• Basic Research •

Comparative analysis of wound healing effect induced by photo-crosslinked adhesive in cornea and conjunctival injuries

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Received: 2026-04-16 Accepted: 2026-07-01

Abstract

● **AIM:** To analyze the healing effect of a new hyaluronic acid (HA) photo-crosslinked ocular adhesive for corneal and conjunctival wounds and evaluate its clinical potential in ocular surface reconstruction.

● **METHODS:** Male New Zealand rabbits were included. A full-thickness corneal wound was induced in the superior corneal limbus, and a 5×5 mm^2 conjunctival flap was dissected while leaving intact the lateral limbus of the cornea. The rabbits were randomly divided into corneal and conjunctival wound groups, each containing three subgroups. Histological findings and transmission electron microscopy (TEM) were used to compare wound healing parameters of inflammation and collagen remodeling at 7 and 14d post-injury.

● **RESULTS:** Thirty rabbits (14–16 weeks old, 3.0±0.5 kg) were included and were randomly assigned to either a corneal injury group (n=15) or a conjunctival injury group (n=15). Each group was further divided into three subgroups: control, enbucrilate, and HA photo-crosslinked adhesive (HPCA) groups (n=5 each). Anterior chamber leakage was observed until the first day in the control group, whereas corneal wound recovery was observed in the other two adhesive groups. Rabbits in the enbucrilate group exhibited significantly higher numbers of infiltrating inflammatory cells compared to HPCA group at both days 7 and 14 post-injury, along with irregular collagen fiber rearrangement. The HPCA group had fewer infiltrating inflammatory cells in the corneal wound area compared to the control (P=0.043, 0.033 on days 7 and 14 post-injury, respectively) and enbucrilate groups (P=0.004, 0.017 on days 7 and 14 post-injury, respectively). It displayed significantly fewer infiltrating inflammatory cells in conjunctival tissue wounds than the other two groups (P=0.043 and 0.033 on day 7 post-injury, both P<0.001 on day 14 post-injury). The regenerated conjunctival tissue in the HPCA group displayed a uniform collagen arrangement, indicating smoother, regular wound healing.

● **CONCLUSION:** The HPCA causes less inflammatory infiltration and more uniform collagen fiber arrangement during corneal and conjunctival tissue wound healing than control and enbucrilate groups, indicating its usefulness for ocular surface reconstruction.

● **KEYWORDS:** ocular injury; corneal wound; photo-crosslinked adhesive; wound healing; rabbit

DOI:10.18240/ijo.2026.11.03

Citation: Jo YJ, Cho MH, Lee JS. Comparative analysis of wound healing effect induced by photo-crosslinked adhesive in cornea and conjunctival injuries. *Int J Ophthalmol* 2026;19(11):2112-2121

INTRODUCTION

The application of tissue-adhesive glues instead of sutures in corneal and conjunctival tissue wounds can reduce surgery time and eliminate suture-related complications and patient discomfort[1-4]. Thus, the demand for the use of adhesive glues in the ophthalmic field has been steadily increasing[5-7]. Extensive research has been conducted on different forms of tissue-adhesive glues that can replace sutures[8]. Tissue adhesive glues are classified into two types, physical adhesives and chemical adhesive glues, based on how they form bonds[9-10]. Physical adhesives form adhesions through physical interactions such as hydrogen and ionic bonding, depending on the components of the adhesive. However, they do not maintain adequate adhesion on the ocular surface in the presence of a relatively weak bonding strength due to constant friction and a

moist environment, limiting their use as adhesive tissue glues for the ocular surface. Therefore, chemical adhesive glues based on chemical bonding or photo-crosslinking can be used as suitable adhesives to maintain adequate tissue adhesion on the ocular surface.

It is necessary to maintain sufficient bonding strength even in a moist environment and withstand physical friction forces such as eyelid blinking. A new tissue adhesive glue that maintains transparency to avoid impairing vision, exhibits excellent biocompatibility, and is biodegradable is required[11]. A transparent form of instant tissue adhesive based on photo-crosslinking bonding has been developed to maintain adequate tissue adhesion on the ocular surface. This medical-grade instant photo-crosslinked adhesive can instantly bond tissue at the required site with a short exposure to low-energy ultraviolet (UV) light (320–500 nm, <5s, <1 J/cm²). Hyaluronic acid (HA) is transparent and highly biocompatible compared with conventional tissue-adhesive glues. The compositional characteristics of HA help with cell proliferation and scar suppression after wound healing[12]. Furthermore, previous *in vitro* experiments have demonstrated that the photo-crosslinked adhesive used in this study rapidly cured with a small amount of light exposure while maintaining sufficient bonding strength and transparency[13]. Therefore, we verified the effect of wound healing on the ocular surface after applying the photo-crosslinked adhesive based on HA formulation to actual ocular surface tissues and assessed its clinical applicability after thorough verification[11,14].

We compared the wound healing effects of traditional treatment materials with photo-crosslinked adhesive materials on corneal and conjunctival wound injuries in rabbits. In addition, we confirmed the wound healing capability and safety of the photo-crosslinked adhesive by analyzing its histological morphology, including transmission electron microscopy (TEM) of the ocular surface tissue.

