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Review Article

Applications of biomaterials in corneal wound healing

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Abstract

Disease affecting the cornea is a common cause of blindness worldwide. To date, the amniotic membrane (AM) is the most widely used clinical method for cornea regeneration. However, donor-dependent differences in the AM may result in variable clinical outcomes. To overcome this issue, biomaterials are currently under investigation for corneal regeneration *in vitro* and *in vivo*. In this article, we highlight the recent advances in hydrogels, bioengineered prosthetic devices, contact lenses, and drug delivery systems for corneal regeneration. In clinical studies, the therapeutic effects of biomaterials, including fibrin and collagen-based hydrogels and silicone contact lenses, have been demonstrated in damaged cornea. The combination of cells and biomaterials may provide potential treatment in corneal wound healing in the future.

Keywords: bioengineered prosthetic devices; biomaterials; contact lenses; corneal regeneration; hydrogels

1. Introduction to the ocular surface and corneal diseases

As a sense organ of light perception, the ocular surface is the outermost part of the eyes, interacting with the outside environment during most of the daytime and protecting the intraocular tissues. The ocular surface is guarded by the eyelid when the eye is closed and comprises transparent cornea centrally with surrounding conjunctival tissues. The conjunctiva is mainly composed of bulbar, palpebral conjunctiva to cover the sclera and the tarsus. The conjunctiva reflects to form a fornix on three sides and a semilunar fold medially. The epithelium of the conjunctiva is continuous with the cornea at the limbus, where it is viewed as the major niche of corneal stem cells for regeneration of epithelial cells.[1–3] The cornea is the most vital refractive medium in the anterior part of the eye, and it is responsible for two-thirds of the total ocular refractive power. From the outer surface to the innermost cellular layer, the cornea consists of the epithelium, Bowman's layer, stroma, Descemet's membrane, and endothelium.[4]

Ocular surface diseases may be induced by extrinsic infectious pathogens, chemical burn, an intrinsic autoimmune reaction, dysregulation of the tear film, and corneal decompensation. A disrupted ocular surface not only leads to severe irritation but also can result in severe visual loss. Here, we introduce some important diseases of the ocular surface and the cornea.

Infectious corneal diseases are mainly derived from bacteria, fungi, viruses, and other rare pathogen invaginations,

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such as acanthamoeba and microsporidia.[5,6] Even with proper treatment, the complications that follow corneal infection, such as scar formation, corneal thinning, and corneal decompensation, may require surgical intervention in order to restore vision. Penetrating keratoplasty, partial layer corneal graft (lamellar keratoplasty), or endothelial keratoplasty can be performed, depending on which part of the cornea tissue is damaged.[7–9]

Chemical injury to the eye may range from mild irritation to complete destruction of the ocular surface and even loss of the eye. Depending on the offending agent, chemical injury can be divided into acidic or alkaline damage, which have different consequences. When acid come in contact with the eye, the proteins of the conjunctival and corneal tissues will become denatured and precipitated to prevent further intraocular penetration of chemical agents. Thus, it will localize the damage in the superficial layer. By contrast, strong alkalis will cause saponification of fatty acids in the cell membrane and result in cellular disruption. They easily penetrate the corneal stroma and rapidly destroy the proteoglycan ground substance and collagen fibers of the matrix. They may also infiltrate the anterior chamber and induce intense intraocular inflammation and tissue destruction. Both acid and alkaline injury to the ocular surface will possibly cause severe tissue necrosis and inflammation. The extensive conjunctival and corneal limbal epithelial damage will result in poor epithelial healing, neovascularization, scar formation, and fornix shortage. The management of complications after chemical injury aims to restore the normal physiological condition of the ocular surface and vision. Several methods can be performed in the reconstruction of the ocular surface, including autologous conjunctival transplantation, limbal stem cell transplantation (LSCT), autologous cultivated oral mucosal epithelial transplantation, and amniotic membrane (AM) transplantation.[10–12] Penetrating keratoplasty or keratoprosthesis can be considered after the inflammation has been controlled.

