



BioHance™

CROSS-LINKED  
HYALURONIC ACID (HA)

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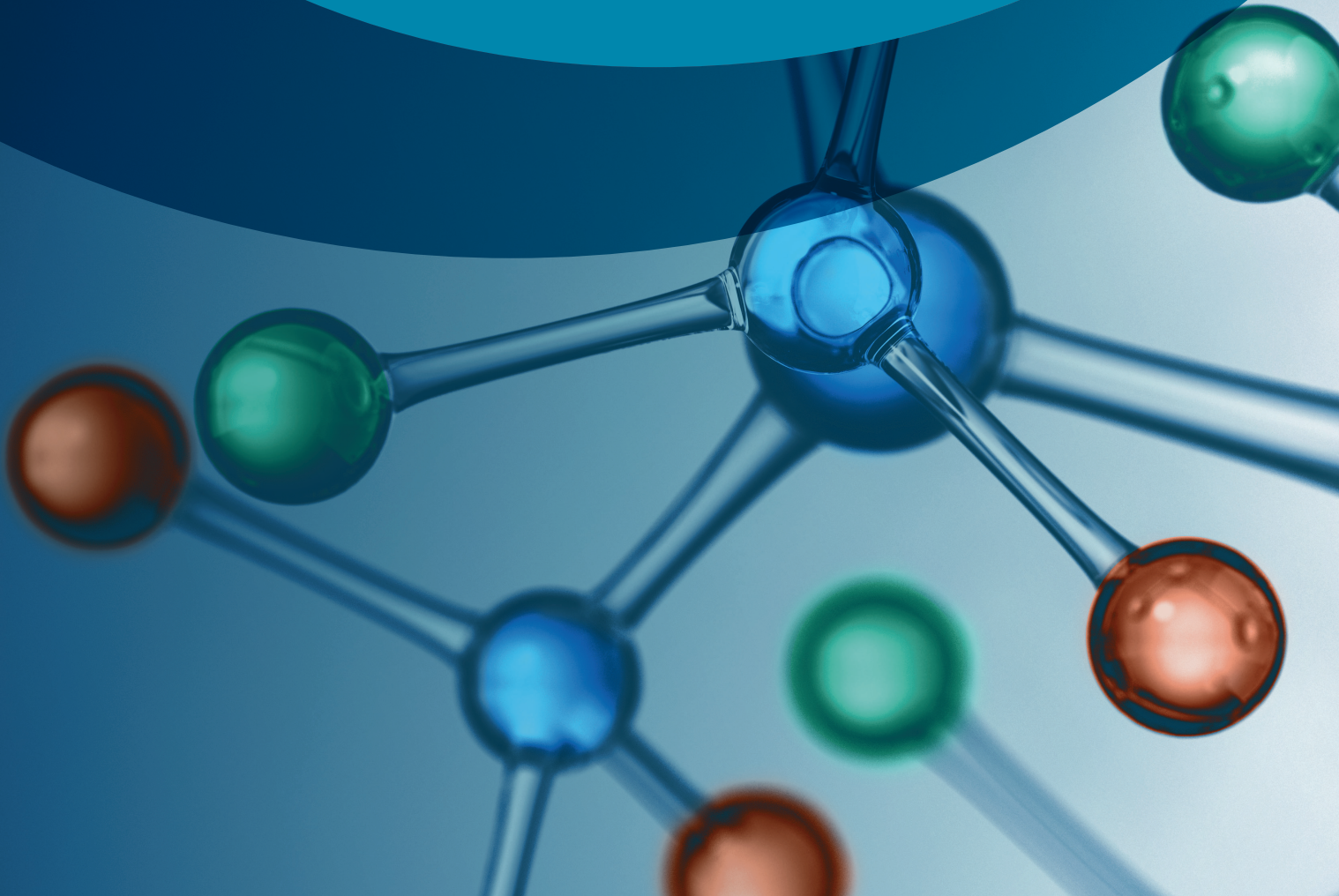
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BioHAnc<sup>TM</sup>

TECHNOLOGY

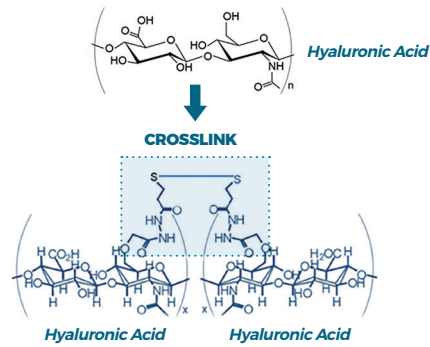


1

## BIOHANCE™ CROSS-LINKED HYALURONIC ACID (HA)

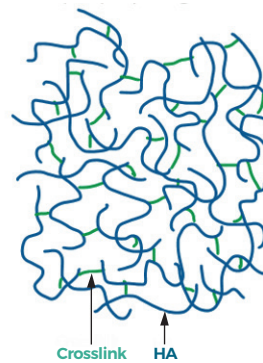
### A patented technology

BioHance™ is a patented technology which uses advanced bioengineering to create a molecular matrix of cross-linked hyaluronic acid (HA). **It produces a molecular scaffolding with unique physical and chemical properties, that enhances hydration, accelerates the body's own healing processes and extends duration on ocular surface.**



