FOR SYSTEMATIC REVIEW

Search strategy

The search strategy for this systematic review aimed to comprehensively identify relevant articles on the advancements of bioengineered corneas. A thorough literature search was

conducted in electronic databases, including PubMed, Web of Science, Scopus, and Google Scholar. The search terms used were a combination of keywords related to "bioengineered corneas," "artificial corneas," "tissue engineering," "corneal transplantation," and "corneal regenerative medicine" [1]. Boolean operators (AND, OR) were utilized to refine the search and ensure the inclusion of all relevant studies up to the date of the search. The search was limited to articles published in English.

Article Selection

Inclusion Criteria: Studies were considered eligible if they reported on the advancements, developments, or applications of bioengineered corneas in human and animal models. Both experimental and clinical studies were included. Additionally, studies that provided information on tissue engineering techniques, biomaterials, cell sources, and regenerative approaches for corneal tissue were also considered for inclusion.

Exclusion Criteria: Articles that were not peer-reviewed, abstracts, conference proceedings, and studies lacking sufficient data were excluded from the review. Studies not related to bioengineered corneas or those focusing solely on non-bioengineered corneal transplants were also excluded.

Quality Assessment

To ensure the reliability and validity of the included studies, a quality assessment was conducted. Two independent reviewers assessed the selected articles using established appraisal tools, such as the Cochrane Risk of Bias Tool for randomized con-trolled trials, the Newcastle-Ottawa Scale

for non-randomized studies, and the ROB-INS-I tool for non-randomized intervention studies. Discrepancies in ratings were re-solved through discussion and consensus between the reviewers.

Data extraction and synthesis

Data extraction was performed independently by two reviewers using a predefined data extraction form. The following information was extracted from each study: author(s), year of publication, study design, sample size, characteristics of the bioengineered cornea (e.g., cell type, scaffold materials), and main findings related to corneal tissue regeneration or transplantation outcomes. Any discrepancies in data extraction were resolved through mutual agreement.

The data from the included studies were synthesized narratively. Thematic analysis was employed to identify common themes and trends in the advancements of bio-engineered corneas. The findings were organized and presented based on the key areas of interest, such as the development of novel biomaterials, cell-based therapies, pre-clinical and clinical outcomes, and challenges in bioengineered cornea implementation.

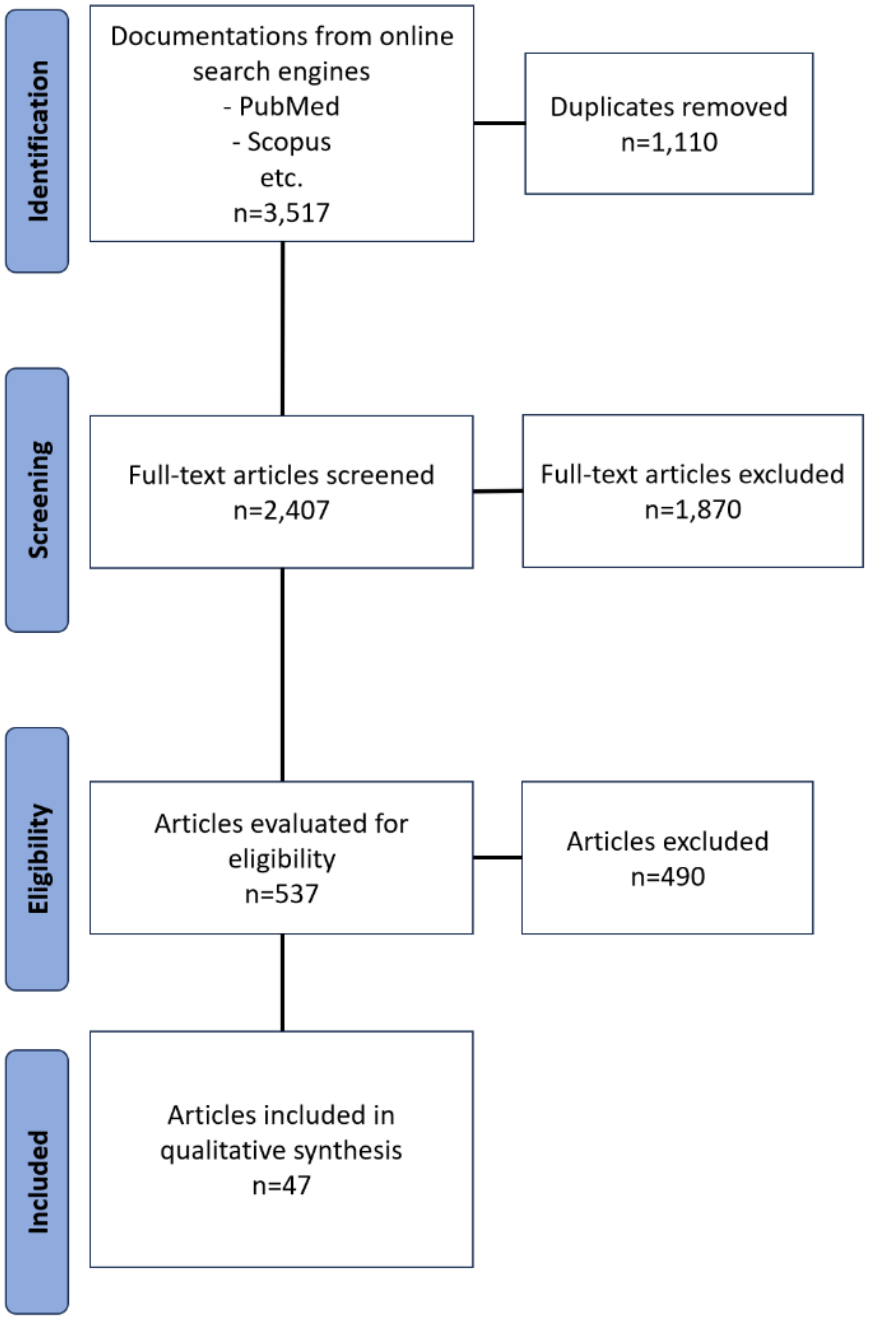

Fig. 1: PRISMA flowchart showing the number of articles and documentation at each step of the systematic review.

