# Evaluating the impact of ophthalmic formulations in a human corneal epithelial cell wound repair *in vitro*


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## Background

- Corneal injuries necessitate effective management to promote rapid and complete wound healing.[1,2]
- Sodium hyaluronate (HA) is widely used in artificial tears or topical ophthalmic formulations due to its hydrating, viscoelastic, and wound-healing properties.
- The performance of HA can depend on its concentration, molecular weight, viscoelastic and quality profiles.
- Eye drops are often evaluated at room temperature, but viscosity at physiological eye temperature is important to understand the HA on-eye performance.
- Limited *in vitro* studies directly compare the wound repair potential of commercially available HA-based eye drops.

## Purpose

The purpose of this study was to evaluate the effects of HA-based ophthalmic formulations with varying viscosities and HA molecular weights on *an in vitro* model for wound repair and assessed their viscosity at room temperature and 37 °C.

## Methods

- For cellular repair following damage and analyses the drops were tested using an in vitro model consisted of immortalized human corneal epithelial cells (HCEC's) cultured in DMEM/F12 media with 1% FBS and 1% penicillin/streptomycin. HCEC's were seeded into 6-well plates and incubated for 24 hours at 37°C and 5% $CO_2$. Once confluent, media was aspirated, and HCEC's were treated with formulations of interest or PBS control for 15 minutes. Formulations were removed, and HCEC's were rinsed briefly with PBS. A p200 pipette tip was used to mechanically scratch a straight line across the cell monolayer to create a cell-free area, simulating a wound (see top row T=0 in Fig 1). Immediately after creating the scratch, cells were gently washed with PBS to remove detached cells and debris. Cells were imaged by brightfield microscopy over time, and image analysis was performed using ImageJ from 24 to 72 hrs.
- Three different ophthalmic formulations (OPTASE® Hylo-Forte™ (OHF), OPTASE® INTENSE (OI) and a Leading Marketed HA Drop (LMHD) SYSTANE® HYDRATION MDPF) with different viscosity profiles were tested. OHF and OI have viscosities of 82 and 75 cps measured at low shear rates while LMHD has a reported viscosity range of 35-40 cps.[3,4] The steady shear viscosity of the eyedrops were characterized with an Anton Paar MCR 502 rotational rheometer using a cone (50mm DIA 1° cone angle) and plate geometry set at RT and 37°C. The steady state shear viscosity measurements were performed over 1-5000 $s^{-1}$.

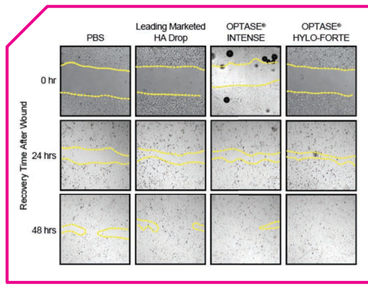


**FIGURE 1.** PBS, OHF, OI and (LMHD), SYSTANE® HYDRATION formulations promote wound repair in damaged HCEC monolayers. Representative images of HCECs with a scratch wound and the repair over 48 hrs after incubation in the formulations. Data are presented as mean ± SD. *p < 0.05 vs. PBS (One-way ANOVA)

## Results

The effects of PBS, OHF, OI and LMHD ophthalmic formulations on the scratch wound and repair of damaged HCEC monolayers are shown in Figures 1 and 2.

- Representative images of HCEC's show the promotion of wound repair in damaged HCEC monolayers for all the formulations tested from 0, 24, 48 and 72 hours. The representative images of HCECs after incubation in the indicated formulations show some important differences. This was evaluated by quantifying the wound closure using image analysis (see Figure 2) and also by calculating the rates of wound closure after treatment plotted over time.
- Figure 2 (below) shows there was a significant increase in wound closure at 24 and 72 hours in cells treated with all three formulations compared to the PBS control ($p < 0.05$). Cells treated with OHF, OI, and LMHD respectively demonstrated superior recovery rates on average of +21.4 points (± 10.8% range), +20.2 points (± 5.4%), and +11.2 points (± 6.4%) at 24 hours compared to the PBS control. This increased wound healing was maintained at 72 hours. The time-lapse wound healing with OHF versus PBS showed statistically significant differences at 24, 48 and 72 hrs ($p < 0.05$) and this is also evidenced by the rate of wound closure in Fig 2 (below).
- The statistical significance indicated with the * in Figure 2 are comparisons made to the PBS CTRL. For example, OHF at 24 hours is statistically significant compared to the control. There is a positive trend of enhanced wound closure for OHF versus the other formulations but more sampling and repeatability are needed for statistical significance interpretation. For example, some P-values (at 24 hours) to illustrate comparisons include:
  - OHF vs PBS, p-value = 0.0399
  - LMHD vs PBS, p-value = 0.1392
  - OHF vs LMHD, p-value = 0.1771
  - OI vs PBS, p-value = 0.0247

