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Surface versus bulk activity of lysozyme deposited on hydrogel contact lens materials *in vitro*

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ABSTRACT

Purpose: To determine and compare the levels of surface versus bulk active lysozyme deposited on several commercially available hydrogel contact lens materials.
Methods: Hydrogel contact lens materials [polymacon, omafilcon A, nelfilcon A, nesofilcon A, ocufilcon and etafilcon A with polyvinylpyrrolidone (PVP)] were incubated in an artificial tear solution for 16 h. Total activity was determined using a standard turbidity assay. The surface activity of the deposited lysozyme was determined using a modified turbidity assay. The amount of active lysozyme present within the bulk of the lens material was calculated by determining the difference between the total and surface active lysozyme.
Results: The etafilcon A materials showed the highest amount of total lysozyme activity (519 ± 8 μg/lens, average of Moist and Define), followed by the ocufilcon material (200 ± 5 μg/lens) and these two were significantly different from each other ($p < 0.05$). The amount of surface active lysozyme on etafilcon and ocufilcon lens materials was significantly higher than that found on all other lenses ($p < 0.05$). There was no active lysozyme quantified in the bulk of the nelfilcon material, as all of the active lysozyme was found on the surface (1.7 ± 0.3 μg/lens). In contrast, no active lysozyme was quantified on the surface of polymacon, with all of the active lysozyme found in the bulk of the lens material (0.6 ± 0.6 μg/lens).
Conclusions: The surface and bulk activity of lysozyme deposited on contact lenses is material dependent. Lysozyme deposited on ionic, high water content lens materials such as etafilcon A show significantly higher surface and bulk activity than many other hydrogel lens materials.

1. Introduction

Any time that biomaterials are exposed to bodily fluids there is potential for proteins to adsorb [1–6]. This adsorption can occur within seconds [7] and proteins may subsequently undergo structural changes (denature) to lower the energy of the protein-substrate system [8]. Proteins denature more readily when they are exposed to a hydrophobic surface compared to hydrophilic surfaces [9]. Denatured proteins on biomaterials can lead to serious complications, including thrombosis, bacterial adhesion, and inflammation [10]. For contact lens wearers, protein deposition on lenses and its subsequent denaturation can cause patient dissatisfaction [11,12], and inflammatory reactions such as giant papillary conjunctivitis (GPC), characterized by mucous discharge, redness, conjunctival swelling, and discomfort [13–15].

Proteins in complex mixtures, such as tears, interact with surfaces, such as contact lenses, whereby the highest mobility proteins are first absorbed. This layer is later replaced by lower mobility proteins that have a higher affinity to the surface. This phenomenon is known as the Vroman effect and first described the interaction of blood serum proteins with artificial surfaces [16,17]. The factors that ultimately influence the level and type of protein deposition are the contact lens material characteristics, such as pore size [18] water content [19,20], hydrophobicity [21] and surface charge [18,22,23].

Over 1500 proteins have been detected in tears by high performance liquid chromatography–mass spectrometry (HPLC–MS) [24], with the major proteins identified as lysozyme, lactoferrin, lipocalin, secretory immunoglobulin A (sIgA) and serum albumin. Lysozyme has been the main focus of protein adsorption studies as it accounts for 30% of all protein in tears [25,26]. Lysozyme is present in nasal secretions, tears and sputum and has been shown to have antimicrobial activity against *Micrococcus lysodeikticus* [27]. Information about the protein state (active versus denatured) of lysozyme extracted from contact lenses can be

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ascertained from the classical micrococcal turbidity assay which measures the activity of the lysozyme on its substrate, the bond between *N*-acetylmuramic (NAM) acid and *N*-acetylglucosamine (NAG), found in the cell wall of *Micrococcus lysodeikticus* [7,23,28–33]. Recently, Hall et al. [7,34] reported on modifications to the turbidity assay that allow discrimination between adsorbed lysozyme (active and denatured) on the contact lens surface and in the bulk of the contact lens material. This is an important distinction, since protein on the surface of the lens material will be more likely to interact with the ocular surface and thus more likely to cause adverse reactions. These studies [7,34] reported on the surface and bulk activity in one or two protein systems, but the tear film is far more diverse and contains many proteins, lipids, and mucins [35,36]. To better understand what happens *in vivo* there is a need to understand how the conformational state of a protein would change when exposed to other tear film components.

Daily disposable lenses (DD), which are removed and discarded at the end of each day, are increasing in popularity and now account for 23–31% of all fits and refits in the US [37] and 22% in the world [37]. Despite the widespread use of DD lenses, many protein adsorption studies focus on materials with 1–4 week replacement schedules [38–45]. The lack of data on deposition of lenses that are replaced every day is likely due to the assumption that DD lenses should have less adverse reactions from deposits due to their short replacement period, but there tends to be no difference in comfort, lens awareness, or dryness compared to extended wear lenses [46,47]. This would suggest that the conformational state of the deposited protein has a greater influence on comfort than the amount of protein deposited [48].