Writing the review

The systematic review was prepared following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure the clarity and transparency of the review process. The results of the quality assessment and data synthesis were integrated into the narrative review, providing a comprehensive over-view of the advancements in bioengineered corneas. The review aimed to present an evidence-based analysis of the current state of bioengineered corneas, discuss their potential applications in clinical practice, and identify areas for future research and development.

BIOENGINEERED CORNEA

Anatomy and Function of the Cornea

The cornea is a transparent, avascular tissue that serves as the outermost layer of the eye. It plays a crucial role in the visual system by refracting light and focusing it onto the retina, enabling clear vision. The cornea's transparent nature is vital for allowing light to pass through and reach the retina, which is essential for visual perception.

Corneal transparency is maintained by the precise arrangement of collagen fibers and the absence of blood vessels, which would otherwise cause light scattering and compromise visual acuity. Additionally, the cornea's shape and curvature contribute to its refractive properties, enabling it to bend and focus light accurately onto the retina, thereby facilitating clear vision.

In summary, corneal blindness poses a significant challenge in the field of ophthalmology, necessitating the exploration of innovative solutions. Bioengineered corneas have emerged as a promising approach to restore vision, providing a potential alternative to traditional corneal transplantation methods. This systematic review aims to assess recent

advancements in bioengineered corneas, focusing on their development, in vivo testing, and clinical outcomes, with the goal of furthering the understanding of their potential for vision restoration. Additionally, understanding the cornea's anatomy and function is crucial for comprehending the significance of its transparency and refractive properties in maintaining clear vision.

Causes and Prevalence of Corneal Blindness

Corneal blindness can arise from various causes, including injuries, infections, and degenerative diseases. Studies have highlighted infectious keratitis and uveitis as significant eye conditions leading to corneal blindness [2]. Additionally, corneal disorders such as keratoconus have been recognized as a cause of vision impairment, with advancements in bioengineered corneal tissue offering a promising treatment option [3]. Furthermore, corneal transplantation has been extensively explored as a means to restore vision; however, the scarcity of corneal grafts has led to investigations into alternative solutions like artificial corneas and stem cells [4].

Corneal blindness remains a significant global health concern, impacting public health and contributing to the burden of visual impairment worldwide. The prevalence of corneal blindness varies across different regions, and prevention strategies, early diagnosis, and effective management are critical in reducing the incidence of vision loss [2]. In particular, keratoconus has been recognized as a condition with considerable impact, and the use of bioengineered corneal tissue, such as BPCDX, has shown positive outcomes, offering hope for minimally invasive vision restoration, particularly in regions with limited access to specialized medical care [3]. The scarcity of corneal grafts for transplantation also highlights the need for advancements

in this field and raises awareness of the challenges in providing effective treatments for corneal blindness on a global scale [4].

Current Treatment Approaches for Corneal Blindness

Corneal transplantation, also known as keratoplasty, is a standard and effective treatment approach for corneal blindness caused by various conditions such as infections, trauma, keratoconus, and other corneal diseases. Traditional corneal transplantation techniques involve replacing the damaged or diseased corneal tissue with healthy donor corneal tissue. However, conventional corneal transplantation has its limitations and challenges, which are important to consider in improving treatment outcomes for patients with corneal blindness.

Limitations and challenges of conventional treatments:

Donor Shortages: Traditional corneal transplantation relies on human donor corneas, which are limited in supply. The demand for corneal grafts often exceeds the availability of suitable donor tissue [2].

Graft Rejection: Despite advancements in corneal matching techniques, graft rejection can still occur. The recipient's immune system may recognize the transplanted cornea as foreign tissue and mount an immune response, leading to graft failure [2].

Complications: Conventional corneal transplantation can be associated with complications, such as infection, glaucoma, and cataract formation, which can affect visual outcomes and patient satisfaction [5].

To address these limitations and challenges, recent advancements in bioengineered corneas have shown promise in overcoming donor shortages and improving treatment outcomes for corneal blindness.

Bioengineered corneas, also known as tissue-engineered corneas, offer an innovative approach to address the limitations of conventional treatments. These bioengineered corneas are created using various biomaterials and cell types, aiming to provide alternatives to cadaveric corneas, improve corneal reconstruction, and facilitate nerve regeneration [6]. They can be produced in a laboratory setting, making them more readily available than human donor corneas.

Recent advancements in molecular therapeutics have also contributed to corneal scar treatment. Targeting molecular pathways, such as TGF-β activation and microRNA therapy, holds promise in reducing corneal scarring and improving visual outcomes [7].

Additionally, research on corneal xenotransplantation, particularly using pig corneas, has been explored as a potential solution to address donor shortages. Advancements in this field focus on safety, efficacy, and compatibility of pig corneas for human trials [8].