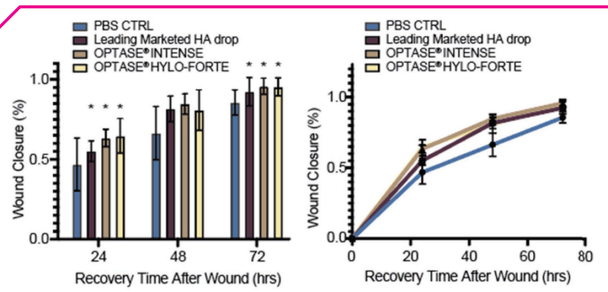


**FIGURE 2.** PBS, Hylo-Forte (OHF), OPTASE Intense (OI) and Leading Marketed HA Drop (LMHD), SYSTANE® HYDRATION formulations promote wound repair in damaged HCEC monolayers. Left: Wound closure was quantified using image analysis for HCECs treated with each formulation. Right: Rates of HCEC wound closure after treatment were plotted over time. Data are presented as mean ± SD. *p < 0.05 vs. PBS (One-way ANOVA).

- The three different ophthalmic formulations OHF, OI and LMHD have different viscosity profiles. To obtain a better understanding of the viscosity profiles the steady state sweep measurements were performed at 25°C and 37°C for the three formulations (Figure 3). The OHF data at 37°C showed unexpectantly higher viscosities at the lower shear rates (< 100 sec-1) indicating there was a thermal influence on the formulation rheology behavior.

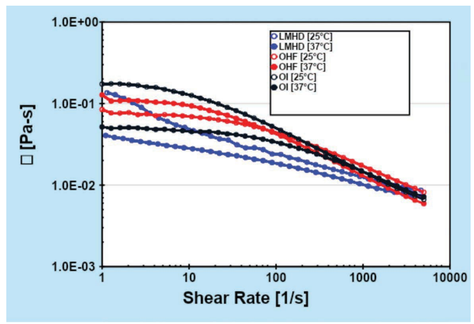


**FIGURE 3.** Steady shear viscosity measurements for (OPTASE® Hylo-Forte™ (OHF) OPTASE INTENSE (OI) and Leading Marketed HA Drop (LMHD), SYSTANE® HYDRATION, at 25°C and 37°C. OHF seemingly resists decrease in viscosity at the higher temperature compared to the other formulations.

## Discussion

- The viscosity of sodium hyaluronate solutions typically decreases as temperature increases.[5,6] This is a typical characteristic of dry eye lubricant drop formulations. The increased thermal energy from the higher temperature should allows for the polymer chains to become more flexible and move more freely and lowering the viscosity.
- The rheology data in Figure for OHF suggests there is a resistance to viscosity changes at the low shear range.
- The in-vitro wound HCEC model (Figures 1 and 2) indicated that OHF supports faster onset of cellular health and hydration recovery in the critical first 24 hours post damage. The data in Figures 1, 2 and 3 indicate that the OHF formulation behaves uniquely compared to other eye drops. The clinical value of the active glycerin and sodium hyaluronate formulations have been reported.[7,8,9,10] OHF contains 0.3% active Glycerin and a 0.3% concentration of a specifically selected HMW HA. It is hypothesized that this combination forms structured hydrogen bonded polymer network that is thermally activated by the eye temperature (Figure 4).

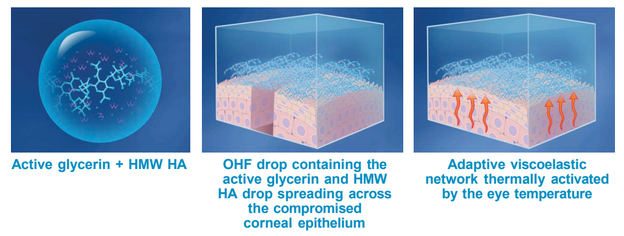


**FIGURE 4.** Schematic representation of how the OHF drop spreads over the eye and forms a thermally activated mesh network composed of the active glycerin and HA to provide ocular protective cushioning and hydration.

## Conclusion

This study presented the development and utilization of an *in vitro* cellular wound repair model to evaluate the effects different topical HA eye drops with varying viscosities under low shear stress on the rate of wound closure. The OHF results suggest that there are other factors besides the viscosity of the HA eyedrops that can influence the wound healing rate. Importantly, this work emphasizes the importance of optimizing formulation viscosity profiles to enhance bioavailability and retention of effect for clinical efficacy needs, taking into consideration factors such as the effect of temperature on lubricant performance properties.

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