

# Comparison of Bacterial Lipase Activity in the Presence of Eye Lid Cleansers

A. Epstein<sup>1</sup>, L. Pang<sup>2</sup>, C. Noorbakhsh<sup>2</sup>, K. Najafi-Tagol<sup>2,3</sup>, R. Najafi<sup>2</sup>, D. W. Stroman<sup>2</sup>, D. Debabov<sup>2</sup>

<sup>1</sup>Phoenix Eye Care – The Dry Eye Center of Arizona, Phoenix, AZ; <sup>2</sup>NovaBay Pharmaceuticals, Inc., Emeryville, CA

<sup>3</sup>Eye Institute Of Marin, San Rafael, CA

5980 Horton Street, Suite 550  
Emeryville, CA 94608  
Phone (510) 899-8828  
ddebabov@novabay.com

## Abstract

**Purpose:** Blepharitis is a generalized inflammation of the eyelid and lid margins, which can include meibomian gland dysfunction (MGD). Meibomian gland dysfunction is characterized by obstruction or a secretion abnormality of the glands that can lead to tear film instability, inflammation and irritation of the lids and ocular surface. The meibomian glands are sebaceous glands found in the lid margins that produce a lipid-rich secretion known as meibum. Excessive bacterial lipase production can alter and degrade meibum on the lid surfaces and within the eye, potentially leading to tear instability and dry eye disease. Saponification breaks down tear lipids into soaps eyelid cleansers including: Cliradex<sup>®</sup>, Johnson's<sup>®</sup> Baby Shampoo, NovaBay<sup>®</sup> Avenova and free fatty acids, which manifests as frothing within the inferior tear meniscus and symptoms of burning. We evaluated the lipase inactivation activity of various commonly used (i-Lid<sup>™</sup> Cleanser), OCuSOFT<sup>®</sup> Lid Scrub<sup>®</sup> Original, OCuSOFT<sup>®</sup> Lid Scrub<sup>®</sup> Plus, and TheraTears<sup>®</sup> SteriLid<sup>®</sup>.

**Methods:** Each eyelid cleanser was added to a solution of 2 mg/mL of *Pseudomonas cepacia* lipase. After an hour of incubation at 37°C, the lipase-eyelid cleanser solutions were diluted 500-fold into lipase buffer. The lipase activity was determined using the Lipase Activity Assay Kit III (Sigma Aldrich<sup>®</sup>). A SpectraMax<sup>®</sup> M5 plate reader was able to continuously incubate the microtiter plate at 37°C and measure the fluorescence ( $\lambda_{ex}/\lambda_{em} = 529/600$  nm) every 5 minutes for 1.5 hours after an initial 10-minute incubation period.

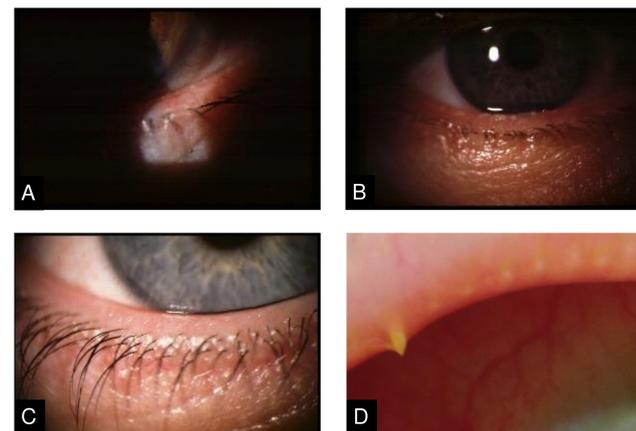
**Results:** In the presence of Cliradex<sup>®</sup>, Johnson's<sup>®</sup> Baby Shampoo, OCuSOFT<sup>®</sup> Lid Scrub<sup>®</sup> Original, OCuSOFT<sup>®</sup> Lid Scrub<sup>®</sup> Plus, and TheraTears<sup>®</sup> SteriLid<sup>®</sup>, bacterial lipase had similar activity to the lipase treated with sterile water, negative control (Figure 3). In the presence of NovaBay<sup>®</sup> Avenova<sup>™</sup>, the bacterial lipase activity was completely inactivated, not distinguishable from no added lipase (Figure 3).

**Conclusions:** Our study compares the inactivation of bacterial lipase by eye care products used to treat blepharitis and MGD. In this *in vitro* study, NovaBay<sup>®</sup> Avenova<sup>™</sup> completely inactivated bacterial lipase activity while all other products had minimal effect.

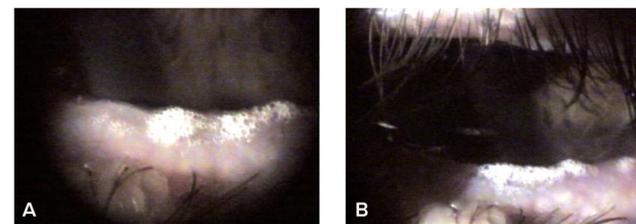
## Introduction

Meibomian gland dysfunction (MGD), often referred to as posterior blepharitis, is a frequent cause of inflammation of the eyelids and ocular surface. MGD is caused by obstruction or abnormal secretion of meibomian glands that run radially within both upper and lower eyelids. Meibomian glands normally secrete meibum, which forms the complex lipid-rich layer of the tear film. Meibum stabilizes the tear structure, reduces evaporation, and can serve as a carbon source for bacteria colonizing lid surfaces. Excessive amounts of bacteria on the lid surfaces can produce sufficient lipase, a fat and oil-reducing enzyme, to degrade the structure of meibum, resulting in inflammation and symptoms of dry eye disease. MGD may alter the corneal reflectivity, impairing vision. In addition, increase in bacterial lipase activity has been shown in patients with meibomian gland abnormalities.<sup>1</sup> Standard treatments aim to remedy MGD by improving the flow of meibomian gland secretions, which are responsible for maintaining tear film.<sup>2</sup> Current treatments include warm compresses, lid hygiene, antibiotics, and anti-inflammatory agents.<sup>2</sup>