MATERIALS AND METHODS

Ethical Approval This study was conducted with the approval of the Pusan National University Hospital Institutional Animal Care and Use Committee (No.2020-162). The experimental procedures adhered to the ethical standards of the National Research Council and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Tissue Adhesive Glues and Animal Model Two tissue adhesive glues were used in this study, enbucrilate (Histoacryl®, B. Braun, Germany) consists of monomeric n-butyl-2-cyanoacrylate, which rapidly polymerizes upon contact with tissue fluid. The HA photo-crosslinked adhesive (HPCA; Mw: 100 kDa, DS: >150%, MA:PA=7:3) was provided from SNVia (Busan, Republic of Korea).

The histological wound-healing effects of the HPCA were evaluated in rabbit corneal and conjunctival incisional wound models. Thirty male New Zealand rabbits (14–16 weeks old, 3.0±0.5 kg) were included in the study. The animals were randomly assigned to either a corneal injury group (n=15) or a conjunctival injury group (n=15). Each group was further divided into three subgroups of five rabbits.

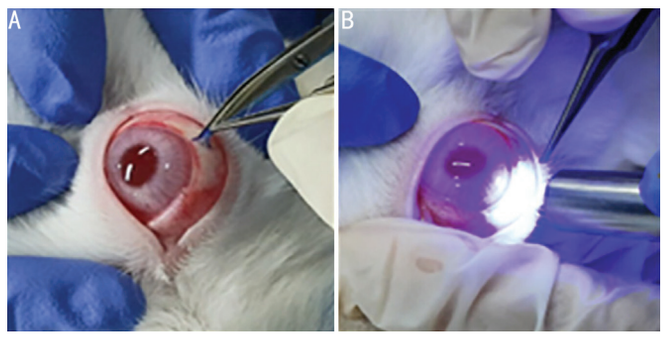


Figure 1 The photo-crosslinking HA glue UV light application device and process A: The conjunctival flap incision was made by Metzenbaum scissors; B: Application of UV light to the conjunctival wound area using the UV application device. HA: Hyaluronic acid; UV: Ultraviolet.

Cornea and Conjunctiva Injury Rabbits were anesthetized by intramuscular injection of ketamine hydrochloride (60 mg/kg) and xylazine hydrochloride (5 mg/kg) before inducing corneal and conjunctival wounds.

Corneal wounds were created by making a 5 mm full-thickness incision at the 12 o'clock position of the superior corneal limbus using a corneal incision knife (BVI; Waltham, MA, USA). In the control group, no additional treatment was applied after wound creation. In the enbucrilate group, the wound area was gently dried with a cellulose sponge. Subsequently, 40 μL of n-butyl-2-cyanoacrylate gel was applied to the wound site for tissue adhesion. In the HPCA group, 40 μL of HA tissue adhesive was applied to the wound site. UV light (365 nm, 35 mW/cm^2) was then applied to the wound site for tissue adhesion. Conjunctival wounds were created using an eyelid speculum and ophthalmic Metzenbaum scissors. A 5×5 mm^2 conjunctival flap was generated at the basal limbus on the lateral side of the cornea. The conjunctival injury groups were divided in the same manner as the corneal injury groups. The light source was positioned 5 cm away from the wound surface during photo-crosslinking (Figure 1).

Tobramycin (Toramicin®; Hanmi, Republic of Korea) eye drop antibiotic was administered thrice daily to prevent infection after inducing corneal and conjunctival wounds, and the rabbits were observed until they were sacrificed.

External Ocular Examination A total of 30 rabbits underwent slit-lamp examinations on days 2, 7, and 14 after inducing the wound to assess the depth of anterior chamber involvement, the formation of anterior chamber inflammation,

and the condition of intraocular conjunctival lesions. Slit-lamp examinations were conducted to assess the recovery status of the corneal wound area and corneal epithelial layer, as well as the presence of corneal leakage using the Seidel test. The degree of conjunctival adhesion was evaluated in rabbits with induced conjunctival wounds; in addition, the inflammatory status at the wound site, formation of neovascularization, and presence of granulation tissue were evaluated.

Histological Examination

Light microscopy examination We next analyzed the histological findings of the cornea, for which rabbits were euthanized on days 7 and 14 post-injury. Their eyes were enucleated and fixed in 10% neutral-buffered formaldehyde for 48h. The fixed tissues underwent paraffin embedding, and 4 μm-thick sections were cut and stained using hematoxylin and eosin staining methods for histological examination. Corneal tissues were observed at 200× magnification to evaluate inflammatory cell infiltration and the organization of the extracellular matrix, including collagen fiber arrangement, among the experimental groups. The conjunctival tissues were examined under an optical microscope at 40× magnification. Different magnifications were used for the cornea and conjunctiva because of their distinct anatomical characteristics. Corneal images were captured at 200× magnification to allow detailed visualization of the corneal layers and cellular responses within the relatively compact tissue structure. In contrast, conjunctival images were obtained at 40× magnification to provide a wider field of view, enabling better assessment of the overall tissue architecture and wound morphology.

Next, we analyzed the extent of inflammatory cell infiltration within the tissues. Optical microscope tissue images of the cornea and induced conjunctival wounds were analyzed using the ImageJ program (ImageJ, National Institutes of Health, Bethesda, MD, USA) to quantify and compare the number of neutrophils on days 7 and 14 post-treatment among the groups. For each specimen, three high-power fields within the central wound area were selected and analyzed. The mean neutrophil count from the three fields was calculated and used for statistical analysis.

