An in vitro evaluation of the effect and protection of artificial tear formulations on human corneal epithelial cells in normal and dry eye disease states

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Abstract

This study developed novel in vitro human corneal epithelial cell (HCEC) assays to comprehensively evaluate the cellular effects of artificial tear solutions (ATS) on ocular cells. Quantitative imaging and assessment of cellular viability in hyperosmotic and desiccation stress demonstrate that ATS products vary in their cytoprotective effect. Finally, the assays presented here provide a rapid and robust framework for evaluating the cytoprotective effect on ocular hydration and protection.

Introduction

- ATS have been effective frontline treatments for dry eye disease (DED), yet current commercially available products often provide only temporary relief, necessitating frequent daily administration [1].
- Despite the clinical importance of ATS, especially those containing HA, current in vitro models often lack the physiological relevance and high-throughput capabilities for comprehensive evaluation of their cytoprotective effects.
- Methods to evaluate ATS have largely focused on human biocompatibility and rheological testing and often overlook the dynamic nature of cellular phenotypes or the protective mechanisms at a cellular level [2].

Purpose

The purpose of this study was to develop a novel in vitro mammalian cell assay to assess the effect of sodium hyaluronate-based artifical tear solutions in ocular hydration and protection.

Methods

Novel in vitro methods to assess HCEC cellular health

ATS Name	Active Ingredient	Manufacturer
OPTASE Intense	0.2% Glycerin	Scope Ophthalmics
Systane Hydration	0.4% Polyethylene Glycol 400 0.3% Propylene Glycol	Alcon
iVIZIA	0.5% Povidone	Théa Pharma, Inc

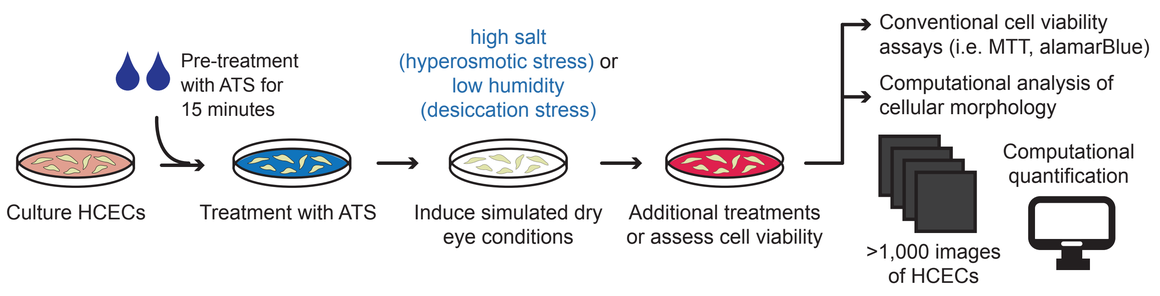

Figure 1. Commercially available ATS were tested on immortalized HCECs cultured in DMEM/F12 media with 1% FBS. HCECs were seeded into 96-well plates at a density of 15,000 cells/well and incubated for 24 hours at 37oC and 5% CO2. Once confluent, media was aspirated, and HCECs were treated with ATS or PBS control for 15 minutes. DED was induced by subjecting cells to either desiccation stress (low humidity, 45%) or hyperosmolar stress (~ 500 mOsM NaCl). Cellular heath and ATS protection were assessed by three methods: (1) cellular viability using alamarBlue™, (2) morphological analysis by time-lapse microscopy, and (3) inflammatory gene expression changes by RT-qPCR.