### BioHance™ cross-linked HA properties

HA is a naturally occurring substance throughout the body of humans and animals, that plays a key role in hydration, tissue lubrication and healing processes. Before BioHance™, the rapid degradation of naturally occurring HA limited its clinical applications and efficacy in the real world. This patented technology makes it possible to chemically modify the HA so it is **more resistant to degradation, while providing an ideal environment to enhance natural healing processes and unique mucoadhesive properties that extend hydration and lubrication.** The concentration of BioHance™ may vary based on the intended use.



#### Cross-linked hyaluronic acid (HA) hydrogel

BioHance™ technology creates a matrix of cross-linked HA that enhances natural healing

### How our cross-linked HA compares to traditional cross-linked alternatives

Our BioHance™ cross-linked HA is a **purified product containing no side components or toxic by-products with a detrimental or irritating effect.** Traditional cross-linking technologies use a process that can result in poor biocompatibility, trigger an elevated immune response, or inflammation.

### A platform technology

In addition, the chemically functionalized polymers (a molecule made from joining together many small molecules) in this **platform technology can be cross-linked to a variety of other molecules.** Not only does this make BioHance™ effective on its own, it can act as a platform technology that we plan to fully leverage **in the future by incorporating other drug actives** in a unique carrier system.

## 2

### HOW IT IS MADE

BioHAnce™ is created by making two modifications to hyaluronic acid. We purchase medical grade linear hyaluronic acid made by a fermentation process, free of animal products, which is modified and purified prior to use in our ocular formulations. Following the final purification, the solution is sterile filtered and aseptically cross-linked to form the final HA gel product.



## 3

### KEY BENEFITS OF BIOHANCE™ CROSS-LINKED HA

- Enhances hydration, lubrication, ocular comfort and helps stabilize the tear film<sup>1</sup>
- Increases residence time (vs linear HA): lasts 2-5 times longer than traditional artificial tears<sup>2,3</sup>
- Requires less applications (BID), thus improving compliance
- Creates a thin barrier that soothes and protects the eye without altering vision
- 0.75% BioHAnce™ cross-linked HA supports faster corneal reconstruction (vs linear HA): up to 34%<sup>4</sup>
- Prolongs the presence of topical treatments on the ocular surface<sup>5</sup>
- Does not bind to antibiotics (unlike autologous serum)<sup>7</sup>
- BioHAnce™ technology has been created specifically for animal health
- Very good tolerance:
  - Preservative free
  - Thiol cross-linking allows for a purified end product (no residual crosslinker which can be irritating or cause inflammation)

## 4

### AVOID THE «GLOB»

#### Comparison of cross-linked vs non cross-linked HA



**Competitor A**  
*Sticky glob on pet's eye*



**Remend® 0.4**  
*Sheer gel drop that spreads a clear, thin film across the eye after each blink*

- **Cross-linking creates a more viscous lubrication at a lower concentration.** It also has mucoadhesive properties that a linear HA molecule does not. Thus, it stays on ocular surfaces longer and does not get cleared from the eye during blinking like traditional HA eye drops do.

- **Once you cross-link HA, it changes its chemical and physical properties.** Thus, you cannot compare the concentration of a linear HA

product to the concentration of a cross-linked HA product. The cross-linking process effectively creates an infinite molecular weight and thus the physical properties are different.

- The higher the molecular weight of a product, the harder it is to get into solution. **Our cross-linked formulation nearly creates an infinite molecular weight but allows for scaling, purification and sterile filtration.**

