Lysozyme adhesion to the four major types of contact lens materials

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Contact lenses are widely used for the treatment of vision problems worldwide. The adhesion of tear proteins to contact lenses contributes to lens contamination and deterioration. There are roughly 60 different proteins in human tears. Five types of proteins predominate: immunoglobulins, albumin, transferrin and lactoferrin, lysozyme, and tear-specific peptidoglycan (tear lipocalin).1-4 The adhesion of tear proteins to contact lenses arises from an interaction between the cornea of the eye and the plastic surface of the lens, leading to the production of tears. Tears are the first and principal refracting surface of the eye, and the surfaces of the ocular conjunctiva and cornea are kept clean and lubricated because of the fluid washing over them, which arises from the corneal epithelium. In addition to proteins, it is estimated that an average 140-300 cells are shed each minute from the basal epithelial layer of the cornea and enter the tear film.5

The frequency of corneal inflammation or infection increases for those wearing contact lenses because of the tear protein deposits on the lenses. This can produce significant discomfort and possible visual impairment. For example, the adherence of Staphylococcus aureus to contact lenses increases when the lenses have lysozyme bound to them.6 One way to ameliorate such problems is to examine the interaction of specific tear proteins with different groups of contact lenses in order to determine which contact lens material has the lowest tear protein adhesion. Studying tear protein interaction with contact lenses provides important and informative findings for optometrists and their patients in their quest to find more durable, safer, and comfortable contact lenses.

The adhesion of proteins to contact lenses is contingent on the type of material used for the contact lens, and fluctuates with tear secretion and pathology.7 There are four types of contact lens materials in use today: FDA Group I (low water, nonionic), FDA Group II (high water, nonionic), FDA Group III (low water, ionic), and FDA Group IV (high water, ionic). Preliminary studies in the authors’ laboratory5,8 have indicated that tear protein adherence depends on the ionic or nonionic nature of the contact lenses. It was also shown that high water ionic (Group IV) lenses accumulate more proteins over time than low water nonionic (Group I) lenses. This difference is probably due to the affinity of lysozyme for negative charges on the contact lens material.9

Preliminary studies using human-worn contact lenses revealed that there is considerable variation in the adhesion of tear proteins to contact lenses, both between and within individuals. Additionally, tear proteins were extracted from the contact lenses using a solution of water and ace-tonitrile (50:50) in small glass vials, and it became a matter of concern whether the extracted proteins would adhere to the walls of the vial, and thus alter the authors’ estimate of tear protein adhesion to lenses. They also wanted to develop a tear protein adhesion assay that could be conducted by undergraduate students as part of an independent study research program.

Subsequently, the adhesion of lysozyme contact lens examinations was examined by incubating never-worn Type IV lenses in a saline solution containing a known concentration of lysozyme for a five-day period in borosilicate glass vials.10,11 The adhesion of the lysozyme to the lenses was monitored using HPLC and bicinchoninic acid (BCA), and the concentration of lysozyme in the vials was monitored using both spectrophotometry at 280 nm and BCA. Results indicated that the lysozyme adhered to the lenses in an up-down pattern, dropping during days two and three, and then climbing again to initial levels by day five. The BCA results were less dramatic than the HPLC-derived results but, in contrast to the latter, were statistically significant (p < 0.05). Likewise, the BCA assay was more precise at measuring lysozyme concentration in the vials than spectrophotometry, and again indicated a statistically significant (p < 0.05) drop in lysozyme concentration in the vials over the five days, presumably due to adhesion to the lenses. The purpose of this experiment was to examine lysozyme adhesion to contact lenses in all four FDA groups.