Transmission electron microscopy examination Next, the corneal and conjunctival wound tissues were observed under TEM. Corneal and conjunctival tissues were obtained on days 7 and 14 post-wounding and fixed in 2% glutaraldehyde for 4h, followed by TEM processing. The histological processing for TEM followed previously reported methods[15]. After initial fixation, the corneal and conjunctival tissues were rinsed with 0.1 mol/L cacodylate buffer (Electron Microscopy Sciences, PA, USA), followed by secondary fixation with 1% osmium tetroxide (Electron Microscopy Sciences, PA, USA) for 2h. Subsequently, the tissues were dehydrated through a graded ethanol series, treated twice with propylene oxide, and embedded in a mixture of propylene oxide and Embed 812 resin (Electron Microscopy Sciences, PA, USA). The embedded specimens were polymerized overnight at 60°C. Ultrathin sections (80 nm) were prepared using a Diatome diamond knife on a Leica UCT ultramicrotome (Leica Microsystems, IL, USA) for TEM analysis.

The sections were stained with uranyl acetate and Sato's lead citrate (Electron Microscopy Sciences, PA, USA), and the tissue structures were observed at a magnification of 6000× under a TEM to confirm the structural rearrangement of collagen fibers in the corneal and conjunctival tissues.

Statistical Analysis Statistical analyses were performed using SPSS software (version 21.0; IBM Corp., Armonk, NY, USA). Data are presented as mean±standard deviation (SD). Normality was evaluated using the Shapiro-Wilk test. To compare differences in neutrophil infiltration among the experimental groups, using one-way analysis of variance, followed by Tukey's post hoc test for multiple comparisons when appropriate. A P-value <0.05 was considered statistically significant.

RESULTS

External Ocular Surface Findings Among the rabbits that underwent corneal wounds, positive findings were observed in Seidel tests in four out of five eyes in the control group on the day of injury and days 1 and 2 (80%), whereas all 10 eyes in the remaining two groups showed negative findings in Seidel tests. However, a very rough and uneven corneal epithelium condition was observed in the enbucrilate group. No anterior chamber leakage was observed in the Seidel test 7d post-injury, and no significant abnormal findings were observed in the anterior chambers of the two tissue adhesive groups. Iris adhesions were sometimes observed in the corneal wound area of the rabbits in the control group.

Conjunctival attachment was observed in all groups after injury in rabbits with conjunctival wounds; however, severe conjunctival edema was observed in the control group. The conjunctival wound attachment area in the enbucrilate group had a relatively heterogeneous form due to the adhesive, and the treated area by the adhesive material was observed to be applied more extensively compared to the wound. However, the adhesive attachment surface was smooth in the HPCA group, with the adhesive observed only over the wound area. Severe conjunctival congestion and granuloma formation were observed in the enbucrilate group on days 7 and 14 post-injury, with cases of enbucrilate adhesive detachment from the cornea surface observed in one out of five eyes, whereas no cases of conjunctival congestion, granuloma formation, or adhesive detachment were observed in the HPCA groups.

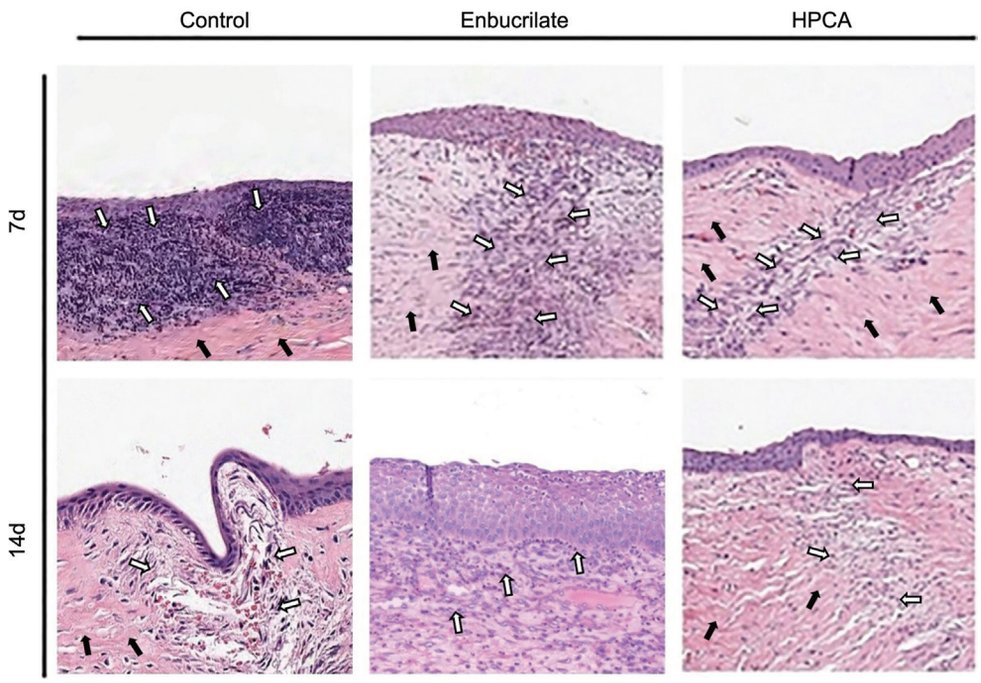


Figure 2 Histological findings of corneal wound healing The hematoxylin and eosin-stained corneal sections from the control, enbucrilate, and HPCA groups at 7d and 14d after corneal incision. Compared with the control and enbucrilate groups, the HPCA group showed fewer inflammatory cells, reduced extracellular matrix edema, and more regular collagen organization. White arrows indicated inflammatory cells, and black arrows indicated collagen distribution. HPCA: Hyaluronic acid photo-crosslinked adhesive.