Moreover, bioengineering approaches have paved the way for corneal regenerative medicine. These approaches involve cell and growth factor delivery, scaffold-based methods, and 3D bioprinting of corneal tissue [9]. Hydrogel materials, in particular, show promise in corneal wound applications and have potential clinical use for corneal substitutes [10].

In specific clinical cohorts, bioengineered collagen implants have demonstrated promise in vision improvement for advanced keratoconus, offering a potential solution for corneal regeneration [3].

In conclusion, recent advancements in bioengineered corneas, molecular therapeutics, and corneal xenotransplantation have shown potential in addressing the limitations and challenges of conventional corneal transplantation techniques. These innovative approaches may pave the way for more effective and accessible treatments for corneal blindness.

Bioengineering Approaches for Cornea Restoration

Corneal disease is a leading cause of visual impairment and blindness worldwide, affecting millions of people. The traditional treatment for corneal blindness is corneal transplantation, which relies on donated corneas. However, the demand for donor corneas far outweighs the supply, leading to a severe shortage and limited accessibility to this treatment [11]. As a result, there has been significant research and advancements in bioengineering approaches for corneal regeneration, aiming to provide effective corneal alternatives and restore vision in affected individuals.

Tissue engineering approaches have shown promise in corneal regeneration. One such method involves the use of a cell-free engineered corneal tissue known as a bioengineered porcine construct, double crosslinked (BPCDX) [3]. In a pilot feasibility study, BPCDX was implanted in 20 subjects with advanced keratoconus, reshaping the native corneal stroma without the need for removing existing tissue or sutures. The 24-month follow-up period demonstrated improvements in corneal thickness, maximum keratometry, and visual acuity. Notably, previously blind subjects regained a mean best-corrected vision and restored tolerance to contact lens wear, highlighting the potential of this approach for vision restoration [3].

Cellular therapies and stem cell-based interventions have also emerged as promising strategies for corneal epithelial defect repair and reconstruction. Human adipose mesenchymal stem cells (ADSC) have been utilized to generate epithelial progenitors via mesenchymal-epithelial transition (MET) for corneal surface reconstruction [12]. In a rat model of limbal stem cell deficiency (LSCD), tissue-engineered MET-Epi cells derived from ADSC demonstrated efficient epithelial healing, suppressed corneal edema, and opacities compared to other control

groups. This study indicates that ADSC-derived epithelial-like cells can potentially be used for autologous epithelial cell-based therapy to address corneal surface disorders [12].

Furthermore, advancements in 3D bioprinting have opened new possibilities for corneal tissue fabrication [11]. 3D bioprinting offers several advantages, such as personalized corneal implants and single or multi-layer corneal equivalents with controllable structure and designed refractive ability. This technology has the potential to overcome the limitations of current corneal alternatives by providing more effective solutions for corneal regeneration [11].

A study on low-energy blue pulsed light-activated injectable materials has also been conducted to restore thinning corneas [13]. These materials, composed of short peptides and glycosaminoglycans, form a hydrogel when exposed to low-energy blue light. The injectable biomaterials have been tested in rat corneas and showed stability in situ without significant inflammation or neovascularization. The ability to rebuild and change the curvature of the cornea tissue using low light intensities makes these materials a promising option for clinical translation [13].

In conclusion, the field of bioengineering corneas for vision restoration has seen significant progress through tissue engineering approaches, cellular therapies, and 3D bioprinting technologies. These advancements offer hope for individuals suffering from corneal diseases and provide potential alternatives to traditional corneal transplantation. However, further research and development are needed to ensure the safety, efficacy, and widespread availability of these innovative approaches for restoring vision in patients with corneal impairments.

Biomaterials and Scaffolds in Bioengineered Corneas

In bioengineered corneas, the choice of biomaterials plays a critical role in providing a suitable substrate for corneal regeneration. One notable type of biomaterial used for corneal substitutes is the bioengineered porcine construct, double crosslinked (BPCDX) [3]. BPCDX is a cell-free engineered corneal tissue that has shown promising results in reshaping the native corneal stroma in advanced keratoconus subjects without the need for tissue removal or sutures. The feasibility study in India and Iran demonstrated improvements in corneal thickness, maximum keratometry, and visual acuity, leading to the restoration of vision in the treated subjects [3].

Another innovative approach involves the development of transparent low-energy photoactivated extracellular matrix-mimicking materials for restoring thinning corneas [13]. These injectable biomaterials are comprised of short peptides and glycosaminoglycans (chondroitin, hyaluronic acid) that assemble into a hydrogel when exposed to low-energy blue light. The biomaterials remain stable in situ without causing significant inflammation or neovascularization, offering a potential solution for corneal thinning conditions [13].

In the quest for suitable biomaterials for corneal regeneration, researchers have explored a wide range of options. Biomaterials that possess sufficient tectonic durability, biocompatibility with cultured cellular elements, transparency, and, in some cases, biodegradability and clinical compliance are considered ideal for corneal substitutes [14]. Synthetic and semisynthetic corneas are being fabricated to model tissue development and disease in vitro and offer promising pharmaceutical screening platforms for regenerative medicine [14].

