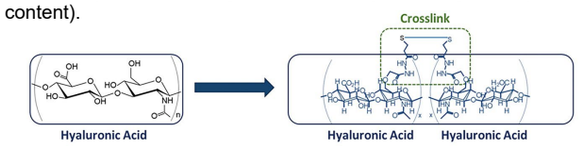
EVALUATION OF CROSSLINKED HYALURONIC ACID GEL DROPS AND THERAPEUTIC COMBINATIONS FOR OPHTHALMIC INFECTIONS (SK Atzet, 1 AD Fankhauser, 1 EK Behan, 1 BK Mann, 1) SentrX Animal Care;1

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 controls 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 APIs 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. E

IN VITRO EFFICACY, DRUG RELEASE RATES, AND CHARACTERIZATION OF CROSSLINKED HYALURONIC ACID GEL DROPS COMBINED WITH COMMON OPHTHALMIC THERAPEUTICS

Introduction

Hyaluronic Acid (HA) is a primary component of the extracellular matrix and can be found throughout the body but especially in connective, epithelial, and neural tissues. Additionally, it is one of the primary lubricants in synovial fluid and contributes to cell migration and proliferation. HA can be chemically crosslinked to create a 3D network often referred to as a hydrogel, or crosslinked HA (XHA). These hydrogels have unique physical properties such as viscosity, shear thinning profiles, and high swelling ratios (water content).



Methods

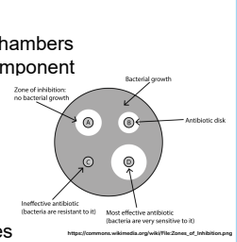
Combination Hydrogel Preparations:
Active Pharmaceutical Ingredients (APIs) with known safety and efficacy were aseptically mixed with crosslinked hyaluronic acid (XHA, Oculenis). Water soluble APIs were simply mixed and poorly soluble APIs were mixed in an emulsion. The following API's were combined with XHA:

- Neomycin, Polymyxin B, Bacitracin
- Ofloxacin
- Gentamicin
- Cefazolin
- Ciprofloxacin
- Gramicidin
- Oxytetracycline
- Tobramycin
- Cidofovir
- Ganciclovir
- Tacrolimus
- Cyclosporin A

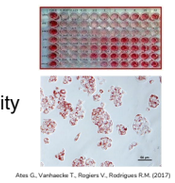
Hydrogel physical properties characterized by:
- Rheometry/Viscosity Profile - shear sweep
- pH
- Total Solids Concentration

API Release:
- UV spectroscopy using medi-dialysis chambers
- Controls using PBS as the aqueous component
- Study time 72 hrs

Zone of Inhibition (ZOI):
- Kirby-Bauer test
- Disc wafer soaked in sample
- Placed on confluent bacteria agar plates
- Area around disc clears due to antimicrobial activity
- Diameter of cleared area indicates activity



Cytopathic Effect Assay:
- Selective Index (SI) measures antiviral activity
- SI>10 indicates moderate antiviral activity
- Crandell Feline Kidney Cells
- Feline Herpes Virus (FHV-1)
- Neutral Red and Visual Assessment to measure reduction in viral effects



Corneal Healing - Rat Model:
- Superficial keratectomy, 5 animals
- 3mm site de-epithelialized by diamond burr
- One eye received saline, one XHA drops bid
- Evaluated by fluorescein stain at T=0, 12, 24, 36, 48, & 72 hours
- Considered healed when no staining was present on the surface
- Histology (H&E) performed after 72 hours

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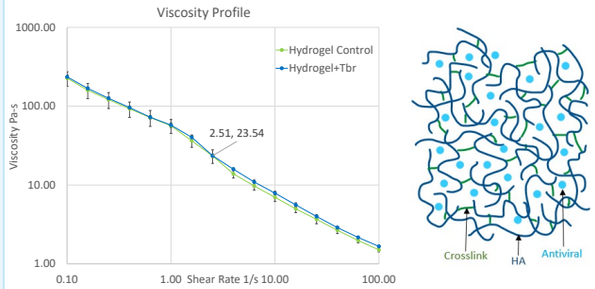
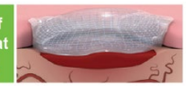
In vitro data shows crosslinked hyaluronic acid (HA) maintains or improves drug efficacy while providing additional benefits of lubrication and promoting healing.

Results and Conclusions

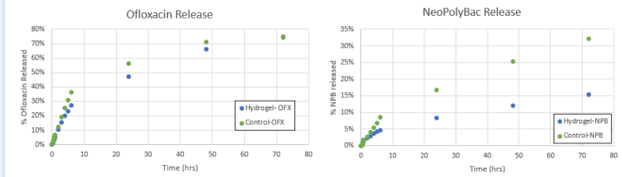
Shear-thinning Profile and Viscosity is maintained



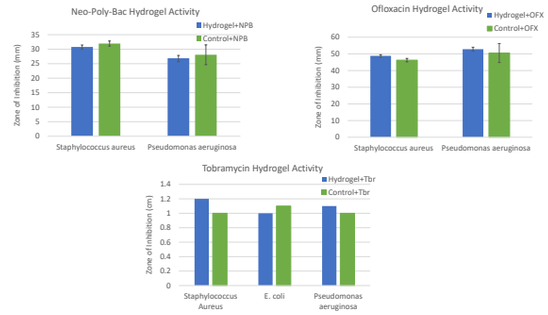
Creates a matrix of crosslinked HA that enhances healing.



Release rates and profiles remain similar



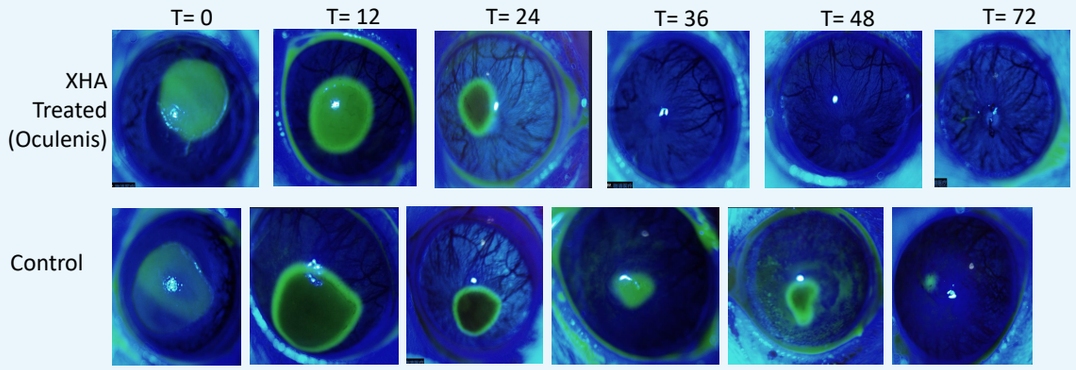
Hydrogels containing antibiotics maintain their efficacy



Ganciclovir shows 3-4 fold increase in potency when combined with hydrogel

Visual Analysis	CC_{50}	EC_{50}	SI
Cidofovir	>100	16	>6.3
Ganciclovir Solution	>100	15	>6.7
Ganciclovir Hydrogel	>100	3.5	>29

Neutral Red	CC_{50}	EC_{50}	SI
Cidofovir	>100	27	>3.7
Ganciclovir Solution	>100	8	>13
Ganciclovir Hydrogel	>100	2.4	>42



XHA treated corneal defects heal faster and better