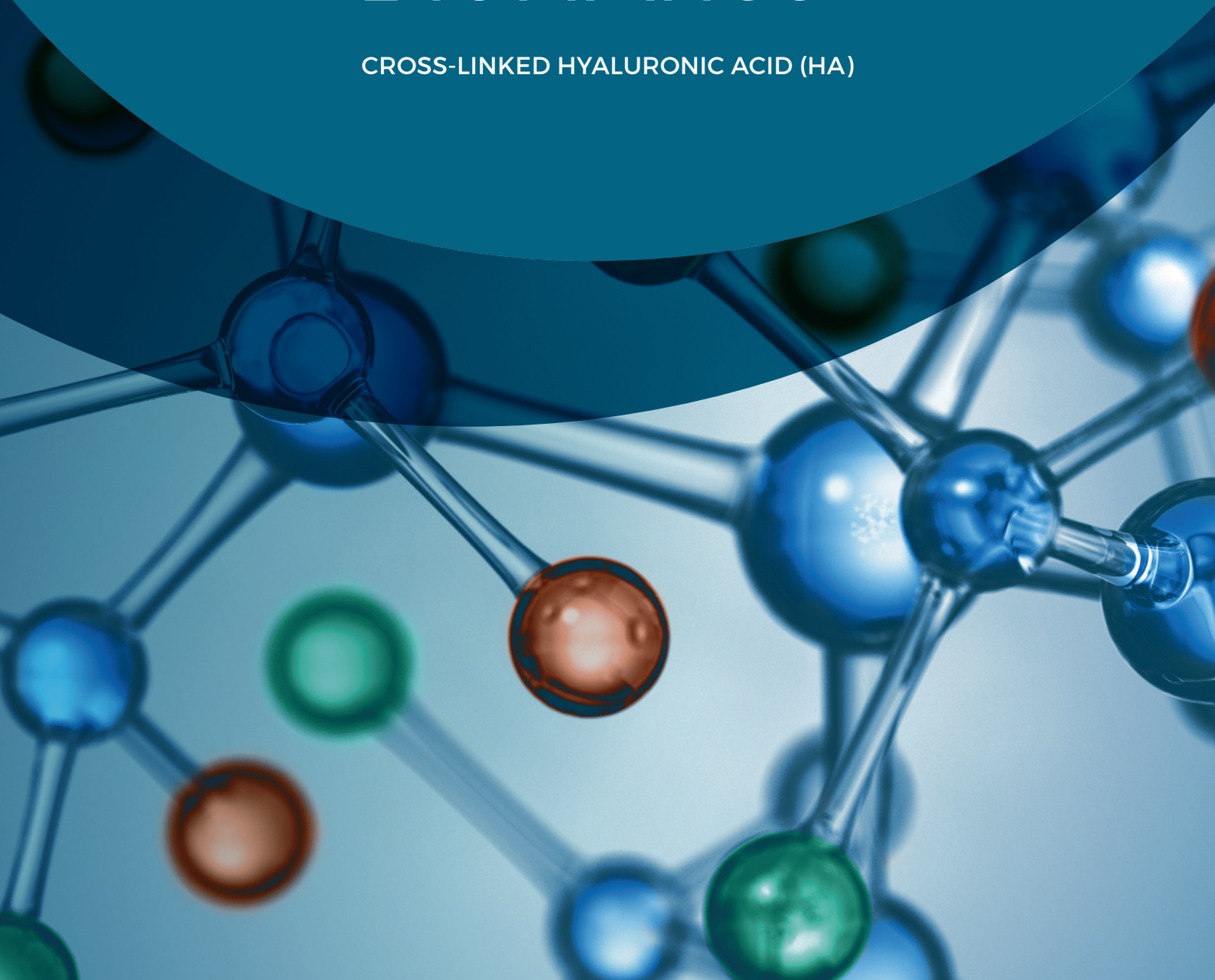


2

STUDIES WITH

BioHAnce™

CROSS-LINKED HYALURONIC ACID (HA)



# 1

## EVALUATION OF TOPICALLY APPLIED CROSS-LINKED HYALURONIC ACID (REMEND®) ON THE OCULAR SURFACE OF CLINICALLY HEALTHY DOGS<sup>1</sup>

ACVO 2022 POSTER SESSION - SUBMITTED FOR PUBLICATION

**Topically applied BioHAnce™ cross-linked HA (Remend®) may improve tear quality, especially tear film stability, in dogs.**

(CE Plummer<sup>1</sup>, BC Martins<sup>2</sup>, C Bolch<sup>3</sup>, PS Martinez<sup>2</sup>, Carbia BE<sup>1</sup>)

1: College of Veterinary Medicine, University of Florida; 2: School of Veterinary Medicine, University of California Davis;

3: Institute for Vision Research, University of Florida

### Purpose

To evaluate effects of a topically applied cross-linked HA (Remend Eye Lubricating Drops® - Bayer Animal Health) on the ocular surface of clinically normal dogs.

### Methods

Twenty dogs with normal ophthalmic examinations received tear ferning tests (TF-M7, TF-M5, and TF-R), Schirmer's tear test (STT-I), tear film breakup time (TFBUT), slit-lamp biomicroscopy, indirect ophthalmoscopy and rose Bengal dye staining (RB) on the first day of examination (day 0), 1 week after initial examination (day 7), and 2 weeks after examination (day 14). Following examination and baseline testing, subjects received cross-linked HA (Remend®) two times a day (BID) on the right eye (OD). The left eye (OS) served as control and received saline BID. Tear fluid samples from both treated and control eyes were evaluated for HA levels by ELISA prior to end at several time points following treatment.

