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 bacteriolytic 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.

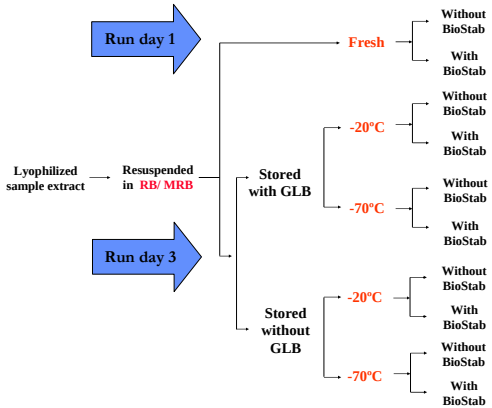
Purpose

- 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).
- The remaining volume was aliquoted and one half of the samples was stored at -20°C and the other half was stored at -70°C. One half of the samples was stored with the addition of Gel Loading Buffer (5% SDS; 100 mM Tris, pH 7.4; 30% 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.
- Immunoreactivity was visualized by incubating with ECL Plus chemiluminescent substrate (Amersham-Pharmacia Biotech). Optical densities of the resulting bands were quantified from digitized images created with a Molecular® Dynamics Storm™ 840 Imager using ImageQuant™ 5.1.
- Four - way analysis of variance was performed on all data to assess differences between Buffer, Stabilizer, Temperature and Gel Loading Buffer.

Figure 1: Schematic of sample processing



Results

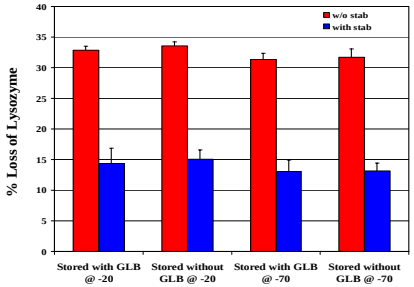


Figure 2: Summary of Percentage Loss of Lysozyme when Resuspended in RB: Graphical representation of the percentage loss of lysozyme following resuspension in **Reconstitution Buffer** and when stored with and without the addition of BioStab/ Gel Loading Buffer.

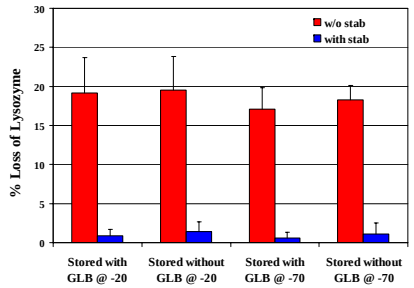


Figure 3: Summary of Percentage Loss of Lysozyme when Resuspended in MRB: Graphical representation of the percentage loss of lysozyme following resuspension in **Modified Reconstitution Buffer** and when stored with and without the addition of BioStab/ Gel Loading Buffer.

- Statistical analysis indicated that buffer composition ($p < 0.001$), storage temperature ($p = 0.04$) and addition of BioStab ($p < 0.001$) were all important in controlling loss of mass over time. However, no significant difference was found when the samples were stored with and without the addition of Gel Loading Buffer ($p = 0.373$).

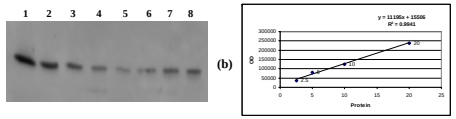


Figure 4: Western Blot and Regression Analysis for Lysozyme Quantification: (a) An example of a lysozyme Western blot. Lanes 1-4 are purified human neutrophil lysozyme {Lane 1 = 20, Lane 2 = 10, Lane 3 = 5, Lane 4 = 2.5 ng/µl; Lanes 5 - 8 are FND lens extracts under four different conditions (lane 5 = w/o BioStab + MRB (stored @-20°C); lane 6 = w/o BioStab + MRB (stored @ -70°C); lane 7 = with BioStab + MRB (stored @ -20°C); lane 8 = with BioStab + MRB (stored @ -70°C)}. **(b)** A regression curve was created by graphing applied concentration of lysozyme standard against the optical density of the resulting band immunoreactivity. Total lysozyme concentration was quantified by extrapolation from this curve.

Conclusions

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

References

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Acknowledgements

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