In this study we aim to quantify the activity of lysozyme deposited on the surface and in the bulk of various daily disposable and frequent replacement hydrogel contact lenses.

2. Materials and methods

2.1. Preparation of contact lenses

The contact lenses evaluated in this study are shown in Table 1. All contact lenses tested were soaked in phosphate buffered saline (PBS; 137 mM sodium chloride, 2.7 mM potassium chloride, 11.9 mM phosphates, pH 7.4) overnight on a shaker to remove any residual blister pack solution. After 24 h of pre-treatment, three to six contact lenses of each type were rinsed in PBS for 10 s, dabbed dry on lens paper, placed in 6 mL glass vials filled with 1.5 mL of the artificial tear solution (ATS, pH 7.4 [49]) detailed in Table 2, and incubated at 37 °C for 16 h. To measure total lysozyme, four replicates of each lens type were incubated in 1.5 mL of ATS (pH 7.4) containing trace amounts of less than 1 μg/ml of ^{125}I radiolabeled lysozyme. Use of radiolabelled proteins has been shown to not alter binding characteristics to biomaterials [50]. At the end of the incubation period, each set of lenses was rinsed twice in PBS, blotted on lens paper, and used to analyze total lysozyme, total lysozyme activity, or surface-adsorbed lysozyme activity.

Table 2
Artificial tear solution components.

Salts (mg/mL)
NaCl (5.26)
KCl (1.19)
Na_2CO_3 (1.27)
$KHCO_3$ (0.30)
$CaCl_2$ (0.07)
$Na_3C_6H_5O_7$ (0.44)
Urea (0.072)
Glucose (0.036)
Na_2HPO_4 (3.41)
HCl (0.94)
ProClin 300 (200 μL/L of solution)
Lipids (mg/mL)
Oleic acid (0.0018)
Oleic acid methyl ester (0.012)
Triolein (0.016)
Cholesterol (0.0018)
Cholesteryl oleate (0.024)
Phosphatidylcholine (0.0005)
Proteins (mg/mL)
Albumin (0.20)
Lysozyme (1.90)
Mucin (0.15)
Lactoferrin (1.80)

2.2. Determination of total lysozyme

Lenses (n = 4) soaked in ATS containing ^{125}I-labeled lysozyme, were placed in sterile 5 mL (12 × 75 mm), non-pyrogenic, polypropylene round bottom tubes. Radioactive counts were determined using a Gamma Counter (Perkin Elmer Wallac Wizard 1470 Automatic Gamma Counter, Wellesley, MA, USA). Radioactive counts per minute were converted to μg lysozyme per lens by interpolation of a standard curve containing known amounts of lysozyme.

2.3. Determination of total lysozyme activity

Lysozyme was extracted from lenses using a 0.2% trifluroacetic acid/acetonitrile (TFA/ACN; 50/50) extraction solution as described previously [20,28,38,51–53]. In brief, all lens materials were placed in 1.5 mL of the extraction solution, with the exception of the two etafilcon A lens types, which were placed in 4.0 mL of the extraction solution due to their high lysozyme deposition. Deposits extracted from all samples were then dried down using a Savant Speed Vac (Halbrook, NY, USA) and stored at −80 °C. Samples were reconstituted in PBS and the activity of the deposited lysozyme was quantified using a micrococcal activity assay, as previously described [34,52]. Briefly, the absorbance of the micrococcal solution (30 °C) was measured at 450 nm every 30 s for up to 15 min. The linear rate of change in absorbance at

Table 1
Conventional hydrogel contact lens materials evaluated in the study.

Conventional Hydrogel							
USAN[a]	nesofilcon A	nelfilcon A	omafilcon A	ocufilcon B	etafilcon A	etafilcon A	polymacon
Manufacturer	Bausch & Lomb	Alcon	Cooper Vision	Cooper Vision	Johnson & Johnson	Johnson & Johnson	Bausch & Lomb
Commercial Brand	BioTrue	Dailies Aqua Comfort Plus (DACP)	ProClear Dailies	ClearSight 1 Day (Biomedics)	1 Day Acuvue Moist (AV Moist)	1 Day Acuvue Define (AV Define)	SofLens 38
Water Content (%)	78	69	60	53	58	58	38
FDA group[b]	II	II	II	IV	IV	IV	I

[a] All lenses studied belong to the daily disposable category (except for polymacon, which belongs to daily wear modality).
[b] FDA classification: Group I: non-ionic, low water content, Group II: non-ionic, high water content, Group III: Ionic, low water content, Group IV: Ionic, high water content.