Review

Research Progress in Corneal Cross-linking Agents

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Abstract

Corneal collagen cross-linking with UVA-riboflavin is currently the only method for preventing the progression of keratoconus from the pathological perspective. Topical application of a direct cross-linking agent is now attracting widespread attention in clinical settings. This article reviews the research progress in the application of indirect or direct cross-linking agents (e.g., riboflavin, glucose, ribose, glutaraldehyde, formaldehyde, glyceraldehyde, short chain aliphatic β-nitro alcohol, and genipin) in the treatment of corneal diseases and analyzes the cross-linking efficacy, toxicity, and merits and disadvantages of each cross-linking agent, providing clinical information for further studies. (***Eye Science 2014; 29:125-128***)

Keywords: cornea; cross-linking agent; keratoconus

A cross-link is a covalent bond that links one polymer chain to another; for example, to consolidate the contraction and stability of a collagen fiber, mainly through physical and chemical cross-linking. Physical cross-linking supplies a low degree of cross-linking, but it could prevent the entry of foreign bodies into collagen. Commonly used physical methods include ultraviolet irradiation and severe dehydration. Collagens with different chemical cross-linking capability could improve the degree of cross-linking, mechanical performance, and biocompatibility. Major chemical agents include glutaraldehye, carbodiimide, diphenyl azidophosphate, and genipin, etc. Different cross-linking methods have varying characteristics and cross-linking techniques have been widely applied in industry and bio-engineering[1-4].

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In recent years, cross-linking has been gradually introduced to treat keratoconus, keratectasia, and other eye diseases.

Keratoconus is a common non-inflammatory, progressive eye disease, which causes formation of bilateral keratectasia lesions and serious injury to visual acuity. Pathological findings revealed that the changes in morphology and arrangement of corneal collagen fibers induce the alteration and thinning of corneal mechanical resistance. Keratoconus mainly occurs during puberty, with an incidence of up to 1/2000. Recently, corneal collagen cross-linking with UVA-riboflavin has emerged as a novel approach for increasing corneal rigidity and preventing the progress of diseases from the pathological aspect. The use of chemical cross-linking is therefore attracting widespread attention in clinical settings.

Indirect cross-linking agent

Riboflavin is a water-soluble vitamin B2 with a molecular weight of 376.37. It is widely distributed in nature and undergoes irreversible decomposition upon exposure to light and ultraviolet irradiation. Thus, riboflavin should be stored in dark. Corneal collagen cross-linking with UVA-riboflavin is a common met photochemical reaction method, which uses riboflavin entering the corneal stroma as a photosensitive reagent. When riboflavin is exposed to ultraviolet radiation with an absorption peak at 370 nm, it generates singlet oxygen and superoxide anion radical, leading to the formation of collagen covalent bonds which prevent the progression of diseases by increasing corneal rigidity and structural stability. This technique does not involve riboflavin as a direct corneal cross-linking agent; instead, riboflavin plays an indirect role in cross-linking following ultraviolet radiation.

Corneal collagen cross-linking with UVA-riboflavin was first proposed by researchers from Dresden University of Technology in 1990s as a novel treatment for progressive keratoconus. In 1997, Spoerl et al. confirmed the potential of corneal collagen cross-linking with UVA-riboflavin and Wollensak et al. conducted further animal studies and clinical trials in 2003 to validate its clinical efficacy. At present, riboflavin has been widely applied in the treatment of keratoconus, keratectasia, and other corneal illnesses in multiple medical institutions around the globe[6-10].

Indirect cross-linking has achieved good efficacy in clinical practice[6,8-10]. However, limitations and adverse events of this technique have also emerged[11-13]: 1.Achievement of an effective riboflavin concentration in the corneal stroma requires removal of the epithelium, which is likely to cause postoperative pain and infection. 2. Keratoconus is characterized by apoptosis of corneal cells, and ultraviolet radiation aggravates the severity of apoptosis, which is the underlying mechanism of the incidence of postoperative corneal edema and corneal haze. 3. To avert ultraviolet-induced corneal endothelial and intraocular tissue injury, only patients with a corneal thickness > 400 μm are able to undergo this treatment. A low osmotic pressure solution of riboflavin was utilized to induce stromal edema and increase corneal thickness before formal treatment. However, the clinical efficacy of cross-linking remains to be investigated. 4. Ultraviolet radiation is likely to cause injuries to corneal limbal stem cells.

A current research focus is transepithelial corneal collagen cross-linking as a means of improving the transepithelial permeability of riboflavin. Clinical applications being investigated include a new penetration enhancer[14], nano-riboflavin[15], ultrasound-guided introduction[16], needle injection[17], and iontophoresis[18-21], *etc*. However, the clinical efficacy of these alternate delivery systems remains to be validated.