Results

Biocompatible ATS products affect HCEC cell morphology

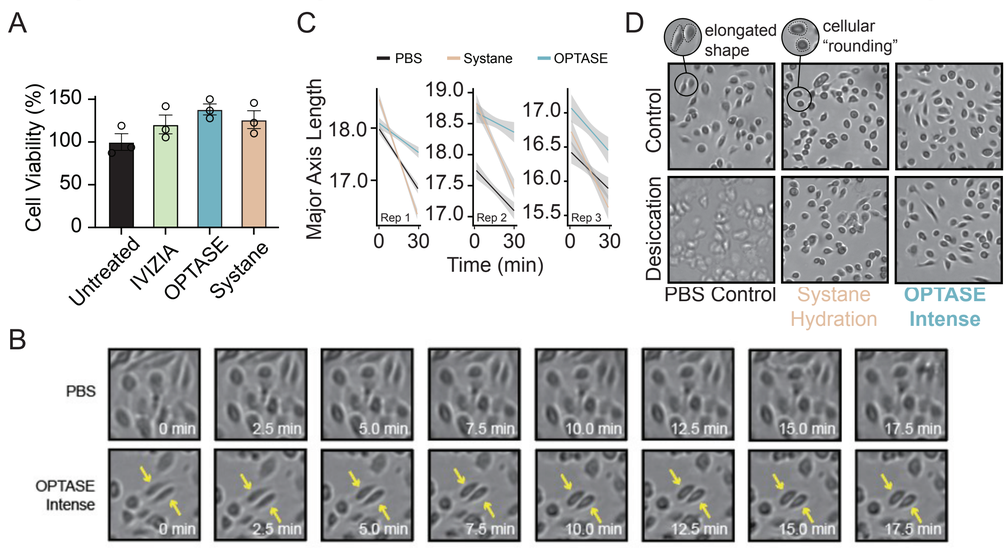

Figure 2. (A) Cell viability of HCECs following 15-minute treatment with PBS control and indicated ATS products. (B) Representative brightfield time-lapse micrographs of HCECs during treatment with PBS control or OPTASE Intense. Yellow arrows indicate cells that are undergoing significant cellular morphology changes during exposure to the ATS product. (C) Computational quantification cellular length changes during incubation with ATS products of interest. (D) Representative images of HCECs and their cellular morphology before and after desiccation conditions (low humidity, 45%) with and without pre-treatment of indicated ATS products.

ATS protects HCEC from desiccation stress

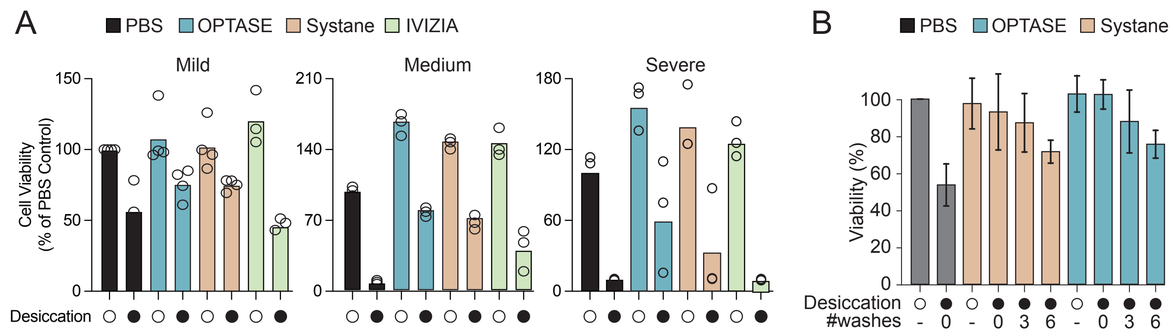

Figure 3. (A) Quantification of cell viability before and after exposure to desiccation stress with different severities, and with and without pre-treatment with the indicated ATS products. Open circles below the plot indicate no desiccation stress (cells remained hydrated) and closed circles indicate 5-minute desiccation stress at 37oC with 5% CO2. (B) Cell viability of HCECs before and after 5-minute desiccation stress, with and without pre-treatment with the indicated ATS products, and with the indicated number of PBS wash steps following ATS pre-treatment and before exposure to low humidity conditions.

