COMPARISON OF PROTEIN EXTRACTION FROM SILICONE HYDROGEL LENS MATERIALS AND CARE SOLUTIONS

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INTRODUCTION

- The tears contain proteins such as lysozyme, lactoferrin, lipocalin, IgA, albumin, transferrin, IgG and IgM that serve antimicrobial roles on the surface of the eye.[1-4]
- When proteins are deposited on contact lenses (CLs), they may become denatured making the eye more susceptible to infection. *Much of the traditional research on protein deposition on contact lenses has centered on the binding of lysozyme.[4-7]
- Protein buildup on CLs also could alter the wettability of the lens surface, or decrease patient comfort and satisfaction.[8]
- Lastly, contact lens wear could lead to the expression of proteins (e.g., inflammatory mediators) that are associated with ocular surface and dry eye disease.

-Thus, it is critical that we fully understand the total contact lens deposition proteome, in addition to differences observed based on lens and care solution chemistry.

Specific Aims

- To determine quantity and identity of tear film proteins inherently attracted to two silicone hydrogel materials.
- To determine quantity and identity of tear film proteins removed from silicone hydrogels by currently available multipurpose "no-rub" care solutions.
- To develop a more complete understanding of the entire CL deposition proteome using a state-of-the-art mass spectrometric-based approach.

METHODS

Study Design and Patient Sample

- Approved by Biomedical IRB in accordance with the tenets of the Declaration of Helsinki.
  - This was a two-armed pilot study involving 10 subjects.
  - Arm 1 (galyfilcon A, Acuvue® Advance™, Vistakon, Inc.)
  - Arm 2 (lotrafilcon B, O_2 Optix™, CIBA Vision, Inc)
  - For both arms, subjects wore lenses on four consecutive days for eight hours. The subjects returned to the clinic for contact lens removal.
  - Each morning, a new pair of CLs was applied.
  - Each day, the 10 contact lenses were pooled and stored in a randomly assigned care solution for 24 hours (lenses were not rubbed or rinsed prior to pooling).

    After the care solution extraction, the 10 lenses were then discarded and all subsequent testing was done on the protein containing solutions.

Proteomic Analyses

- Extraction and Precipitation
  - As mentioned above, proteins were extracted by the contact lens care solutions.
  - Protein was precipitated from the care solutions by the addition of trichloroacetic acid or acetone.
  - Precipitated protein was pelleted by centrifugation and resuspended in water.
- Quantification: Bradford Assay
  - Coomassie Blue G250 was added to an aliquot of each sample.
  - The total concentration of protein was determined using a spectrophotometer by measuring absorbance at 595 nm.
  - Total protein quantities were determined by extrapolating the concentration data.
  - Data were averaged for each solution given the number of lenses.
- Identification: Chromatography and Mass Spectrometry

  1) Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis (SDS-PAGE)
     - One-dimensional gel electrophoresis was used on each sample to analyze the distribution of protein extracted by the CL care solutions.
     - The gels were subsequently stained using the Invitrogen SilverQuest™ Silver Staining Kit.
     - The result was a side-by-side comparison of visible bands of protein present in each sample solution. (See figure 2).

  2) Liquid Chromatography Tandem Mass Spectrometry (nano LC-MS/MS)
     - This technique was used to examine bands of interest that arose from SDS-PAGE.
     - The bands were excised from the gel, treated with trypsin and analyzed by nano LC-MS/MS for mass analysis and fragmentation of the peptide to generate sequence information.
     - Sequence information from the MS/MS data was processed by converting the raw data files into a merged file (.mgf) using MGF creator (merge.pl, a Perl script).
     - The resulting mgf files were searched using Mascot Daemon by Matrix Science (Boston, MA). Data processing was performed following published standard guidelines.9
     - Assigned peaks had a minimum of 10 counts (S/N of 3). The mass accuracy of the precursor ions was set to 2.0 Da given that the data were acquired on an ion trap mass analyzer and the fragment mass accuracy was set to 0.5 Da.
     - Considered modifications (variable) were methionine oxidation and carbamidomethyl cysteine.
     - Protein identifications were checked manually, and proteins with a Mascot score of 40 or higher with a minimum of two unique peptides from one protein having a -b or -y ion sequence tag of five residues or better were accepted.

RESULTS

Quantification

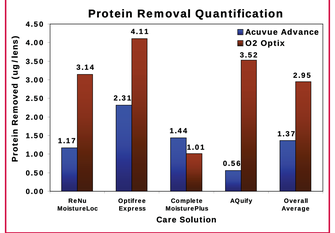

Figure 1. The figure shows the average protein amount per lens removed from each lens type with each solution as determined by Bradford Assay. Results are reported in units of µg/lens.

Identification

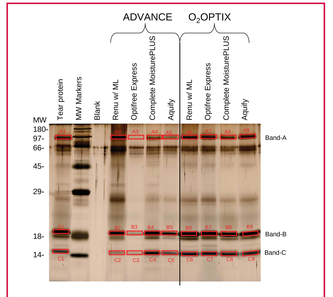

Figure 2. 1-D SDS-PAGE followed by silver staining yielded the gel displayed. The protein bands within each sample are separated on the basis of molecular weight and are compared to the standard tear protein and molecular weight columns on the far left. The eight columns on the right represent proteins extracted by the four care solutions for each lens type. The outlined bands were excised for trypsin digestion/LC-MS/MS.