Direct cross-linking agent

1. Conventional cross-linking agent

As early as 1998, Spoerl et al. statistically compared the clinical efficacy between chemical and ultraviolet radiation cross-linking and confirmed that aldoses (including glucose and ribose) which play a role in glycation functions also caused a low hardening effect, which required a long time (up to 14 days). Glutaraldehye, formaldehyde, and glyceraldehyde yielded a high efficacy similar to ultraviolet cross-linking, but the toxicity of these compounds may limit their clinical application[22,23].

2. Aliphatic alcohols

Paik et al from Columbia University of New York investigated the safety of a short chain aliphatic β-nitro alcohol in bovine corneal endothelial cells and found a toxicity lower than that seen with alternative medicines such as fluoroquinolone antibiotics, antiproliferation drugs, and benzalkonium chloride, which validated the clinical value of short chain aliphatic β-nitro alcohol in the treatment of corneoscleral diseases[24]. In 2009, the same group conducted further clinical efficacy studies including in vitro cross-linking experiments using short chain aliphatic β-nitro alcohol in fresh porcine corneal strips and corneoscleral grafts. The experimental temperature was 34℃, pH 7.4, and the tissues was immersed for 96 h. Determination of shrinkage temperature indicated that aliphatic alcohols had a higher cross-linking efficacy compared with ultraviolet radiation cross-linking[25]. Nitrate was released in a time-dependent manner and formation of formaldehyde was observed; these compounds were regarded as the triggers for the chemical cross-linking[26].

In 2013, Wen et al. reported permeability values for aliphatic alcohols in rabbit corneal epithelia and thoroughly confirmed that aliphatic alcohols could enter stroma and increase cross-linking efficacy via a transepithelial pathway[27]. Subsequently, Li et al. reported that aliphatic alcohols had chemical stability under storage conditions and evaluated the possibility of applying aliphatic alcohols in clinical practice[28]. Previous in vitro experimental studies investigated the cell toxicity, degree of cross-linking, corneal permeability, and storage stability of cross-linking using aliphatic alcohols and validated that the cross-linking efficacy of aliphatic alcohols was similar to or higher than UVA-cross-linking, yielded mild cell toxicity, and showed good corneal epithelial penetrability. Nevertheless, cross-linking using aliphatic alcohols has a long reaction time and yields yellow products that decreased the penetrability. In vitro an-

imal experiments required that the corneal strip be immersed for 96 h. These methods still require further modification.

3. Genipin

Genipin is a type of natural cross-linking agent extracted from the fruits of Cape jasmine; it shows extremely low cell toxicity and excellent biocompatibility. In 2010, Avila et al. from National University of Colombia found that different concentrations of genipin as cross-linking agents could significantly enhance the biomechanical properties of porcine corneal strips and improve the activity of a bacteria-resistant collagen enzyme[29]. In an experiment conducted in 2012, genipin was administered as eye drops to fresh porcine eyeballs every 5 min for 20 min. The efficacy of cross-linking using genipin was similar to that of UVA-cross-linking and it showed low toxicity to endothelial cells[30]. However, this study failed to observe whether surrounding tissues such as conjunctiva, sclera, and trabecular meshwork were affected by genipin treatment.

4. Advantages and limitations of direct corneal cross-linking

1. A direct cross-linking agent is clearly superior to indirect cross-linking through riboflavin and ultraviolet radiation. Avoids ocular injury caused by ultraviolet radiation; 2. No need to remove the corneal epithelia; 3. Convenient to operate; 4.Corneal thickness is not limited; 5.Feasibility of multiple rounds of treatment.

Nevertheless, cross-linking using direct cross-linking agent is still in a primitive stage and a variety of problems remain to be resolved. One drawback is that chemical cross-linking agents probably lead to adverse events, staining reactions, and biocompatibility. Another problem is the influence of topical application of chemical agents upon tissues surrounding the cornea. Neighboring tissues are subject to the influence of cross-linking agents via the tear film and anterior chamber. Tear film patterns involve the eyelid, meibomian gland, conjunctiva, and lacrimal duct. Anterior chamber patterns affect the trabecular meshwork, lens, and anterior capsule. The presence and severity of cross-linking in surrounding tissues could be a pivotal factor determining the feasibility of application of cross-linking agents clinically.

Prospect and outlook

Currently, cross-linking techniques have taken huge strides forward. The preliminary efficacy of iontophoresis and transepithelial corneal cross-linking with riboflavin and UVA has been evaluated. Direct cross-linking agents, especially natural cross-linking agents, have become a research focus in recent years. A variety of cross-linking agents including genipin, oleuropein, procyanidine, tea polyphenol, and tannic acid which show good biocompatibility and low to even no toxicity, have now attracted widespread attention. If the administration of cross-linking agents could be modified to avoid involvement with surrounding tissues, satisfactory clinical efficacy may be achieved. Consequently, corneal cross-linking stands as an extremely promising technique for the treatment of keratoconus and keratectasia and will be applied globally.

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