ATS protects HCECs from hyperosmotic stress

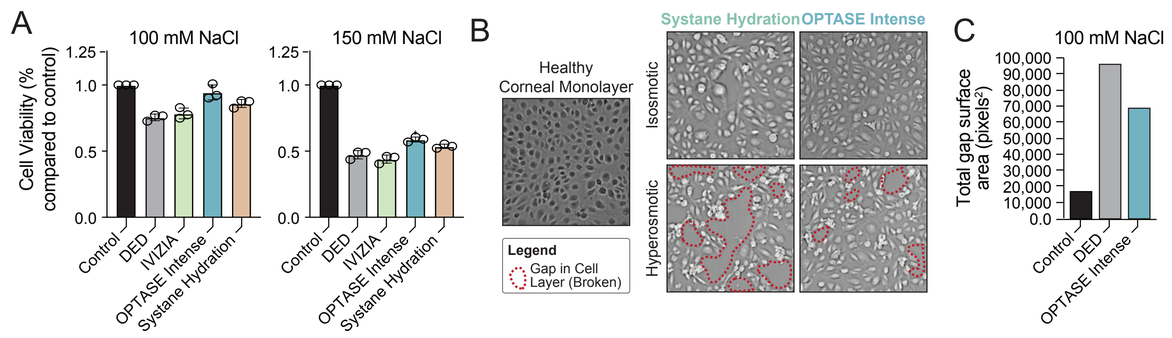

Figure 4. (A) HCEC viability for cells without pre-treatment with indicated ATS products and high salt conditions for 16-24 hours. (B) Representative brightfield micrographs of HCECs grown in a monolayer in standard conditions (left) and in hyperosmolar conditions, with and without pre-treatment with indicated ATS products (right). Dotted red line indicates regions in the monolayer where there are observable gaps due to breakage of the cellular monolayer. (C) Quantification of the surface area (in pixels squared) of gaps and HCEC monolayer breakage in hyperosmolar conditions in the indicated conditions.

OPTASE protects HCECs from inflammation due to desiccation and hyperosmotic stress in vitro models

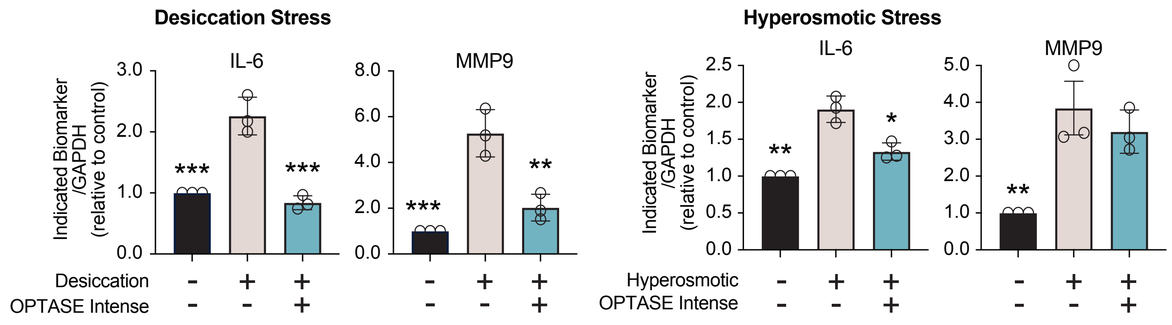

Figure 5. Inflammatory biomarkers were measured by RT-qPCR in HCECs before and after the indicated DED conditions, either by treatment with desiccation stress or hyperosmolar stress. Significance tests are comparisons to the DED treatment. Statistical significance determined by one-way ANOVA with Tukey multiple comparisons test * $p < 0.05$, *** $p < 0.005$.

Conclusions and Future Directions

- Despite being marketed for similar purposes, this study demonstrated that commercially available ATS products can exhibit differences in their toxicity and protective efficacy, which needs to be considered for personalized patient care.
- Extending beyond simple product evaluation, the assays developed here have uncovered a previously underappreciated cellular response to in vitro modelled DED stresses, particularly the breakage of the cellular monolayer following hyperosmotic stress.
- The models used in this study recapitulate the pro-inflammatory cellular response commonly observed in dry eye disease, providing support that the assays established can be used to evaluate ATS toxicity and protection.
- The insights gained from these assays lay the groundwork for future research and development that will be critical in guiding the next-generation ATS development.

References

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[2] Kaluzhny Y, Klausner M. In vitro reconstructed 3D corneal tissue models for ocular toxicology and ophthalmic drug development. In Vitro Cell. Dev. Biol. - Anim. 2021;57(3):207–237. doi:10.1007/s11626-020-00533-7.

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