DISCUSSION

Lens Comparisons

- O_2Optix lenses average more than twice as much protein removed per lens (2.95 µg/CL) compared with Acuvue Advance lenses (1.37 µg/CL).
  - These quantities correspond with a recent in vitro lysozyme experiment by Subbaraman et al. which used a radioactive gamma counter method to determine protein mass bound to SH lenses after 12 hours (0 to 2µg/lens).[7]
- Although more total protein was removed from O_2Optix, Acuvue Advance (total unique proteins = 12) was associated with slightly more individual (non-redundant) proteins than O_2Optix (n = 10).
- Several factors may be related to these findings:
  - The O_2Optix polymer may interact and bind with certain tear proteins more readily than the Acuvue Advance polymer.
  - Proteins may be more difficult to remove from Acuvue Advance than O_2Optix (i.e., the proteins bind deeper or at greater strengths).
  - While it is difficult to say which of these theories is more likely, previous studies on hydrogel lenses indicate that when more protein is deposited on a CL, a greater quantity will also be extracted.[4] This observation is more consistent with the theory that O_2Optix is prone to slightly greater protein deposition.

Solution Comparisons

- OPTI-FREE® EXPRESS® removed the greatest quantity of protein per lens with both lens materials.
  - Mok et al. also showed that OPTI-FREE® EXPRESS® appeared more efficacious when compared to ReNu MultiPlus and Solo Care Plus in removing total protein.[10]
- OPTI-FREE® EXPRESS® and ReNu® with MoistureLoc® both yielded 11 unique proteins (across both materials) and also yielded the highest quantity of protein removed (again, across both materials).
  - The higher protein quantities might be due to the removal of these additional proteins.

Deposition Proteome

- Gel Electrophoresis (Figure 2)
  - The distribution of contact lens extracted proteins present in many samples closely resembled that of tears.
  - However, gel electrophoresis results need to be interpreted with caution relative to identification. Bands of interest should be excised and identified by mass spectrometry for confirmation.
    - As discussed below, many proteins were present within the bands (on the lenses) that were not present in a representative tear sample.
- Mass Spectrometry (Table 1)
  - In total, 16 unique individual proteins were identified in this study.
  - The results of LC-MS/MS were moderately consistent with those of a tear protein standard and were consistent with molecular weights as matched on the gels.
  - In some instances LC- MS/MS showed no proteins identified, while in other bands, two or three additional proteins were recorded that were not present in the standard tear sample.
  - Lactoferrin, lipocalin, and lysozyme were consistently present in the protein samples removed from both lens types.
  - Care solutions more consistently removed these three proteins from O_2Optix lenses, whereas more unique proteins were associated with Acuvue Advance (as noted above).
  - Keratin complexes are also consistently identified at varying MWs (possibly due to fragments or homopolymers) mostly on O_2Optix. These keratin complexes are not identified in the analogous galyfilcon lipocalin bands, suggesting a stronger binding affinity to O_2Optix than Acuvue Advance.
  - O_2Optix, which has a higher modulus and less lubricity compared to galyfilconA, may be more prone to mechanical interaction with the conjunctival epithelium leading to the deposition of keratin complexes.
  - This study also indicated that after only 8 hours of wear, many known tear proteins are already deposited on SH lenses in measurable quantities such that upon removal and subsequent testing, protein spectra very similar to a standard tear sample are observed.

Table 1. Nano LC-MS/MS identification of protein in bands excised from gel samples.

MW (amu)	Standard tear sample	ReNu® with MoistureLoc®		OPTI-FREE® EXPRESS®		COMPLETE® MoisturePLUS™		AQUIFY®	
		ACUVUE® ADVANCE™	O_2OPTIX™	ACUVUE® ADVANCE™	O_2OPTIX™	ACUVUE® ADVANCE™	O_2OPTIX™	ACUVUE® ADVANCE™	O_2OPTIX™
97	• Lactoferrin	• Lactoferrin • Poly Ig Receptor • Ig Alpha	• Lactoferrin	• Keratin 2a • Keratin 10 • Keratinocyte proline-rich protein • Ifapsoriasin	• Lactoferrin • Keratin 1 • Keratin 6	• Lactoferrin	• Lactoferrin	• None Identified	• Lactoferrin • Poly Ig receptor
18	• Lipocalin	• Lipocalin • Prolactin induced protein	• Lipocalin • Keratin 1 • Cytokeratin 9 • Prolactin-induced protein	• Lipocalin	• Lipocalin • Cytokeratin 2	• Lipocalin	• Lipocalin	• Lipocalin	• Lipocalin • Cytokeratin 9 • Keratin complex 2
14	• Keratin 1 • Lysozyme • Keratin 6	• Keratin 1 • Keratin 10 • Lysozyme • Fatty acid binding protein	• Lysozyme	• None Identified	• Lysozyme	• Lysozyme	• Lysozyme	• Lysozyme	• Lysozyme
TOTAL UNIQUE (Non-redundant) PROTEINS		9	6	6	6	3	3	2	6
		11		11		3		6	

*16 unique proteins identified in total (12 from Acuvue Advance and 10 from O_2Optix).
*Lactoferrin, lipocalin, and lysozyme were the most commonly observed proteins (observed in at least 6 of 8 identifications) in this one day (8 hour) study.

SUMMARY

To our knowledge, this sequence of testing and analysis for the deposition proteome of tear proteins on contact lenses has not been reported in literature.

This study shows new insights into proteins present in the lens deposition proteome, in addition to showing quantitative differences associated with lenses and care solutions.

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