Histological Findings Ocular tissue extraction was performed on rabbits with corneal and conjunctival wounds at 7d and 14d post-injury to verify the histological differences in wound healing responses. The corneal wounds in the control group reported a significant increase in the infiltration of inflammatory cells, particularly beneath the corneal epithelium, by day 7 post-injury. A partial reduction in the number of inflammatory cells, such as neutrophil infiltration by day 14, yet a relatively uniform distribution of collagen fibers was observed.

Inflammatory cell infiltration beneath the corneal epithelium was observed by day 7 post-injury in the enbucrilate group, with the widest and most severe neutrophil infiltration around the wound site among the three groups. Inflammation cell infiltration beneath the corneal epithelium had gradually decreased by day 14, although the rearrangement of the extracellular matrix collagen appeared somewhat heterogeneous.

Conversely, optical microscopy at 200× magnification on day 7 post-injury demonstrated significantly less neutrophil infiltration beneath the corneal epithelium in the HPCA group compared with the other two groups. Inflammatory cell infiltration beneath the corneal epithelium was mostly absent by day 14, and a uniform distribution of collagen fibers was observed in the healing tissues of corneal wounds. Finally, the photo-crosslinked adhesive facilitated histologically complete attachment to the corneal epithelial layer, causing relatively fewer neutrophils and inflammatory cell infiltration. This caused uniform formation of the extracellular matrix, indicating efficient wound recovery (Figure 2).

On the 7th day after making the corneal wound, compared to control group, the enbucrilate group showed more severe inflammatory response around the corneal wound. The HPCA group revealed less inflammatory reaction and irregular rearrangement of collagen than those of the enbucrilate group (Figure 2). On the 14th day after making the corneal wound, compared to the control group, the enbucrilate group showed less inflammatory response around the corneal wound. The HPCA group showed less inflammation, and relatively uniform arrangement of regenerated collagen than the enbucrilate group (Figure 2).

An analysis was conducted using the ImageJ software to determine the number of neutrophil infiltrations in the obtained corneal tissues. The control group exhibited 964.5±29.0 neutrophils on day 7 post-injury in a high-magnification field of view, whereas the enbucrilate group had 1047.0±38.8 neutrophils. In contrast, HPCA group reported the least number, with 664.0±25.2 neutrophils among the three groups. The control group reported 795.0±25.1 neutrophils by day 14 post-injury, the enbucrilate group had 890.5±19.2 neutrophils, and HPCA group demonstrated the least number with 449.0±21.5 neutrophils.

Statistical comparison among the three groups revealed that the number of inflammatory cell infiltrations in the wound

area was statistically lower in HPCA group compared with the control (day 7 post-injury, *P*=0.043; day 14 post-injury, *P*=0.033) and enbucrilate groups (day 7 post-injury, *P*=0.004; day 14 post-injury, *P*=0.017; Figure 3).

Histological findings of conjunctival wounds in the control group revealed that complete adhesion between the conjunctival tissue and Tenon's capsule did not occur even on day 7 post-injury, and a mesh-like histological structure, along with tissue edema, was observed. Complete adhesion between the conjunctiva and Tenon's capsule was still not achieved on day 14 post-injury, and metaplasia of the conjunctival epithelium, along with an increased density of neovascularization within the conjunctival tissue, was confirmed.

Partial loose mesh-like edema patterns were observed between the conjunctiva and Tenon's capsule in the enbucrilate group on day 7 post-injury, with pronounced metaplasia of conjunctival epithelium and significant neutrophil infiltration around the wound area, which was the most severe among the three groups. Conjunctival edema had decreased by day 14 post-injury; however, metaplasia of the conjunctival epithelium, infiltration of inflammatory cells beneath the conjunctival epithelium, and neovascularization of the extracellular matrix were still observed.

However, partial adhesion between the conjunctiva and Tenon's capsule was observed from day 7 post-injury in the HPCA group, and the infiltration of inflammatory cells beneath the conjunctival epithelium was lower than that in the other groups. No metaplasia of the conjunctival epithelium or neovascularization was observed by day 14 post-injury, indicating histological recovery of the conjunctival tissue comparable to pre-wound conditions (Figure 4).

ImageJ software was used to analyze the inflammatory cell infiltration of the conjunctival tissue. The control group exhibited 878.0±39.7 neutrophils on day 7 post-injury, whereas the enbucrilate group reported 2231.5±62.3 neutrophils. In contrast, the HPCA group demonstrated the lowest number, with 615.0±35.2 neutrophils among the three groups. The control group had 743.0±36.2 neutrophils by day 14 post-injury, the enbucrilate group had 1430.0±73.8 neutrophils, and finally, the HPCA group reported 615.5±63.2 neutrophils, again the lowest among the three groups. Statistical comparison among the three groups revealed that the number of infiltrated inflammatory cells in the conjunctival tissue wounds was significantly lower in the HPCA group than in control and enbucrilate groups on days 7 and 14 post-injury (day 7, *P*=0.043, 0.033; day 14, both *P*<0.001; Figure 5).