In addition to the biomaterials used, the design of scaffolds is crucial for successful corneal regeneration. A study introduced a bio-orthogonally crosslinked hyaluronate-collagen hydrogel as a potential scaffold for corneal defect repair [15]. This hydrogel can be applied in

situ without the need for sutures, initiators, or catalysts. It exhibited high water content and excellent light transmittance, making it a promising candidate for biomaterials in corneal regeneration. The mechanical properties, refractive index, morphology, biocompatibility, and corneal re-epithelialization capacity of the hyaluronate-collagen hydrogel were extensively investigated, showcasing its potential for cornea repair and regeneration [15].

In conclusion, biomaterials and scaffolds play a pivotal role in bioengineering corneas for vision restoration and corneal regeneration. The use of bioengineered porcine constructs, light-activated biomaterials, and bio-orthogonally crosslinked hyaluronate-collagen hydrogels are promising advancements that offer potential solutions to the global shortage of donor corneas and the limitations of conventional corneal transplantation. These innovative approaches hold the potential to significantly impact the field of ophthalmology and provide hope for individuals suffering from corneal diseases and impairments. Further research and development in this area will continue to advance the field and bring these bioengineered corneal substitutes closer to routine clinical application.

Advancements in Cell Sourcing and Cultivation

To address shortage and explore alternative treatments, researchers have been working on various bioengineering techniques to develop bioengineered corneal tissues. These advancements aim to provide functional alternatives to human donor corneas and improve the outcomes of corneal transplantation procedures.

In cases of limbal stem cell deficiency, adipose mesenchymal stem cells (ASCs) have been investigated as an alternative autologous cell source for ocular surface reconstruction. Researchers have successfully differentiated ASCs into corneal epithelial lineage using limbal

explant condition media. Additionally, ASC sheets have been developed to facilitate the effective delivery of these cells to the damaged site. This approach offers a novel therapeutic strategy for treating bilateral limbal deficiency conditions and represents a significant advancement in the field of corneal surface therapies [16].

Advances in bioengineered tissue-cellular products have also shown promise for the treatment of corneal diseases. These products include tissue-cellular products that have received regulatory approval, are used off-label in clinical practice, or are in active use in clinical trials [17]. For example, cultivated limbal epithelial transplantation (CLET) offers a promising alternative for the treatment of limbal stem cell deficiency. Engineered tissue matrices and porcine-derived corneas are being explored as potential substitutes for human donor tissue in anterior lamellar keratoplasty and intrastromal transplants for advanced keratoconus [17].

Moreover, researchers are investigating the use of Pax6-induced bone marrow mesenchymal stem cells (BM-MSCs) as a source for generating bioengineered corneal epithelium. By overexpressing Pax6, BM-MSCs can differentiate into limbal epithelial stem cell-like cells, offering a potential treatment option for corneal damage [18].

Despite the progress made in bioengineering corneal tissues, the mechanical properties of these constructs remain critical for their success in clinical applications. Poor biomechanical performance has been associated with the failure of some bioengineered stromal implants in clinical trials [19]. Therefore, future developments in bioengineered corneal tissues should take into account the biomechanical properties to improve their integration and performance in vivo [19].

In summary, advancements in cell sourcing and cultivation strategies have opened up promising avenues for addressing the global shortage of donor corneas and improving treatments

for corneal diseases. From bioengineered porcine constructs to 3D bioprinting technology and the use of Pax6-induced BM-MSCs, researchers are continuously pushing the boundaries of regenerative medicine in ophthalmology. With ongoing progress and further research, these innovative approaches may revolutionize corneal transplantation and offer improved outcomes for patients with corneal diseases.

Bioactive Factors and Growth Promoters

Growth factors and cytokines have also been explored for the treatment of limbal stem cell deficiency (LSCD), a condition that can cause vision loss or blindness. Limbal stem cells play a crucial role in maintaining corneal integrity, and their deficiency can lead to corneal abnormalities [16]. Researchers have investigated the use of adipose mesenchymal stem cells (ASCs) as an alternative cell source for treating bilateral LSCD. ASCs were differentiated into corneal epithelial lineage and developed into cell sheets to facilitate their effective delivery to the damaged site [16]. The use of growth factors and cytokines in combination with ASCs may enhance their regenerative potential and promote the restoration of corneal transparency and function.

Mechanical properties of bioengineered corneal stromal constructs have been identified as critical factors in their success in clinical applications [19]. The incorporation of growth factors and cytokines in the design of bioengineered stromal scaffolds may influence their biomechanical performance and tissue integration, thus affecting their overall success as corneal substitutes.

Furthermore, the development of precision-guided non-surgical therapies that modulate pathological signaling pathways and factors has shown promise in treating corneal disorders,

including corneal scar/haze, inflammation, and angiogenesis [20]. These therapies may involve the use of growth factors and cytokines to regulate cellular processes and promote corneal tissue healing and repair.

Innovative approaches have also utilized human corneal tissues discarded during allogeneic corneal transplantation surgery to construct allogeneic cornea-derived matrix (ACM) scaffolds. These scaffolds contain preserved collagen and glycosaminoglycan levels and serve as a promising source for the fabrication of corneal stromal implants with favorable optical properties and structural strength [21]. Growth factors and cytokines may be incorporated into these scaffolds to enhance their regenerative potential and promote corneal tissue healing and transparency.

Extracellular matrix (ECM)-scaffold-based engineering has emerged as a new perspective in corneal regenerative medicine. Stromal laminar tissues obtained from lenticular-based refractive correction procedures, such as SMall Incision Lenticule Extraction (SMILE), have been investigated as a source of collagen-rich ECM scaffolds for corneal regeneration. These lenticules can be customized and decellularized to serve as acellular scaffold niches for repopulating with cells, including stromal keratocytes and stem cells [22]. The incorporation of growth factors and cytokines within these ECM scaffolds may support cell growth, proliferation, and differentiation, leading to the regeneration of native-like corneal stromal tissue and restoration of corneal transparency.