### Results

For the duration of the study, there was no statistically significant difference in aqueous tear production (STT-I) or RB retention between study and control eyes. There was a statistically significant improvement in TFBUT between study and control eyes on Day 7 ( $p < 0.001$ ) and Day 14 ( $p < 0.001$ ). There was a statistically significant improvement in TF-M7 scores ( $p < 0.02112$ ) and TF-R scores by Day 14 ( $p < 0.01097$ ). HA was present in measurable quantities in the tear fluid at 30 minutes and one hour after topical application in treated eyes.

## CONCLUSIONS

Topically applied cross-linked HA may improve tear quality, especially tear film stability, in dogs. Supported by Bayer Animal Health.



2

## FLUOROMETRIC EVALUATION OF CROSS-LINKED VS LINEAR HYALURONIC ACID EYE LUBRICANTS<sup>2</sup>

ACVO 2022 POSTER SESSION - SUBMITTED FOR PUBLICATION

**BioHance™ cross-linked HA exhibited a broader ocular surface coverage and a significantly increased ocular surface contact time (up to 180 min) compared with linear HA (up to 36 min).**

(F Montiani Ferreira<sup>2</sup>, SK Atzet, AD Fankhauser<sup>1</sup>, EK Behan<sup>1</sup>, DJ Haeussler<sup>3</sup>)

1: SentrX Animal Care; 2: Veterinary Medicine Department, Federal University of Paraná; 3: Animal Eye Institute

### ● Purpose

This study evaluated the residence time of linear versus cross-linked hyaluronic acid (XHA) on the canine ocular surface, using covalently labeled fluorescent compound. This allows for evaluation of the actual presence of XHA, as opposed to the bulk medium (water).

### ● Methods

Linear HA and XHA were covalently modified using AlexaFluor 488 reactive moieties. Physical properties of the solutions were also evaluated for concentrations, viscosity and shear thinning profiles. Eye drops were applied to eyes of 18 dogs that were previously assessed and determined to have normal baseline ocular health (STT, slit-lamp biomicroscopy, tonometry and funduscopy). Using a blue light filter (450-490 nm), digital images were obtained, from instillation to 180 minutes. Images were analyzed assessing the percent of the total ocular area covered with green fluorescence at various time points.

### ● Results

All HA samples were successfully modified with approximately 5 mol% Alexa-Fluor. Viscosity varied from 0.4 to 32 Pa-s and all samples exhibited shear thinning. Linear HA quickly migrated to the tear meniscus and could be quantified up to 36 min. XHA exhibited a dual phase behavior: a wide surface coverage first, lasting up to 50 min, then accumulating in tear film meniscus and medial canthus in the second phase, remaining in contact with the ocular surface up to 180 min.

## CONCLUSIONS

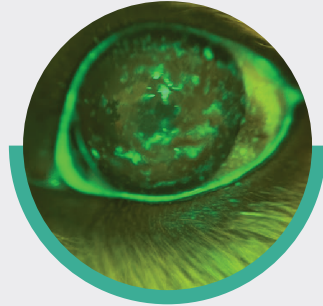
XHA exhibited a broader ocular surface coverage and a significantly increased ocular surface contact time compared with linear HA. Not only could this indicate extended lubrication but, potentially, could be used as a topical sustained-release drug application method.

Supported by SentrX Animal Care.



BioHance™ cross-linked HA exhibited a dual phase behavior: a wide surface coverage first, lasting up to 50 min, then accumulation in the tear film meniscus and medial canthus in the second phase, allowing contact with the ocular surface for up to 180 min.

1<sup>ST</sup> PHASE Wide surface coverage



BioHance™ cross-linked HA (0.75%)

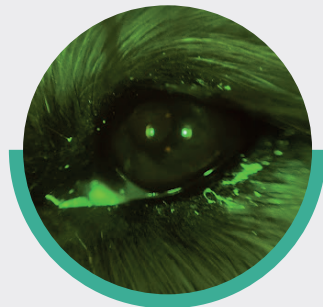
0 min



BioHance™ cross-linked HA (0.75%)

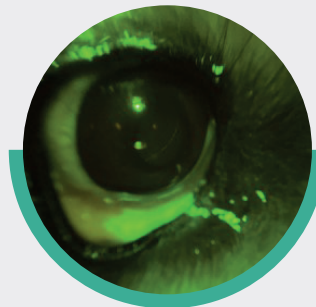
5 min

2<sup>ND</sup> PHASE Medial canthus deposit phase (slow release)



BioHance™ cross-linked HA (0.75%)