Graft-versus-host disease (GVHD) is one of the most severe complications after allogenic hematopoietic stem cell transplantation (HSCT) in patients with mainly hematopoietic dysfunction or hematologic and lymphoid malignancies.[13] In the pathogenesis of GVHD, it is supposed that grafted cells attack the recipient's tissues, such as the skin, lungs, liver, gastrointestinal tract, and eyes. If the intervention of immunemodulators or steroid therapy is inadequate or delayed, inflammatory conjunctiva with or without fibrosis, keratoconjunctivitis sicca from the destroyed lacrimal gland, and corneal scarring due to limbal stem cell deficiency (LSCD) will occur. Some studies demonstrated that cytokines in tear film, such as interleukin-6 (IL-6) and interferon-gamma (IFN-γ), are strongly correlated to the severity of dry eye in GVHD patients.[14] Thus, anti-inflammatory agents may help in the treatment of ocular GVHD. Topical cyclosporine has recently been proposed as an effective agent to control ocular inflammation and dryness in patients with severe dry eyes and chronic GVHD.[15] After stabilization of GVHD treated with anti-inflammatory agents and frequent lubricants, corneal or limbal transplantation with reconstruction of the ocular surface are necessary to rescue vision.[16]

Although the detailed etiology of ocular cicatricial pemphigoid (OCP) or mucous membrane pemphigoid remains unknown, autoantibodies with activated complements are believed to mediate cytotoxic (type II sensitivity) effects on the basement membrane and the subsequent breakdown of the conjunctiva. Some proinflammatory cytokine imbalances have been detected in patients with OCP, such as elevated macrophage migration inhibitory factor and macrophage colony stimulating factor in tear film,[17,18] increasing tumor necrosis factor-α and decreasing IL-6 levels in serum.[19] OCP is clinically characterized to involve the mucous membrane, including the mouth, oropharynx, kidney, gastroenteric tract, and genital tract. Based on the severity of OCP, subepithelial fibrosis (Stage I), loss of goblet cells, fornice shortening (Stage II), symblepharon (Stage III), and surface keratinization and extensive adhesion of the lid to the globe (Stage IV) will occur sequentially. In addition to conventional therapy with topical steroids and lubricants, biotherapies with interferons, immunoglobulins, monoclonal antibodies, rituximab, and mycophenolate mofetil are generally investigated.[20–22]

Erythema multiforme major, the so-called Stevens–Johnson syndrome (SJS), is induced by several medications, including sulfonamides, anticonvulsants, salicylates, penicillin, ampicillin, and isoniazid; or pathogens such as herpes simplex virus, mycoplasma, streptococcus, and adenovirus. The pathogenesis of SJS is the deposition of immune-complex in the dermis and conjunctival stroma, leading to severe desquamation of the skin, subepithelial bullae, fibrosis, and scarring of the conjunctiva. Symblepharon is a severe complication because the necrotic conjunctivas adhere to each other. The main treatment for SJS is supportive care using lubricant, ointment, and prophylactic antibiotic drops along with selective steroid administration to reduce inflammation and protect the cornea and the conjunctiva from drying out or becoming infected.[23] Treatment outcomes of late sequelae with cicatricial changes and severe dry eye have been disappointing. Therefore, early topical treatment in the acute stage is the main goal to reduce the risk of late complications.[24–26]

LSCD may result from congenital diseases, such as congenital aniridia, ectodermal dysplasia, and sclerocornea; or acquired injuries, including chemical burn, thermal injury, chronic contact lens wearing, ocular surgery, and chronic cicatricial conjunctivitis. The re-epithelialization of cornea is based on the availability of healthy LSCs to renew the corneal epithelium and, at the same time, to prevent pannus formation across the cornea from conjunctival vascular intrusion. Maintaining a healthy corneal surface requires 25–33% intact limbus; accordingly, transplantation with autologous, allogenic, and even cadaveric limbal tissue provides convincing effects in restoring the corneal surface.[27,28]

2. The AM and LSCs

The limbus is a unique structure that serves as a barrier between the cornea and the conjunctiva. Cumulated evidence has shown that corneal stem cells reside in the limbal epithelium and possess characteristics intermediate between corneal and conjunctival epithelial cells. The stroma of the LSC niche is full of fibroblasts and melanocytes and is nourished by limbal vasculatures. In the normal corneal renewal and healing process, the basal cells of the limbal epithelium may first differentiate into transient amplifying cells with finite mitotic times and then develop into differentiated cells to replenish the corneal epithelium.

