Quantity and Conformation of Lysozyme Deposited on Conventional and Silicone Hydrogel Contact Lens Materials Using an *in vitro Model*

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Introduction & Purpose

- Protein deposits can result in discomfort, reduced visual acuity and giant papillary conjunctivitis. [1-4]
- Silicone hydrogel (SH) lenses exhibit differing deposition profiles to that seen with conventional lenses. [5-9]
- In our previous work investigating protein deposition on SH materials, [5-7] we have used an extraction buffer developed by Keith and colleagues. [10] However, this buffer is not compatible with all SH materials (data on file).
- The purpose of this study was to:
  - determine the quantity and activity of hen egg lysozyme (HEL) deposited on conventional and SH materials using an *in vitro* model.
  - investigate the ability (and compatibility) of a new modified extraction buffer consisting of 50:50 acetonitrile: 0.02% trifluoroacetic acid to extract protein from certain SH contact lens materials.

Methods & Materials

- Conventional Materials:
  - Acuvue® 2 - Etafilcon A; Group IV; AV2
  - Proclear® Compatibles - Omafilcon A; Group II; PC
- SH Materials:
  - Acuvue® Advance™ - Galyfilcon A; Group I; AA
  - Acuvue® OASYS™ - Senofilcon A; Group I; AO
  - Focus® Night & Day™ - Lotrafilcon A; Group I; FND
  - O_2Optix™ - Lotrafilcon B; Group I; O2
  - PureVision™ - Balafilcon A;Group III; PV
- Lenses (n=6) were doped *in vitro* in PBS (pH 7.4) containing HEL (Sigma; 2mg/ml) for 17 days at 37°C with constant shaking.
- Following doping, lenses were rinsed briefly with 1X PBS to remove any residual HEL.
- Rinsed lenses were placed in Kimble vials filled with the extraction buffer, as seen in Table 1.

Table 1: Optimal extraction buffer and volumes

Lens	Buffer	Volume (ml)
Acuvue 2	ACN:0.2% TFA	4
PureVision	ACN:0.2% TFA	1.5
Focus Night&Day	ACN:0.2% TFA	1.5
O2 Optix	ACN:0.2% TFA	1.5
Acuvue Advance	ACN:0.02% TFA	1.5
Acuvue OASYS	ACN:0.02% TFA	1.5
Proclear	ACN:0.2% TFA	1.5

- The vials with the lenses were stored in the dark for 24 hours to allow for extraction of HEL from the lenses.
- AO, FND, and O2 were also extracted with 1.5ml buffer containing 200μg bovine serum albumin, due to the low mass of protein present.
- Extracts were lyophilized to dryness and quantified for total HEL protein and activity.
- Lyophilized extracts were re-suspended in dilution buffer (DB; 10mM Tris, pH 8.0 plus 1mM EDTA).
- Percent activity was assayed using a micro *Micrococcus lysodeikticus* assay with HEL as the standard (HEL standard was subject to the same conditions as the lenses).
- Western blotting was performed to determine total HEL protein using HEL as the standard, as seen in Figure 1.
- Immunoreactivity was visualized with ECL Plus chemiluminescent substrate. Optical densities of the resulting bands were quantified from digitized images on a Molecular® Dynamics Storm™ 840 Imaging System using ImageQuant™ 5.1.

Figure 1: Schematic of method

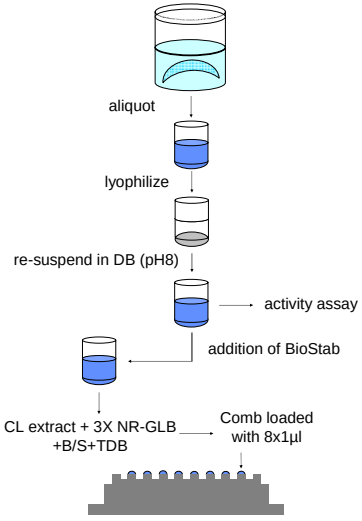
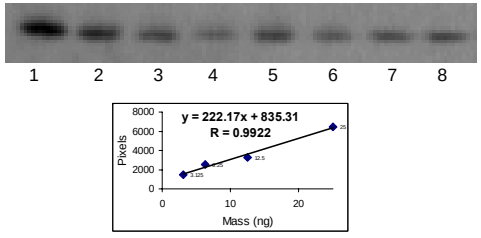


Figure 2: Typical Western Blot Showing Amount of HEL extracted, with standard curve plot below using lanes #1-4 for HEL standard (Lane 1: 25ng; Lane 2: 12.5ng; Lane 3: 6.25ng; Lane 4: 3.125ng). Lanes 5-8 consist of Acuvue OASYS samples. The standard curve plot below was used to quantify total HEL adsorbed onto contact lenses.



Results

- Lysozyme deposited on AV2 exhibited the greatest activity (91±5%) and this was statistically different from all other lens types ($p<0.001$), as shown in Figure 3.
- The lowest activity of the lysozyme deposited was found on FND (24±5%) and O2 (23±11%). Lysozyme deposited on other lens materials exhibited intermediate activity (AA, 60±15; AO, 51±9; PV, 58±8; and PC, 38±3%.
- In terms of total lysozyme accumulation, AV2 showed the most, with 1800μg, PC and PV the next with 68μg and 44μg respectively. FND deposited the least, with 2μg. AO, O2, and AA accumulated similar amounts of lysozyme, approximately 6 - 9μg, as shown in Figure 4.

Figure 3: Percent Activity

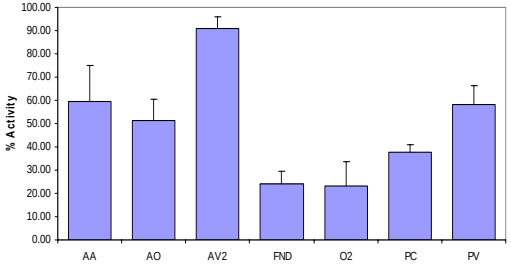
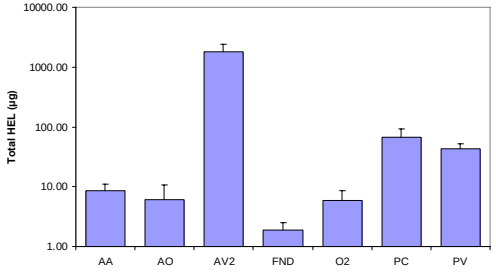


Figure 4: Total Lysozyme



Conclusions

- Silicone hydrogels deposit lower amounts of lysozyme than either conventional Group II (PC) or Group IV (AV2) lenses, and the level of lysozyme denaturation varies with the composition of the SH material.

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