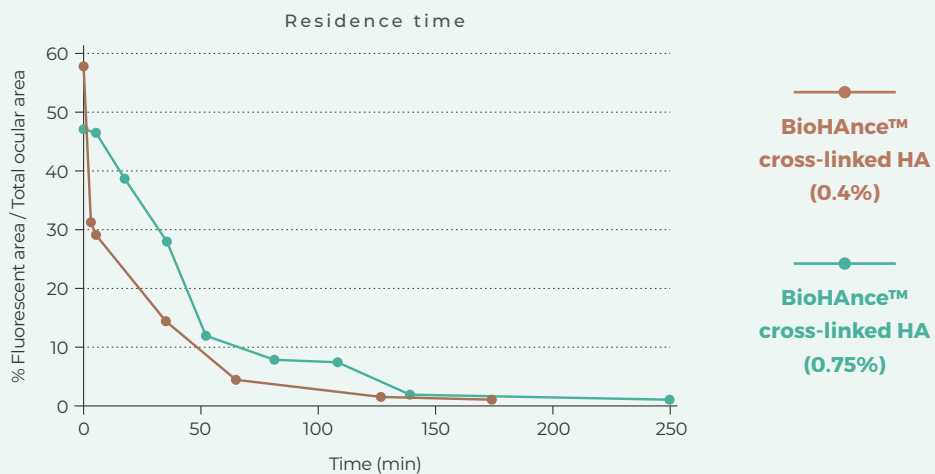
180 min



Everted eyelid showing deposit  
BioHance™ cross-linked HA (0.75%)

180 min

Additional privately held study performed by SentrX Animal Care with healthy dog eyes



3

## PROOF OF CONCEPT STUDY COMPARING HEALING RATES OF BIOHANCE™ CROSS-LINKED HYALURONIC ACID HYDROGEL VS STERILE SALINE AND AMNIOTIC EYE DROPS

PREPUBLICATION DATA ACVO 2022

**In this proof-of-concept study, BioHance™ cross-linked HA showed reduced healing time (30-50%) in comparison to sterile saline drops and amniotic eye drops in a murine model with a standardized central corneal epithelial injury. This result warrants further study into the comparative efficacy of hydrogels in corneal healing time in dogs.**



### Introduction

Hyaluronic acid and amniotic-based hydrogels have been shown to enhance corneal healing. Considering the importance of faster reepithelialization in corneal repair and patient comfort, the aim of this study was to compare the efficacy of BioHance™ cross-linked HA vs sterile saline and amniotic eye drops as well as to understand study group size to power future work.

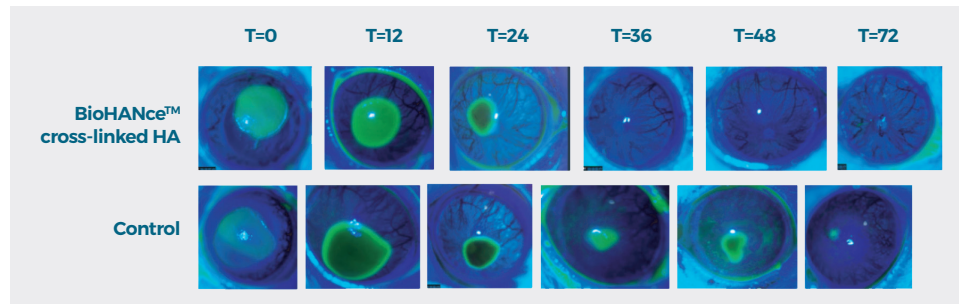
### Materials & Methods

Efforts were made to minimize the pain and discomfort of the rats according to the guidelines of the Association for Research in Vision and Ophthalmology (ARVO) Statement for Use of Animals in Ophthalmic and Vision Research. In addition, the investigation was approved by «Pequeno Príncipe Hospital Complex's Ethics in Animal Use Committee, Curitiba-PR, Brazil». In this model five rats were anesthetized via intramuscular administration of 10% ketamine hydrochloride at a dose of 50mg/kg and 2% xylazine hydrochloride at a dose of 10 mg/kg. They also received topical application of 0.4% oxybuprocaine hydrochloride eye drops. Following anesthesia, superficial keratectomy surgery was performed on both eyes.

The size of the corneal epithelial injury was pre-established and performed using a corneal trephine 3.0 mm in diameter. The area bounded by the trephine was then de-epithelialized with a corneal diamond burr (AlgerBrush II), thus generating a central corneal defect, as previously described by Portela 2021<sup>6</sup>. Corneal defects were then evaluated by fluorescein staining and imaged under a blue light immediately following the surgery (time point T=0), then at 12, 24, 36, 48, and 72 hours. Each animal had one eye that served as a control and received sterile saline drops at the same frequency as hydrogel drops. The other eye, the treated group, received 1 drop twice daily of BioHance™ cross-linked HA as prepared as previously described and provided in sterile eye drop bottles.