Electron Microscopy Analysis The TEM histological differences between the groups were assessed based on corneal and conjunctival wound healing, and the ocular tissue was extracted on days 7 and 14 post-injury.

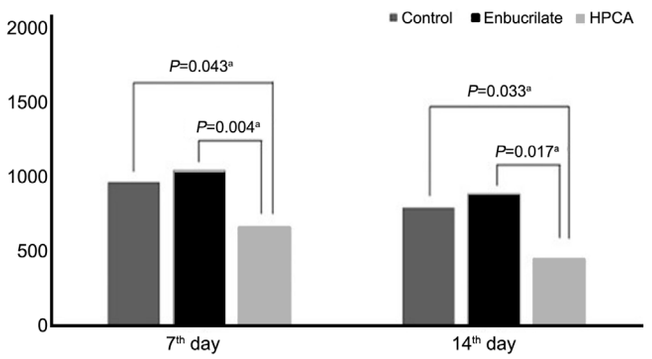


Figure 3 Comparison of infiltrating inflammatory cell counts in corneal tissue on days 7 and 14 after treatment Seven days after creating corneal wound, the HPCA group exhibited a statistically lower cell count than the other groups (*P*=0.043, 0.004). Similarly, on day 14 after treatment, the HPCA group showed a statistically lower cell count compared to the other groups (*P*=0.033, 0.017). HPCA: Hyaluronic acid photo-crosslinked adhesive. [a]Statistically significant difference.

TEM findings of corneal wounds on day 7 post-injury in the control group revealed that the distribution of collagen fibers in the corneal tissue was relatively homogeneous, with partial cytoplasmic vacuolation of corneal stromal cells observed along with infiltration of inflammatory cells. Irregular arrangement and swelling of collagen fibers within the corneal tissue were observed by day 14 post-injury, indicating ongoing regeneration. Numerous curved collagen fibers and infiltration of inflammatory cells were observed on day 7 post-injury around the corneal wound site in the enbucrilate group. By the 14th day post-injury, it was noted as the group with the most severe reshaping of curved collagen fibres and significant infiltration of inflammatory cells among the three groups. The morphological arrangement, thickness, and spacing of collagen fibers showed relatively heterogeneous characteristics in the corneal stroma on day 7 post-injury in HPCA group. However, a decrease in inflammatory cell infiltration within the corneal stroma was observed by day 14 post-injury, maintaining a uniform collagen fiber structure (Figure 6).

Pronounced curvature of collagen fibers and numerous aggregates, along with inflammatory cells, were observed in the conjunctival tissue wounds in the control group on day 7 post-injury. Collagen fibers in the conjunctival tissue were arranged in a relatively regular pattern by day 14 post-injury. Infiltration of inflammatory cells was observed on both days 7 and 14 post-injury, along with an observation of collagen fibers with unclear boundaries in the enbucrilate group. Aggregates and inflammatory cells were observed on day 7 post-injury in the HPCA group. However, compared with the other two groups, a more uniform arrangement of conjunctival collagen fibers was observed. The collagen fibers in the conjunctival tissue were regularly arranged by day 14 post-injury, indicating recovery in fiber distribution and thickness (Figure 7).

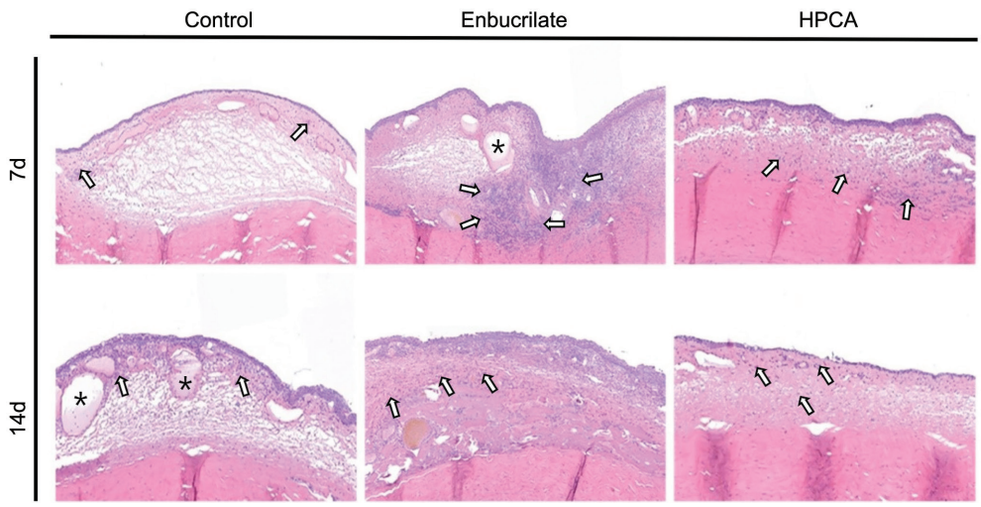


Figure 4 Histological findings of conjunctival wound healing The hematoxylin and eosin-stained conjunctival sections from the control, enbucrilate, and HPCA groups at 7d and 14d after conjunctival incision. HPCA group exhibited less inflammatory cell infiltration and more organized extracellular matrix structure compared with the control and enbucrilate groups. White arrows indicated inflammatory cells, and black star indicated vesicle or bullae formation. HPCA: Hyaluronic acid photo-crosslinked adhesive.