Overall, the utilization of growth factors and cytokines in bioengineered corneal tissues and scaffolds holds great promise for enhancing cellular proliferation, tissue healing, and ultimately improving corneal regeneration outcomes. By leveraging the regenerative potential of

these bioactive factors, researchers aim to overcome the limitations of traditional corneal transplantation and offer more effective treatments for corneal diseases and visual impairment.

Assessing Efficacy and Safety of Bioengineered Corneas

Before conducting clinical trials in human subjects, preclinical studies using animal models play a crucial role in evaluating the safety and efficacy of bioengineered corneas. These studies provide valuable insights into the potential outcomes and risks associated with the use of these innovative treatments.

One study investigated the use of bioengineered cornea versus human donor cornea in the treatment of fungal corneal ulcers [23]. This preclinical study utilized a porcine acellular corneal matrix as a substitute for human donor corneas. The results demonstrated a significant control rate of infection in both groups, indicating the potential efficacy of the bioengineered corneas in treating fungal corneal ulcers. However, the study also highlighted that the visual acuity and graft transparency were significantly better in the human donor cornea group compared to the bioengineered cornea group, indicating the need for further improvement and optimization in bioengineered corneas.

Clinical trials are essential to evaluate the safety and efficacy of bioengineered corneas in human subjects. These trials provide crucial data on the performance of these substitutes in real-world scenarios and their potential benefits over traditional treatments.

One study focused on evaluating the safety and efficacy of a bioengineered corneal implant using femtosecond laser-assisted anterior lamellar keratoplasty for superficial corneal opacities [24]. The clinical trial included six patients with superficial corneal stromal opacities,

and the results showed improved corrected distance visual acuity, decreased refractive astigmatism, and reduced flat keratometry at 12 months postoperatively. The study demonstrated the effectiveness of the bioengineered corneal implant in improving vision quality and reducing the need for human donor tissue, thus potentially addressing the issue of donor tissue shortage.

Moreover, a review article provided an overview of the advancements in bioengineered corneas, emphasizing their potential as substitutes for human corneas in restoring vision in patients with corneal opacity or irregularities [25]. The review highlighted various approaches, including cell-based therapies, endothelial replacement, 3D printing of the corneal stroma, and cell-free pro-regeneration implants, all of which have shown promise in clinical trials. These innovative approaches offer potential solutions to overcome the global deficit of donor corneal tissue and improve accessibility to treatment for patients in lower-income nations.

Another study evaluated the efficacy of acellular porcine corneal stroma (APCS) transplantation in treating infectious keratitis, focusing on graft survival and risk factors influencing graft failure [26]. The clinical trial involved 39 patients with progressive infectious keratitis who underwent therapeutic lamellar keratoplasty using APCS. The results indicated an increase in visual acuity postoperatively and demonstrated that APCS transplantation can be a viable alternative for eyes with medically unresponsive infectious keratitis. However, the study also identified risk factors for graft failure, such as herpetic keratitis and graft size larger than 8 mm, underscoring the importance of careful patient selection and postoperative management to achieve favorable outcomes.

Overall, the combination of preclinical studies in animal models and clinical trials in human subjects provides valuable evidence for assessing the efficacy and safety of bioengineered corneas as potential substitutes for human donor corneas. While the results are promising,

ongoing research and optimization are essential to fully realize the potential of bioengineered corneas in restoring vision and addressing the global shortage of donor corneal tissue.

Biomechanical and Optical Properties of Bioengineered Corneas

One of the key challenges in developing bioengineered corneas is ensuring appropriate mechanical strength and stability to mimic the natural corneal stroma. Poor biomechanical performance has been identified as a major factor leading to the failure of bioengineered corneal stromal constructs in clinical trials [19].

The corneal stroma is responsible for providing mechanical strength to the cornea, which is essential for maintaining its shape and transparency. It is composed of approximately 200 collagen lamellae that criss-cross in different directions, making up nearly 90% of the corneal thickness [27]. Therefore, any bioengineered cornea must replicate this intricate collagen arrangement and ensure sufficient tensile strength to withstand normal physiological stresses and pressures.

To address this challenge, researchers have been developing various approaches for bioengineering corneal stromal constructs [19]. These approaches involve the use of different biomaterials and fabrication techniques to mimic the natural structure of the corneal stroma. However, the mechanical properties of these constructs must be carefully evaluated and optimized to achieve successful clinical outcomes. Biomechanical testing of these bioengineered corneas is essential to assess their tensile strength, elasticity, and stability under physiological conditions [19].

Furthermore, hydrogel materials have shown promise in replacing partial or full-thickness pathological corneas [10]. With the aid of 3D bioprinting technology, hydrogels can be

shaped to refined and controllable forms, making them attractive candidates for corneal reconstruction research [10]. These hydrogel-based bioengineered corneas must also undergo rigorous biomechanical testing to ensure they possess sufficient mechanical strength to withstand the stresses and forces within the eye.

In addition to biomechanical properties, the optical characteristics of bioengineered corneas are critical for achieving successful visual outcomes. The cornea's transparency and refractive properties are crucial for focusing light onto the retina and ensuring clear vision [27].