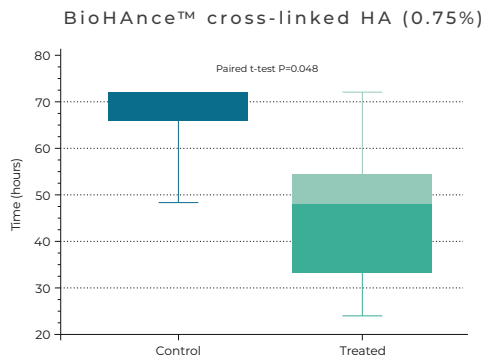
## Data Analysis & Results

- The cornea was considered healed when there was no fluorescein staining on the corneal surface (Figure 1).

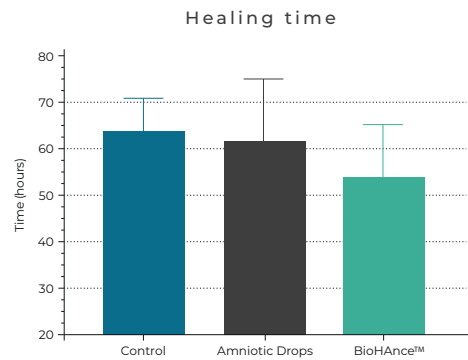


**Figure 1.** Fluorescein imaging of corneal defects treated with cross-linked hyaluronic acid compared with control (no treatment) immediately following surgery in a rat. T=0 and at time points of 12, 24, 36, 48, and 72 hours.

- A paired t-test statistical analysis was performed to compare the control group with the treated group. The treated group had a mean healing time of  $48 \pm 18$  hours and the control group had a mean healing time of  $67 \pm 11$  hours, although in several animals healing was not complete in the control group at the last time point. The healing time was significantly reduced ( $p=0.048$ ) in the eyes treated with cross-linked hyaluronic acid (Figure 2).



**Figure 2.** Box plot of time to healing comparing control (no treatment) with cross-linked hydrogel (treated). Statistical significance from a paired t-test with a p value of 0.048, n=6. Study concluded at 72 hours, so no whisker variation is shown beyond that point.

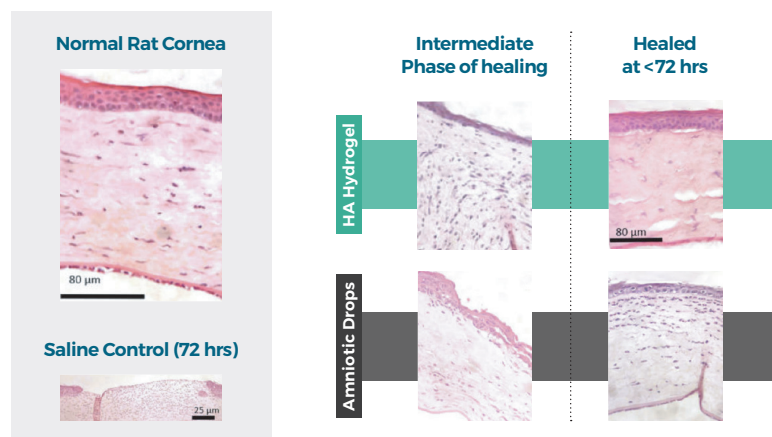


**Figure 3.** Time to healing comparison of control to two different treatment groups (BioHANCE™ cross-linked HA and amniotic eye drops).

## Discussion

This proof-of-concept study supports that BioHANCE™ cross-linked HA contributes to increased healing rates (30-50%) versus control. Indeed, it is also worth noting that measurements for the study were formally stopped at 72 hours while it took over 100 hours to heal the cornea in the control group.

BioHANCE™ cross-linked HA showed a reduced healing time in comparison to amniotic eye drops. The difference was statistically significant. This result warrants further study into the comparative efficacy of hydrogels in corneal healing time in dogs.



**Figure 4.** H&E stained sections of normal rat cornea with no injury, saline treated corneal injury, intermediate phase of healing and fully epithelialized cornea injuries treated with either cross-linked HA hydrogels, BioHANCE, or amniotic eye drops.

4

## TOPICAL CROSS-LINKED HA-BASED HYDROGEL ACCELERATES CLOSURE OF CORNEAL EPITHELIAL DEFECTS AND REPAIR OF STROMAL ULCERATION IN COMPANION ANIMALS

WILLIAMS DL, WIROSTKO BM, GUM G, MANN BK (2017). INVEST OPHTHALMOL VIS SCI. ; 58:4616-4622.

