Stabilization of Lysozyme Mass Extracted from Silicone Hydrogel Contact Lenses

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Introduction

- One of the major problems with hydrophilic contact lenses is that they are susceptible to spoilage from the constituents of the tear film, which include a wide variety of proteins, lipids and mucins. 1,2
- These deposits may result in reduced comfort, vision and overall satisfaction. 3
- The predominant tear film protein deposited on traditional contact lens materials is lysozyme. 4 Lysozyme is a bactericidal enzyme derived from the lacrimal gland. It is relatively small (14.5kDa) and has a large net positive charge, which enables it to adsorb on negatively charged substrates.
- Data from our laboratory demonstrates that novel Silicone Hydrogel (SH) lens materials deposit extremely low levels of protein compared to conventional hydrogel lenses. 5,6
- Results in our laboratory demonstrated that lysozyme deposits extracted from SH contact lens materials demonstrated a loss in total mass as a function of storage time when assessed by Western blotting, which represents a potential source of error when quantifying total lysozyme deposition.

Purpose

- The purpose of this study was to devise a method whereby lysozyme mass would be preserved over time and would be compatible with our previously published Western Blotting procedure. 7

Methods & Materials

- Lysozyme deposits from 12 human worn lotrafilcon contact lenses were extracted using a 50:50 mixture of 0.2% trifluoroacetic acid and acetonitrile. 8
- Extracts were lyophilized to dryness, then resuspended in either Reconstitution Buffer (RB) (10mM Tris-HCl, 1mM EDTA) or Modified Reconstitution Buffer (MRB) (RB + 0.9% saline).
- BioStab Biomolecule Storage Solution (Sigma-Aldrich) (1 in 4 parts) was added to one half of the samples from each buffer group.
- 1µL of each of the samples was immediately subjected to SDS-PAGE followed by Western blotting to PVDF membranes using the PhastSystem™ (Amersham-Pharmacia Biotech).

Results

- The remaining volume was aliquoted and one half of the samples was stored at -20ºC and the other half was stored at -70ºC. One half of the samples was stored with the addition of Gel Loading Buffer (5% SDS; 100 mM Tris, pH 7.4; 50% Glycerol; 1 mM EDTA, 0.02% bromophenol blue), while the remaining half was stored without the addition of Gel Loading Buffer (see Figure 1 for a summary of sample processing).
- All the stored samples were subjected to electrophoresis and western blotting procedures after 48 hours of storage.
- Lysozyme was identified using a rabbit anti-human lysozyme polyclonal antibody (Calbiochem), followed by a peroxidase conjugated goat anti-rabbit secondary antibody (Sigma-Aldrich).
- Individual standard curves of purified human neutrophil lysozyme (Calbiochem) were run on each gel to facilitate regression analysis.

Figure 1: Schematic of sample processing

Figure 2: Summary of Percentage Loss of Lysozyme when Resuspended in RB: Graphical representation of the percentage loss of lysozyme following reuspension in Reconstitution Buffer and when stored with and without the addition of BioStab/ Gel Loading Buffer.

Figure 3: Summary of Percentage Loss of Lysozyme when Resuspended in MRB: Graphical representation of the percentage loss of lysozyme following reuspension in Modified Reconstitution Buffer and when stored with and without the addition of Gel Loading Buffer.

Conclusions

- We have optimized a procedure using Modified Reconstitution Buffer, BioStab Biomolecule Storage Solution and storage at -70ºC, whereby the extracted mass of lysozyme deposits found on SH lenses can be preserved without loss to facilitate accurate quantification via our WB procedure.

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References