One of the main challenges in bioengineering corneas is replicating the corneal transparency, which is dependent on the specific characteristics of the cornea, including its immune and angiogenic privilege [27]. Angiogenic privilege involves a balance of proangiogenic and anti-angiogenic factors to maintain corneal avascularity and transparency. Bioengineered corneas must preserve this immune and angiogenic privilege to avoid complications such as inflammation, neovascularization, and limbal deficiency, all of which threaten corneal clarity [27].

Moreover, bioengineered corneas must be optically transparent to allow the passage of light through the cornea without scattering or aberrations. The corneal curvature and refractive properties must be carefully controlled and optimized to achieve accurate vision correction and prevent refractive errors. Optical coherence tomography (OCT), very-high-frequency ultrasound (VHF-US), and Brillouin optical microscopy have been utilized to assess in vivo biomechanical measurements and characterize the optical properties of the cornea [28].

Therefore, successful bioengineered corneas must not only possess appropriate biomechanical strength and stability but also replicate the optical characteristics of the natural cornea to ensure favorable visual outcomes [27]. To achieve these goals, a comprehensive

understanding of the biomechanical and optical properties of bioengineered corneas is essential, and continuous advancements in measurement techniques and fabrication methods are crucial for further progress in this field.

Immunogenicity and Graft Rejection

Graft rejection is a significant concern in corneal transplantation, whether using donor corneas or bioengineered substitutes. To ensure the long-term viability and success of bioengineered corneas, minimizing immune responses and graft rejection is of paramount importance.

1. Strategies to Minimize Immune Responses and Graft Rejection

One approach to minimize immune responses and graft rejection in bioengineered corneas is through rational peptide design and self-assembly. Peptides, especially collagen-like peptides and peptide amphiphiles, have shown promise in corneal tissue engineering and regeneration due to their hierarchical self-assembling propensity, allowing for the creation of desired nano- to macroscale 3D architecture [29]. By carefully designing and controlling these peptides, researchers aim to create extracellular matrix mimetic implants that reduce the risk of immune reactions and improve tissue integration.

Furthermore, the use of decellularized corneas as scaffolds for corneal remodeling has gained popularity as a potential alternative to donor corneal grafts [30]. However, decellularized corneas can still elicit immune responses due to the exposure of certain antigenic sites after distortion of collagen fibrils during decellularization. To address this, cross-linking decellularized corneas with chondroitin sulfate (CS) has been proposed as a strategy to restore the fibrous matrix's conformation and reduce graft rejection [30]. This cross-linked

decellularized cornea exhibited the least immune response and seamless graft integration in animal studies.

Moreover, advancements in culture, expansion, and molecular understanding of corneal endothelial cells in vitro have been explored to engineer human corneal grafts with healthy endothelium [31]. Corneal endothelial dysfunction can lead to blindness, and transplantation of donor corneas containing healthy endothelium has been the primary treatment. However, there is a severe global shortage of donor corneas, making tissue engineering a potential solution. By understanding the behavior of corneal endothelial cells and optimizing their culture and expansion, researchers aim to develop successful corneal grafts that minimize immune responses and graft rejection.

2. Long-term Viability and Durability of Bioengineered Corneas

The long-term viability and durability of bioengineered corneas are crucial for their clinical success. Several studies have explored the use of various biomaterials and tissue engineering approaches to create functional corneal substitutes.

Alginate-based composites have been investigated as potential biomaterials for corneal regeneration due to their adaptability, transparency, and low immunogenicity [32]. These composites offer the necessary mechanical properties and degradation features to mimic the corneal tissue, and their blending with other functional compounds allows researchers to control their properties and improve their functionality [32]. By optimizing the properties of these biomaterials, bioengineered corneas may achieve better long-term performance.

The use of stem cell therapy and tissue-engineered stem cell scaffolds has also shown promise in corneal regeneration strategies [33]. Stem cells, due to their self-renewal capacity, can be used to regenerate corneal epithelial defects and promote corneal repair. The combination of

stem cells with suitable biological scaffolds, such as amniotic membranes, fibrin, and hydrogels, can provide the necessary signals for stem cell proliferation and differentiation [33]. These innovative approaches offer potential solutions to enhance the long-term viability and durability of bioengineered corneas.

In conclusion, strategies to minimize immune responses and graft rejection, along with optimizing the long-term viability and durability of bioengineered corneas, are crucial aspects of advancing corneal tissue engineering and providing effective alternatives to donor corneas. Continuous research and advancements in biomaterials, stem cell technology, and tissue engineering techniques hold the promise of overcoming current limitations and meeting the growing demand for corneal replacements worldwide.

Ethical Considerations in Bioengineering Corneas

The field of bioengineering corneas presents several ethical considerations that must be carefully addressed to ensure patient safety, respect for donors, and the ethical use of stem cells and tissues.

1. Ethical Challenges in Using Human Cells and Tissues

The scarcity of healthy donor corneas poses significant ethical challenges in corneal transplantation [34]. This shortage raises ethical questions about how to prioritize patients in need and allocate limited resources fairly and equitably.

Additionally, the use of stem cells in bioengineering corneas raises ethical concerns. Pluripotent stem cells, such as embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC), have shown promise in differentiating into corneal endothelial cells (CEC) for corneal regeneration [35]. However, the use of ESCs has faced ethical debates due to the destruction of

human embryos during their isolation [35]. Moreover, iPSCs require viral vectors for their production, raising safety concerns that limit their clinical application [35].