**0.75% BioHance™ cross-linked HA administered TID in addition to 0.5% chloramphenicol significantly accelerated (up to 34%) the closure of acute corneal stromal ulcers in dogs and cats compared with a 0.3% linear HA solution. Furthermore, topical administration of the cross-linked HA aided in healing chronic (>2 weeks) corneal ulcers in dogs.**



### ● Purpose

The purpose of this study was to determine the safety of topical ocular administration of a cross-linked, modified hyaluronic acid (xCMHA-S) hydrogel, and its effectiveness in accelerating repair and closure of acute and nonhealing corneal ulcers in companion animals as a veterinary treatment and its utility as a model for therapy in human corneal ulceration.

### ● Methods

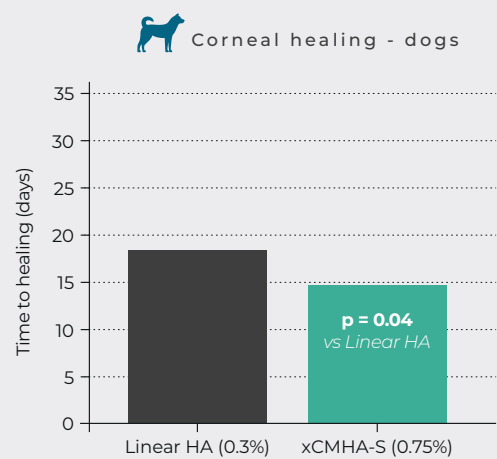
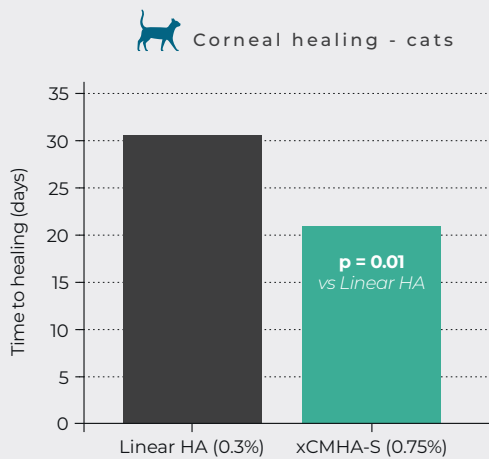
Two concentrations of xCMHA-S (0.4% and 0.75%) were topically administered to the eyes of rabbits six times daily for 28 days to assess safety. Then, 30 dogs and 30 cats with spontaneous acute corneal ulcers were treated with either xCMHA-S (0.75%) or a non-cross-linked HA solution (n = 15 per group for each species), three times daily until the ulcer had healed. Finally, 25 dogs with persistent nonhealing corneal ulcers were treated with xCMHA-S (0.75%) twice daily until the ulcer had healed.



### ● Results

Both concentrations of the xCMHA-S hydrogel were well tolerated, safe, and nontoxic in the 28-day exaggerated dosing study in healthy rabbits. Topically applied xCMHA-S significantly accelerated closure of acute corneal stromal ulcers in dogs and cats compared with a non-cross-linked HA solution. Furthermore, topical administration of the xCMHA-S aided in closure of nonhealing corneal stromal ulcers in dogs.

## CONCLUSIONS

Hyaluronic acid has previously been shown to aid in corneal wound repair. This study demonstrates that a cross-linked, modified HA hydrogel provides additional benefit by accelerating time to corneal wound closure compared to a non-cross-linked HA solution in companion animals.



	xCMHA-S (0.75%)	iDrop linear HA (0.3%)	p
 CATS	n=15 21.0 +/- 11.0 days	n=14 31.8 +/- 10.3 days	0.01
 DOGS	n=15 14.8 +/- 4.1 days	n=15 18.3 +/- 4.9 days	0.04

Mean time to healing of acute corneal stromal ulcers in 30 dogs and 30 cats with topical chloramphenicol and three-times daily BioHance™ cross-linked HA (0.75%) or linear HA (0.3%)<sup>†</sup>



5

## EVALUATION OF CROSS-LINKED HYALURONIC ACID GEL DROPS AND THERAPEUTIC COMBINATIONS FOR OPHTHALMIC INFECTIONS<sup>7</sup>

ACVO 2022 POSTER SESSION

The physical properties (viscosity, shear thinning, and concentration) of BioHance™ cross-linked HA are maintained when combined with antibacterial or antiviral drugs. *In vitro* results suggest that the efficacy of tobramycin is maintained, and that the efficacy of ganciclovir is improved when combined with BioHance™ cross-linked HA.

(SK Atzet<sup>1</sup>, AD Fankhauser<sup>1</sup>, EK Behan<sup>1</sup>, BK Mann<sup>1</sup>)<sup>1</sup>: SentrX Animal Care

### Purpose

To evaluate the *in vitro* efficacy and physical properties of combining antibacterial and antiviral drugs with a patented cross-linked hyaluronic acid (XHA) based eye drops.