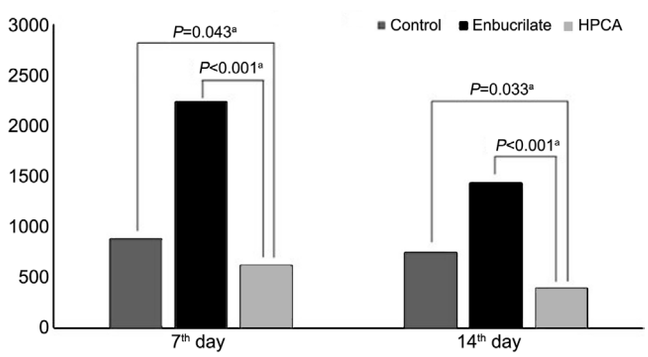


Figure 5 Comparison of infiltrating inflammatory cell counts in conjunctival tissue on days 7 and 14 after treatment Seven days after conjunctival wound creation, the HPCA group exhibited a statistically lower cell count than the other groups (P=0.043, <0.001). Similarly, on day 14 after treatment, the HPCA group showed a statistically lower cell count compared to the other groups (P=0.033, <0.001). HA: Hyaluronic acid. [a]Statistically significant difference.

DISCUSSION

Tissue adhesives maintaining strong adhesion and transparency in a moist ocular surface environment have been increasingly studied through the development of different formulations[16-19]. However, the clinical utility of the widely used ophthalmic tissue adhesive glue remains limited. For instance, the application of enbucrilate, containing cyanoacrylate, is limited by drawbacks such as histological toxicity and potential complications, severely restricting its usage[20]. Furthermore, fibrin tissue adhesives, known for their high biocompatibility, are suitable for application to ocular surfaces with different physical frictional forces owing to their rapid degradation and low tissue stability[21].

The ideal characteristics of tissue adhesives suitable for clinical use on ocular surfaces, such as the cornea and conjunctiva, include several considerations[22-24]. First, these should be convenient to use on ocular surface tissues and polymerize rapidly. Second, these must possess sufficient flexibility to maintain the physiological structure of lesions requiring tissue adhesion while also maintaining appropriate mechanical strength on curved surfaces. Third, these should maintain high bonding strength even on moist ocular surfaces. Finally, these should uphold high biocompatibility on ocular surfaces, minimizing inflammatory reactions and the impact on vision.

Previous research on the therapeutic effects of existing tissue adhesives primarily focused on mechanical strength and bonding strength to ocular surfaces; however, research on histological biocompatibility is lacking[25-26]. Therefore, we developed a novel photo-crosslinked adhesive with appropriate mechanical and adhesive properties, high biocompatibility, and transparency for optical advantages. In addition, we verified its histological therapeutic effects through animal experiments to assess its clinical utility as a tissue-adhesive glue for appropriate applications.

The characteristics of the photo-crosslinked adhesive based on the newly developed instant HA formulation used in this study are multifaceted. The photo-crosslinked adhesive used consisted of a gelatin methacrylate prepolymer, distinguishing it from previously developed formulations. Its characteristic depends on its ability to form network structure of photo-crosslinking chemical bonds upon exposure to weak UV light, thereby imparting physical strength[27].

Experiments conducted using animal models confirmed that the photo-crosslinked adhesive hardened rapidly within 2–3min of exposure to UV light[28]. However, exposure to strong UV light for curing tissue adhesives may cause photodamage to ocular surfaces such as the cornea, conjunctiva, or retina. Nevertheless,