To overcome these ethical challenges, researchers have explored alternative sources of stem cells, such as dental pulp stem cells (DPSC), derived from the patient's own tissues [35]. DPSCs offer a potential solution as they can differentiate into CEC and avoid issues related to immunological rejection and ethical concerns associated with ESCs and iPSCs.

2. Informed Consent and Patient Perspectives

In the context of bioengineering corneas, obtaining informed consent from donors and patients is a critical ethical consideration. Donor corneas that are unsuitable for transplantation in their original state can be recycled and used as carriers for engineered endothelial grafts [34]. Ethical guidelines must be followed to ensure that donor tissues are used in a manner consistent with the donor's wishes and that informed consent is obtained from the donor or their legal representatives.

Moreover, patients undergoing bioengineered corneal treatments should be fully informed about the procedures, potential risks, benefits, and alternatives to make autonomous decisions [2]. Informed consent is essential to respect patients' autonomy and ensure they are aware of the implications of the treatment they are receiving.

Patient perspectives on bioengineered corneas are also critical in guiding ethical considerations. Patients' values, beliefs, and preferences should be taken into account when developing and implementing bioengineered corneal therapies [2]. Understanding patient perspectives helps ensure that the treatments align with patients' needs and expectations and that ethical principles such as beneficence, non-maleficence, and justice are upheld.

In conclusion, ethical considerations in bioengineering corneas encompass the equitable allocation of resources, responsible use of stem cells and tissues, obtaining informed consent from donors and patients, and taking patient perspectives into account. Addressing these ethical challenges is essential to advance the field responsibly and ethically, ensuring the best outcomes for patients with corneal diseases and visual impairment.

Future Perspectives and Challenges

Corneal tissue engineering has shown promising advancements in recent years, offering potential solutions to address the challenges posed by corneal diseases and the shortage of donor corneas. However, several future perspectives and challenges remain to be addressed to fully realize the potential impact of this technology and further enhance its applications.

1. Potential Impact on the Field of Ophthalmology and Vision Restoration

The development of bioengineered corneas has the potential to revolutionize the field of ophthalmology and vision restoration [36]. With over 10 million people worldwide suffering from bilateral corneal blindness, the demand for corneal transplantation far exceeds the supply of healthy donor corneas [37]. Bioengineered corneas offer a promising alternative for patients with high-risk indications and those facing complications with traditional corneal transplantation [38]. These engineered corneas could provide safer and more effective solutions, minimizing the risk of immune rejection and infections [37]. Furthermore, the use of 3D bioprinting technology allows for the creation of customized corneal grafts tailored to individual patients, supporting the concept of personalized medicine [39]. This personalized approach could lead to improved patient outcomes and decreased side effects [39].

In addition to restoring vision, bioengineered corneas hold potential for other applications in ophthalmology, such as ocular drug delivery [40]. 3D bioprinting techniques can be utilized to fabricate drug delivery systems and dosage forms with complex geometries, enabling targeted and personalized treatments for retinal and corneal diseases [40]. This convergence of tissue engineering and drug delivery opens new avenues for therapeutic interventions and improved patient care.

2. Identifying Research Gaps and Areas for Future Investigation

Despite the progress in corneal tissue engineering, several research gaps and challenges remain to be addressed [36]. One of the major challenges is the reconstruction of the complex corneal stroma, which requires optimization of bioprinting approaches to satisfy all demands, including mechanical properties and cell-cell interactions [36]. Current bioprinting techniques show promising results, but further advancements are needed to match the complexity of the human corneal stroma [36].

Another area for future investigation lies in the development of ideal biomaterials for corneal tissue engineering [37]. The choice of biomaterials is critical in ensuring biocompatibility, biodegradability, and proper cell-scaffold interactions [37]. Moreover, the biomechanical qualities of scaffolds should be thoroughly assessed to guide cell migration and behavior, replicating the natural corneal endothelium as closely as possible [37]. Understanding mechanotransduction and how cells respond to biophysical signals from substrates will be essential in optimizing scaffold design [37].

In the context of corneal endothelial repair, research should focus on improving the properties of biomaterials with particular emphasis on biomechanical qualities [37]. Addressing

these research gaps will help create biomaterials that closely mimic the native cornea and provide optimal support for corneal endothelial cell growth in vitro [37].

3. Translation to Clinical Application

Moving forward, the translation of corneal tissue engineering from the laboratory to clinical application poses a significant challenge [36]. The development of bioengineered corneas must undergo rigorous preclinical testing and validation to ensure safety and efficacy before clinical trials [38]. Regulatory approval and ethical considerations regarding the use of human cells and tissues will also need to be carefully addressed [36].

4. Bioengineered Corneas and Early Corneal Blindness Detection

Corneal diseases, such as keratitis, are leading causes of corneal blindness worldwide. Timely detection and treatment of keratitis are crucial to prevent vision loss [41]. However, the shortage of skilled ophthalmologists, particularly in resource-limited settings, poses challenges in early diagnosis [41]. To address this issue, recent advancements in artificial intelligence (AI) have led to the development of a deep learning system that can automate the classification of keratitis and other cornea abnormalities based on slit-lamp images [41]. This AI system exhibits remarkable performance in detecting keratitis, showing comparable sensitivity and specificity to experienced cornea specialists [41]. Moreover, the system can be applied to both digital slit lamp cameras and smartphones, making it accessible for early diagnosis and treatment of keratitis, ultimately preventing corneal blindness [41].