### Methods

Several active pharmaceutical ingredients (Neomycin, Polymyxin B, Bacitracin, Gentamicin, Cefazolin, Ciprofloxacin, Gramicidin, Oxytetracycline, Tobramycin, Cidofovir, and Ganciclovir) were aseptically mixed with XHA, which, with its unique extracellular matrix, serves both as a delivery vehicle and eye lubricant. The resulting combined hydrogels were then evaluated for changes in physical properties (e.g., viscosity and shear thinning). Tobramycin hydrogels were evaluated for antimicrobial activity using a zone of inhibition assay. Ganciclovir hydrogels were tested for antiviral efficacy using a cytopathic effect assay (CPE) with Feline Herpesvirus 1 (FHV-1). Both were compared with the same drugs diluted in saline serving as controls.

### Results

The addition of active ingredients resulted in no significant changes to the viscosity or shear thinning profile of XHA hydrogels. Tobramycin hydrogel and tobramycin control exhibited equivalent zone of inhibition against three strains of bacteria. XHA ganciclovir solution was found to have a 4.3 and 3.2 fold reduction of viral activity as compared with saline solutions of Ganciclovir.



## CONCLUSIONS

*In vitro* results suggest that both the unique physical properties (viscosity, shear thinning, and concentration) of XHA and efficacy of tested active pharmaceutical ingredients are maintained or improved in the case of Ganciclovir. Future work will include target animal efficacy and disease state clinical studies along with application and dosing requirements based on potential synergistic effects from the XHA's increased residence time. Supported by SentrX Animal Care.

## 6

## CROSS-LINKED HYALURONIC ACID ENHANCES TEAR FILM CONCENTRATIONS OF CEFAZOLIN SODIUM IN CANINE EYES<sup>5</sup>

ACVO 2023 CONFERENCE ABSTRACT - SUBMITTED FOR PUBLICATION

**0.75% BioHance™ cross-linked HA increased the duration of high concentrations of antibiotic in the tear film of 10 healthy dogs. The tear film concentration of cefazolin sodium was increased and higher tear film antibiotic concentrations were maintained longer (up to 8 hours) when mixed with 0.75% BioHance™ cross-linked HA compared to a less viscous eye lubricant.**

(L Sebbag, E Ortaeskinaz, Y Goncharov, R Ofri, D Arad) Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel

### ● Purpose

To compare tear film concentrations of cefazolin sodium when formulated with two different excipients/ lubricants.

### ● Methods

Cefazolin sodium was compounded as a 5% solution using either 1.4% polyvinyl alcohol (PVA; Refresh®, Allergan) or 0.75% cross-linked hyaluronic acid (XHA; Oculenis®, Sentrx animal care). Ten ophthalmologically healthy dogs (5 brachycephalic, 5 non-brachycephalics; n = 20 eyes) received one drop of cefazolin-PVA in one randomly selected eye, and one drop of cefazolin-XHA in the other eye. Tear fluid was collected with 2- $\mu$ l capillary tubes at times 0 min, 1 min, 5 min, 10 min, 15 min, 30 min, 60 min, 120 min, 240 min, 360 min and 480 min. Cefazolin tear concentrations were measured with UV-Vis spectrophotometry.

### ● Results

No significant differences were noted in tear cefazolin concentrations between brachycephalic and non-brachycephalic dogs with either formulation at any time point ( $P \geq 0.086$ ). In all eyes, mean tear film concentrations were significantly higher with XHA than PVA at all time points ( $P \leq 0.049$ ) except for baseline and t = 60-120 min ( $P \geq 0.105$ ). Tear film kinetics of cefazolin-XHA were somewhat 'biphasic', with drug levels decreasing from 0-120 min, then slightly increasing from 120-360 min prior to declining again until the end of the experiment (480 min). The area under the time-concentration curve ( $AUC_{0-480}$ ) was 2.7 greater with XHA than PVA ( $P = 0.002$ ).

## CONCLUSIONS

XHA greatly improved tear film concentrations of cefazolin sodium when compared with PVA, a less viscous excipient/lubricant. To dictate dosing regimens and determine clinical efficacy, future experiments should assess XHA-cefazolin in dogs with bacterial keratitis and determine clinical breakpoints for cefazolin against common bacterial pathogens of dog eyes. LS serves on the scientific advisory committee for the company that funded this study (Sentrx animal care).



# EYE EXPERTS

As an international reference in ophthalmology, Dômes Pharma is committed to providing veterinarians, nurses, and pet owners with:

- An extensive range of innovative ophthalmic products, from daily care and prevention to diagnostics and therapeutics
- Our teams' scientific and technical expertise
- A broad range of services, including disease management guidelines and innovative educational experiences.

## References

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