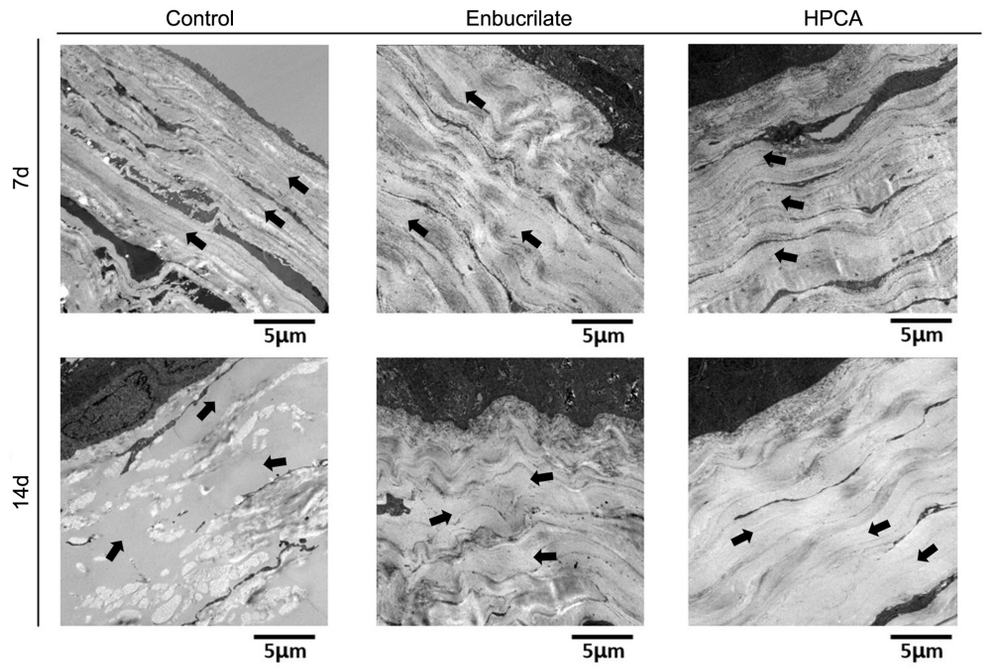


Figure 6 TEM images of the corneal stroma from the control, enbucrilate, and HPCA groups at 7d and 14d after incision In control and enbucrilate groups, the extracellular matrix showed edema, loosening, and irregular collagen fiber arrangement. In contrast, HPCA group demonstrated more regularly arranged collagen lamellae, with less extracellular matrix edema and better collagen reorganization. These ultrastructural findings support improved stromal repair in HPCA group. Black arrows indicated collagen reorganization and extracellular matrix collagen arrangement. Scale bar=5 μm. TEM: Transmission electron microscopy; HPCA: Hyaluronic acid photo-crosslinked adhesive.

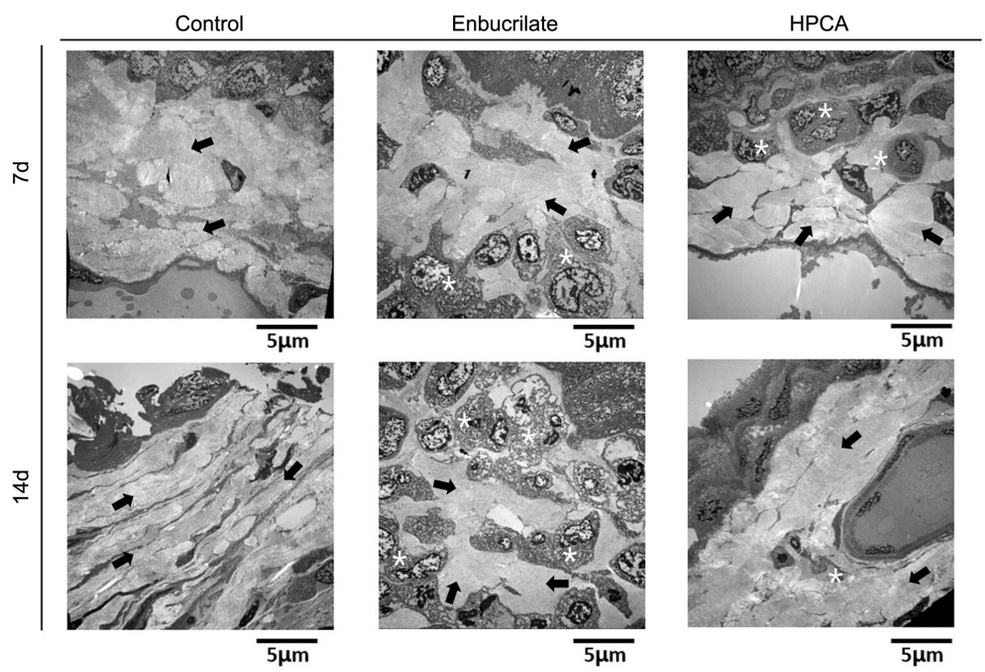


Figure 7 TEM images of the conjunctiva structure from the control, enbucrilate, and HPCA groups at 7d and 14d after incision The enbucrilate groups revealed relatively numerous inflammatory cells and edematous extracellular matrix with irregular collagen organization. The cut surface of the enbucrilate showed consistent with an inflammatory cell, likely a macrophage-like cell. In comparison, HPCA group showed fewer inflammatory cells and a more compact, regularly organized collagenous extracellular matrix, consistent with more favorable tissue remodeling. White stars indicated inflammatory cells, likely a macrophage. Black arrows indicated collagen reorganization and extracellular matrix collagen arrangement. Scale bar=5 μm. TEM: Transmission electron microscopy; HPCA: Hyaluronic acid photo-crosslinked adhesive.

we used a wavelength of 365 nm UV light, which is commonly used in ophthalmic treatment methods such as corneal cross-linking for the management of keratoconus. A lower intensity and considerably shorter exposure time of light were used

compared to those used in treatment, suggesting no stability issues when using UV light on the ocular surface. In addition, we confirmed that no damage to the corneal or conjunctival tissue was observed in the histological examinations of rabbits subjected to UV irradiation using photo-crosslinked adhesive.

Next, it is essential for a tissue adhesive designed for a curved ocular surface to maintain sufficient flexibility to preserve the physiological structure of the lesion requiring tissue adhesion without causing damage while simultaneously maintaining adequate mechanical strength. No structural deformities of corneal or conjunctival lesions due to reduced physical strength were observed in the photo-crosslinked adhesive group while evaluating the strength of the tissue adhesives across groups on ocular surface of rabbits, including a comparison with the widely used enbucrilate adhesive.

Particularly for tissue adhesives intended for use on the ocular surface, characteristics such as blinking and eye movement can create varying shear forces, potentially causing adhesive failure or reduced adhesion capabilities. However, observed cases of adhesive detachment with enbucrilate during wound healing after injury, whereas no cases of adhesive detachment were noted in the instant photo-crosslinked adhesive group. In addition, the photo-crosslinked adhesive formed a relatively smooth and transparent surface when applied to the ocular surface, whereas enbucrilate formed a rough and opaque adhesive surface, making it challenging to evaluate wound healing progress[29].