The AM is the covering layer of the fetus and is composed of three parts: the epithelium, the basement membrane, and the stroma. The epithelium monolayer consist of cuboidal cells which rely on their basal lamina. Evidence shows that several growth factors, including epidermal growth factor (EGF), transforming growth factor (TGF)-α, keratinocyte growth factor, hepatocyte growth factor, fibroblast growth factor, TGF-β1, and cytokines are present in the epithelial layer of the amnion.[29] The basement membrane serves as a scaffold in all transplant therapy for epithelial cells to repopulate. The loose connective tissue of the stroma provides its elasticity. Suppression of TGF-β1 and IL-8 were found in AM-based culture of corneal, conjunctival, and limbal fibroblasts, providing evidence that the AM has an inhibiting effect on subconjunctival fibrosis.[30] The stromal layer of the AM also possesses vital anti-inflammatory and anti-angiogenic effects, providing therapeutic effects when applied to the inflammatory corneal surface. The clinical effects of the whole AM include enhanced epithelialization by providing a basement membrane for cell migration, reinforcing adhesion of basal epithelial cells, promoting epithelial differentiation, inhibition of inflammation, neovascularization, and antimicrobial properties. Thus, the AM may be utilized in repairing corneal and conjunctival defects, in reconstruction surgery, and in LSC culture.[9,11,12,24,26]

Although LSCT provides an option for the treatment of LSCD, the shortage of donor tissue is still a major issue for patients with bilateral ocular stem cell deficiency, such as those with OCP and SJS. The finding that autologous LSCs could be cultivated and expanded *in vitro* on the AM suggests a simple method to repopulate corneal LSCs and considerably improve the possibility of LSC therapy.[31]

3. Hydrogels for corneal wound healing

Several types of hydrogels, including collagen, gelatin, alginate, chitosan, and fibrin, are used at the preclinical and clinical stages of therapy for corneal regeneration.[32] Fibrin hydrogels are composed of fibrinogen and thrombin, which have been widely used for various biomedical applications including drug delivery and tissue engineering.[33] Recent studies showed that fibrin hydrogels provide appropriate microenvironments to culture LSCs without losing their phenotype. Fibrin hydrogels increase the survival rate of LSC and progenitor cells during the entire culture period.[34,35] Moreover, fibrin hydrogels can degrade within 24 hours after transplantation, which is important for clinical application.[34] In a clinical study, patients with corneal damage regained useful vision after treatment with LSCs that had been cultured on fibrin hydrogel.[35] Mesenchymal stem cells (MSCs) are currently being investigated in corneal regeneration. MSCs can be differentiated into corneal epithelial-like cells *in vivo* and *ex vivo*. In one *in vivo* study, a rabbit's damaged corneal surface could be successfully reconstructed by delivery of MSCs that had been cultured on fibrin hydrogels. The results suggested that MSCs may participate in the healing process of injured corneal epithelium.[36–38] Collagen is the most abundant protein in the human cornea.[39] In cornea regeneration, collagen hydrogels are mainly used for limbal epithelial cell (LEC) encapsulation. Collagen hydrogels are biocompatible and biodegradable and can maintain the proliferation and differentiation of LECs. However, collagen hydrogels have an inherently weak structure. Recent studies showed that the gel strength of collagen can be increased by crosslinking.[40–42] In clinical studies, corneal re-epithelialization occurred in all patients who had significant vision loss after treatment with a crosslinked collagen hydrogel.[43,44] Gelatin hydrogels have been used as cell carriers, including corneal endothelial and stromal cells, and sustained ocular drug release delivery for cornea regeneration.[45–47] An animal study showed that transplantation of fibroblast precursors combined with gelatin hydrogel into the corneal stroma may promote cornea wound healing.[47] Alginate is an anionic polysaccharide composed of 1,4-linked β-D-mannuronic acid and its C-5 epimer α-L-guluronic acid. Alginate hydrogels have found numerous applications in biomedical engineering, including drug delivery and tissue engineering, however, only a few studies have been performed in the field of ophthalmology.[48,49] Recent studies showed that alginate-based hydrogels can be used for LEC and corneal endothelial cell cultivation. The results suggested that alginate-based hydrogels may have potential in corneal regeneration applications.[50,51] Chitosan is a linear copolymer composed of D-glucosamine and *N*-acetyl-D-glucosamine by (1,4)-linkage. Chitosan-based biomaterials have been investigated for ocular drug and cell delivery.[52–54] A chitosan membrane has been used to promote wound healing and decrease scar tissue formation in a rabbit corneal alkali burn model.[55] Chitosan-based hydrogels combined with induced pluripotent stem cells have been shown to be effective in the treatment of surgical abrasion-injured corneas *in vivo*.[56]