Various eyelid treatments, commonly used to treat MGD, were evaluated *in vitro*. These products include OCuSOFT<sup>®</sup> Lid Scrub<sup>®</sup> Original and OCuSOFT<sup>®</sup> Lid Scrub<sup>®</sup> Plus (both products containing polyaminopropyl biguanide [PHMB] and additional components such as surfactants and pH stabilizing solutions), Avenova<sup>™</sup> containing a novel pure 0.01% hypochlorous acid in a 0.9% saline solution at pH 4 (Neutrox<sup>™</sup>), Cliradex<sup>®</sup> (containing terpinen-4-ol), TheraTears<sup>®</sup> SteriLid<sup>®</sup> (containing linalool and tea tree oil), and Johnson's<sup>®</sup> Baby Shampoo (containing surfactants, salts, preservative, fragrance and other additives). Only NovaBay Avenova<sup>™</sup> (i-Lid<sup>™</sup> Cleanser) inactivated the lipase completely, while other products had negligible inactivation effects.



**Figure 1.** A. Advanced MGD with inspissation and lid inflammation. B. Thickened saturated lipids on lid margin. C. Meibomian seborrhea and anterior blepharitis. D. A pouting meibomian gland.



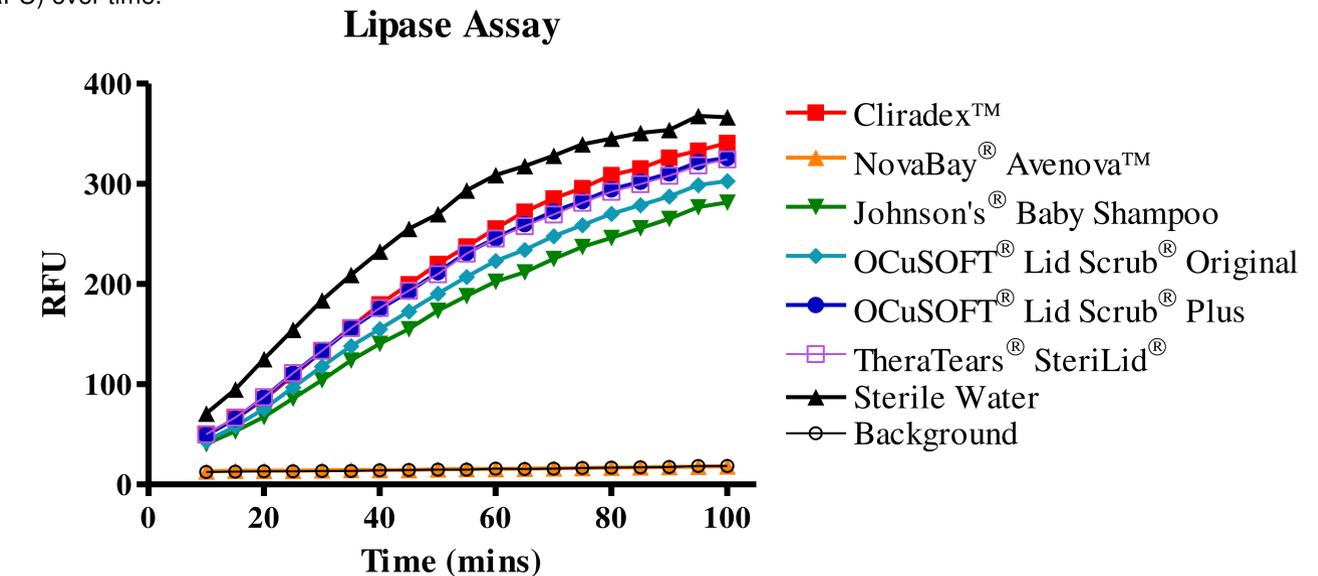
**Figure 2. A-B.** Examples of frothing associated with saponification due to the action of lipase on meibum.

## Materials & Methods

Each eyelid cleanser was added to a solution of 2 mg/mL of *Pseudomonas cepacia* lipase (Sigma Aldrich<sup>®</sup>, St. Louis, MO). After an hour of incubation at 37°C, the lipase-eyelid cleanser solutions were diluted 500-fold into lipase buffer. The lipase activity was determined using the Lipase Activity Assay Kit III (Sigma Aldrich<sup>®</sup>). A SpectraMax<sup>®</sup> M5 Microplate Reader was used to continuously incubate the microtiter plate at 37°C and measure the fluorescence ( $\lambda_{ex}/\lambda_{em} = 529/600$  nm) every 5 minutes for 1.5 hours after an initial 10-minute incubation period.

## Results

**Figure 3.** Activity of bacterial lipase in the presence of various eyelid cleansers measured in relative fluorescence units (RFU) over time.



## Conclusions

Our study compared the inactivation of bacterial lipase by eye care products used to treat blepharitis and MGD. In this *in vitro* study, NovaBay<sup>®</sup> Avenova<sup>™</sup> completely inactivated bacterial lipase activity while all other products had minimal effect.

## References

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- Qiao J, Yan X. Emerging treatment options for meibomian gland dysfunction. Clin Ophthalmology. 2013;7:1797-803.

## Disclosures

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K. Najafi-Tagol, L. Pang, C. Noorbakhsh, R. Najafi, D. Debabov, D. W. Stroman: NovaBay Pharmaceuticals employees.