Furthermore, patients often require therapeutic lens wear to minimize discomfort when using enbucrilate tissue adhesive due to irregularities on the adhesive surface. In contrast, the photo-crosslinked adhesive, primarily composed of HA and water, can maintain a smooth and slippery surface even after application, eliminating the need for therapeutic lens wear on the ocular surface. Moreover, moisture content levels similar to those of the Food and Drug Administration-approved ReSure and Europe-approved OcuSeal for treating corneal incisions during surgery indirectly demonstrate that therapeutic lens wear is not necessary when using photo-crosslinked adhesives[30].

In addition, it is crucial for a tissue adhesive intended to maintain a high bonding strength for its application on a moist ocular surface[14]. The enbucrilate tissue adhesive used in this study solidifies immediately upon exposure to a moist environment, thereby decreasing the adhesive strength and potential detachment from wet surfaces. However, the photo-crosslinked adhesive glue used in this study demonstrated consistent characteristics on moist ocular surfaces without changes, maintaining a high bonding strength until the end of the experiment, which was 14d after UV treatment to initiate photo-crosslinking. No cases of detachment of adhesive components were observed. Enbucrilate tissue adhesive is indicated only when corneal perforations are less than 1 mm in size; leakage due to adhesive detachment from the lesion occurs in 30% to 50% of patients in cases where perforations occur, necessitating re-application using tissue adhesive after the first application or reporting of corneal suturing for treating corneal perforation[20,31].

The adhesive should maintain high biocompatibility on the ocular surface, inducing minimal inflammatory response and minimizing impact on vision[11,32]. Compared to the control and enbucrilate groups, photo-crosslinked adhesive demonstrated more uniform arrangement of remodeling shape on the ocular surface of rabbits.

Corneal and conjunctival tissues of rabbits treated with control, enbucrilate, and photo-crosslinked adhesives were studied and compared using light and TEM prossecing to observe the arrangement and distribution of extracellular matrix collagen fibers alongside the degree of inflammatory cell infiltration. After corneal injury, the most severe inflammation and cell infiltration around the wound site were observed on day 7 in the control and enbucrilate groups, whereas the HPCA group exhibited the least inflammation. Control and enbucrilate groups demonstrated persistent inflammation and loose attachment between the conjunctiva and Tenon's capsule in the conjunctival tissue, generating a mesh-like conjunctival edema. Conversely, the HPCA group displayed improved conjunctival attachment starting from day 7 of treatment, with comparatively reduced inflammation. Previous reports have suggested that higher infiltration of inflammatory cells in the conjunctival tissue may cause fibrosis and granuloma formation[33-34], a finding consistent with the results of the present study. Severe conjunctival edema and neovascularization occurred after 7d of injury in the control and enbucrilate groups, specifically, whereas HPCA group exhibited minimal conjunctival edema and inflammation, causing complete conjunctival attachment and reduced neovascularization. TEM revealed distinctive changes in the extracellular matrix collagen fiber morphology among the three groups, particularly in the corneal and conjunctival tissues, revealing differences in fiber arrangement, diameter, and spacing. Therefore, the use of photo-crosslinked adhesives caused more uniform morphology of collagen fibers after corneal injury compared to the use of control and enbucrilate, suggesting a relationship between the uniformity of collagen fiber curvature and morphological arrangement with peripheral inflammatory cell infiltration. Reduced inflammatory cell infiltration and response to tissue injury possibly contributed to the observed differences in histological outcomes following wound healing[35].

Several limitations of this study should be acknowledged. First, although histological examination, quantitative neutrophil counting, and TEM analysis were performed to evaluate wound healing, collagen-specific histological staining, such as Masson's Trichrome staining, was not included. Therefore, collagen deposition and stromal remodeling were assessed primarily based on collagen fiber organization and ultrastructural findings. Second, immunohistochemical analyses using specific inflammatory and fibrotic markers, such as myeloperoxidase, CD68, and α-smooth muscle actin, were not performed. Although inflammatory cell infiltration was quantitatively evaluated by neutrophil counting, these additional markers could provide more detailed information regarding inflammatory cell populations, myofibroblast activation, and fibrosis during the wound-healing process. Future studies incorporating collagen-specific staining and immunohistochemical analyses may further elucidate the mechanisms underlying ocular surface wound healing following treatment with HPCA.

Another limitation of this study is the absence of normal control groups. Although the primary aim was to compare different wound closure methods under identical injury conditions, the inclusion of normal controls would have provided additional baseline information regarding physiological tissue characteristics and wound-healing responses.

In conclusion, the HPCA used for corneal and conjunctival wound treatment in rabbits offers several advantages over the enbucrilate tissue adhesive. No serious complications or adverse effects were observed. Therefore, we believe that it has clinical utility as an ophthalmic tissue adhesive for reconstructing ocular surfaces following human corneal and conjunctival injuries, similar to rabbit animal models.

ACKNOWLEDGEMENTS

Authors' Contributions: Conceptualization, investigation, and formal analysis: Jo YJ, Cho MH; Methodology: Jo YJ, Lee JS; Writing-original draft: Jo YJ, Lee JS.

Data Availability Statement: The data generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

AI-Generated Content Disclosure: The authors declare that no generative AI was used.

Foundation: Supported by Biomedical Research Institute Grant (No.20230020), Pusan National University Hospital.

Conflicts of Interest: Jo YJ, None; **Cho MH,** None; **Lee JS,** None.

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