4. Bioengineered prosthetic devices

Corneal transplantation/keratoplasty is a therapeutic intervention used in most patients with corneal blindness. However, the main problem with corneal transplantation is the shortage of donor organs.[57] Several artificial cornea products, including osteo-odonto keratoprosthesis (OOKP), AlphaCor, and Boston keratoprosthesis, have been used clinically in recent years.[57] The Boston type I keratoprosthesis is most widely used clinically. Patients showed rapid visual recovery after implantation of the Boston type I keratoprosthesis.[58,59] Although synthetic keratoprosthesis provides a treatment intervention for patients who suffer from corneal blindness, there still remain some difficulties to overcome for its successful development.[57] In recent years, bioengineered corneas have been developed to mimic the corneal architecture and replace damaged corneas.[40,41,60,61] The ideal bioengineered cornea should be as similar as possible to native corneas, including possessing an epithelialized artificial button, optically functional core, and similar chemical composition, flexibility, and oxygen permeability.[62] Collagen-based implants have been shown to promote cell and nerve repopulation in a rabbit corneal alkali burn model.[60] In a clinical study, the therapeutic effects of collagen-based implants have been demonstrated in 10 patients.[43] Recently, several novel biomaterials were developed for corneal tissue engineering. Recent studies showed that rheological properties of fibrin with 0.1% agarose are similar to the native cornea.[63] The gelatin–chondroitin sulfate scaffold has been used to encapsulate rabbit corneal keratocytes for corneal stromal tissue engineering. The results suggested that the porous structure of gelatin could provide a suitable nutrient transport to the cells and showed good biocompatibility *in vitro*.[64] Arg–Gly–Asp (RGD)-functionalized silk with human cornea fibroblasts have been studied for corneal tissue engineering. The results revealed that the RGD-functionalized silk promoted the attachment, proliferation, and alignment of cells; moreover, the expression of corneal stroma differentiation markers was also increased.[65]

5. Contact lenses and drug delivery

Persistent epithelial defects (PED) of corneas occur when a damaged area of superficial cornea fails to show re-epithelialization in the expected time.[66] There are several types of contact lenses that have been developed to treat PED, including soft, rigid gas-permeable, and scleral contact lenses.[67] Soft silicone hydrogel contact lenses have higher oxygen transmission and water content than those of soft conventional contact lenses, which have gained a lot of attention in recent years.[67,68] The major disadvantage of rigid gas-permeable contact lenses is the size, which is smaller than that of soft contact lenses. The mobility of small contact lens on the eye may increase the risk of abrasions to the cornea, which affect the healing process of PED.[67] Recent studies demonstrated that scleral contact lens could be beneficial in patients with PED. Moreover, the scleral contact lens has been used as an alternative tarsorrhaphy to manage combined exposure and neurotrophic keratopathy.[69] Combinations of cells and contact lenses for ocular surface reconstruction have been studied in patients with LSCD. Limbal-derived epithelium cultured on silicone contact lenses displayed a progenitor-like phenotype. The results of clinical studies showed that re-epithelialization occurred in all patients after treatment.[70,71]

In corneal wound healing, there are several approaches for drug delivery through contact lenses, including soaking, particle-laden contact lenses, molecular imprinting, and ion ligands.[72] EGF has been encapsulated in hydrogel contact lenses to treat abraded corneas of rabbits. The results demonstrated that the overall healing rate in the EGF-treated contact lenses was significantly higher than in control eyes.[73] Hydrogel contact lenses have been used as sustained release carriers of high-molecular-weight polymer for corneal wound healing. Hyaluronic acid (HA) was incorporated in contact lenses through an imprinting method. The results demonstrated that the hydrogel contact lenses sustained the release of HA *in vitro*.[74] A recent study showed that vitamin E-loaded silicone contact lenses can increase the release duration of hydrophilic drugs; moreover, they can decrease the corneal damage that results from exposure to UV light.[75]

Hydrogels
- Collagen
- Gelatin
- Alginate
- Chitosan
- Fibrin

Bioengineered prosthetic devices
- Osteo-odonto keratoprosthesis (OOKP)
- AlphaCor
- Boston keratoprosthesis

Contact lenses
- Rigid gas-permeable contact lens
- Soft silicone hydrogel contact lens
- Scleral contact lens

Fig. 1. Applications of biomaterials in corneal wound healing.

In conclusion, to date, the AM is the most widely used carrier for cell transplantation in corneal wound healing. However, there are still some limitations with the clinical use of the AM. In recent years, novel biomaterials have attracted a great deal of interest in corneal wound healing. Biomaterials have uniform structures and their physical properties can be easily modified. Both collagen and fibrin hydrogels have been used for corneal regeneration in clinical studies. In addition, gelatin-, alginate-, and chitosan-based hydrogels, bioengineered prosthetic devices, and contact lenses are currently under investigation for corneal regeneration *in vitro* and *in vivo*. We have summarized the recent developments of biomaterials in this article (Fig. 1). We hope these promising biomaterials will have great outcomes in treating harmful ocular surface disorders in the future.

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