AI has also shown significant potential in the detection and diagnosis of other corneal diseases, including microbial keratitis, keratoconus, dry eye syndrome, and Fuchs endothelial dystrophy [42]. The recent progress in AI-driven diagnostic tools is primarily attributed to the development of deep learning algorithms based on various imaging modalities [42]. These

algorithms have been tailored to differentiate microbial keratitis classes and quantify microbial keratitis features, aiding in more accurate disease detection [42]. For keratoconus, AI has the potential to assist in early detection and staging, enabling timely intervention to preserve vision [42]. Additionally, AI-driven approaches have been developed to detect, segment, and quantify features associated with dry eye syndrome and Fuchs endothelial dystrophy, contributing to improved diagnostic precision and management strategies [42].

In conjunction with AI-driven detection, image segmentation and ROI selection have emerged as crucial techniques for analyzing slit-lamp images to detect corneal diseases [42]. Image segmentation is essential for identifying and delineating specific structures and abnormalities within corneal images [42]. It allows for the precise localization and measurement of features associated with corneal diseases, aiding in their accurate diagnosis and monitoring [42]. Moreover, ROI selection is integral to the analysis of slit-lamp images, as it enables researchers and clinicians to extract useful statistics from specific anatomical regions of interest [43]. The process of ROI selection, when done manually, can be laborious and subject to variability. However, recent advancements in deep learning, such as the DeepImageTranslator (DIT) V2 software, provide a user-friendly tool for multimodal medical image segmentation analysis [43]. This tool combines the preexisting machine-learning features of DeepImageTranslator and allows for the selection of ROIs based on deep learning-generated segmentation maps, facilitating accurate statistics computation for these ROIs [43, 44]. By utilizing AI-driven image segmentation and ROI selection, clinicians can obtain precise and standardized measurements, enhancing the diagnostic process and enabling data-driven treatment decisions [49, 50].

In recent years, single-cell nuclei RNA sequencing (snRNA-seq) has emerged as a powerful and transformative tool in the field of biomedical research. This innovative technique allows for the comprehensive analysis of gene expression profiles at the single-cell level, offering valuable insights into the cellular heterogeneity and functional diversity within various tissues and organs of the human body [45, 46, 47, 48, 51, 52]. In the context of bioengineered cornea research, snRNA-seq has been successfully applied to elucidate the transcriptional landscape of corneal cells, shedding light on their distinct subpopulations and molecular signatures [48]. By leveraging this cutting-edge technology, researchers have gained a deeper understanding of the cellular composition and complexity of the cornea, paving the way for the development of more refined and tailored bioengineered corneas with improved biocompatibility and functionality. Through systematic review of the literature, the application of snRNA-seq on the cornea and other tissues will undoubtedly provide valuable evidence and crucial insights into the advancements in the field of bioengineered corneas, ultimately driving progress towards safer and more effective treatments for corneal diseases and vision restoration.

In summary, bioengineered corneas hold immense promise in transforming the field of ophthalmology and vision restoration. By addressing the challenges and research gaps, this technology has the potential to overcome the limitations of traditional corneal transplantation and offer personalized solutions for patients with corneal diseases. Advancements in 3D bioprinting, biomaterials, and understanding of cell behavior will pave the way for the future of corneal tissue engineering, bringing hope to millions of individuals suffering from corneal blindness worldwide. However, translation to clinical application and ethical considerations remain critical aspects that must be carefully navigated to ensure safe and effective treatments for patients in need.

CONCLUSION

1. Summary of Key Findings and Advancements in Bioengineered Corneas

The systematic review presented herein provides a profound insight into the progress of bioengineered corneas, a vital advancement in vision restoration. Through a detailed analysis of various methodologies including tissue engineering, cell-based therapies, 3D bioprinting, and regenerative medicine techniques, the significant achievements in restoring visual acuity for those afflicted with corneal blindness are outlined. The careful evaluation of preclinical and clinical studies reaffirms the safety, efficacy, and long-term viability of these innovative solutions, while simultaneously drawing attention to areas that require further refinement.

2. Promising Outlook for Vision Restoration through Innovative Approache

The burgeoning field of bioengineered corneas offers a transformative and hopeful path for the future of ophthalmology. By addressing existing limitations in traditional corneal transplantation techniques and advancing the use of biomaterials, cell sourcing, biomechanical optimization, and commercialization strategies, the pursuit of effective and accessible bioengineered corneal solutions has arrived at an encouraging juncture. Ethical considerations, immunogenicity, and regulatory landscapes have been thoughtfully addressed, ensuring a responsible and sustainable approach. Despite the existing challenges, the remarkable progress made underscores the tremendous potential for large-scale implementation and the bright prospect of extending a new lifeline to individuals across the globe suffering from corneal blindness. Further research, collaboration, and technological innovation will only accelerate the realization of this promising frontier, steering us towards a future where vision restoration is not just an aspiration but a widespread reality.

In conclusion, this review not only celebrates the triumphs in the field of bioengineered corneas but also emphasizes the essential path forward. The intricate synthesis of advancements, challenges, ethical responsibilities, and future perspectives forms a holistic view that encourages continued exploration and commitment. The ability to replicate and restore one of the most vital human senses – sight, through bioengineered solutions is no longer confined to science fiction but is an emerging reality, reflecting the unparalleled ingenuity and determination of modern scientific endeavors.

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ADDITIONAL INFORMATION

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