Experimental

Lysozyme (Sigma Chemical Co., St. Louis, MO) was weighed to the nearest 0.1 mg and dissolved in 90 mL of sterile saline to create a solution containing 2.0 mg/mL. Then, 5.0 mL of the lysozyme solution was placed in each of 15 6.0-ml borosilicate glass vials (Cole-Parmer Instrument Co., Vernon Hills, IL). The vials were divided into five groups of three replicates. The first group of three vials (1A, 1B, and 1C) did not contain lenses, and served as controls for the experiment. The second, third, fourth, and fifth groups of vials (2A–2C, 3A–3C, 4A–4C, and 5A–5C) received contact lenses. Following are the contact lenses used in the experiment. Type I: Optima FW disposable lenses (Bausch and Lomb, Inc., Rochester, NY); Type II: Soflens one-day disposables (Bausch and Lomb); Type III: PureVision continuous-wear disposables (Bausch and Lomb); and Type IV: AcuVue disposables (Vistakon, Jacksonville, FL). For the BCA assay, aliquots (10 µL) of the contents of the vials were added to 1,990 mL of distilled water for a final volume of 2.0 mL. Then, 1.0 mL of this mixture was added to 1.0 mL of BCA reagent (Pierce Chemical Co., Rockford, IL) for color development. Lenses were placed in 1.0 mL of distilled water, and then 1.0 mL of BCA reagent was added for color development. These mixtures were incubated at 60 °C for 1 h to allow color development, and then the absorbance was measured at 562 nm using a model 8453 UV-VIS spectrophotometer (Agilent Technologies, Palo Alto, CA). The lysozyme content of each vial was monitored on day 1 of the experiment. On day 2, the protein content of each vial was determined, and the lenses were removed from the vials in group 2 for quantification of lysozyme adhesion. These lenses were then monitored daily until the conclusion of the experiment. A similar procedure was followed on days 3, 4, and 5 with the respective groups of vials. Incubation of vials and lenses during the experiment was at room temperature (25 °C). The mean ±SD was determined for each group of vials and lenses. A lysozyme standard curve was used to relate the absorbance of the

Figure 1 Lysozyme concentration in borosilicate glass vials over a representative five-day experiment. Although the average age concentration of lysozyme in some groups of vials changed, no changes were significant (p > 0.05).

Figure 2 Lysozyme adhesion to FDA Group I contact lenses during a period of five days (average of three contact lenses). Lenses kept in 2.0 mg/mL lysozyme for one day had the equivalent (lysozyme amount of adhesion as did lenses kept three to four days (p < 0.05), while lenses kept in lysozyme for two days had lower lysozyme adhesion (p < 0.05).
BIA assay to lysozyme content. Students’ t-tests were used to test for statistical significance between paired means.

Results
A calibration curve was used to relate the absorbance of the BCA assay to lysozyme concentration (not shown). Figure 1 displays the lysozyme concentration in the replicate groups of vials over a representative five-day experiment. On the first day, all vials contained the same amount of lysozyme. Vials in group 1 contained no contact lenses, and vials in groups 2–5 contained lenses. On day 2, lysozyme concentration was determined in all vials, and the lenses in the vials that contained lysozyme were examined to determine the amount of lysozyme adsorbed (see Figure 2). The same procedure was followed on days 3–5, and for the vials for all groups analyzed for lysozyme adsorption on day 5. As can be seen, there were no significant changes in lysozyme content in any of the groups (p > 0.05).

Figure 2 shows the adhesion of lysozyme to FDA Group I contact lenses in the vials over the five-day experiment. Lenses kept in 2.0 mg/mL lysozyme for 2–4 days had lysozyme adsorption equivalent to lenses kept for one day (p > 0.05), while lenses kept in lysozyme for two days had lower lysozyme adsorption (p < 0.05). Figure 3 shows the adhesion of lysozyme to FDA Group II contact lenses. Lysozyme adhesion to these lenses followed a complex pattern, with the lenses that were kept in lysozyme for four days had significantly more lysozyme than similar lenses kept in lysozyme for three days (p < 0.005) and had a similar amount of lysozyme as one-day lenses (p > 0.05).

Discussion
The results of this study show that the concentration of lysozyme in the vials and its adhesion to contact lenses varied over time and between contact lens types. In some experiments where the lenses adsorbed large amounts of lysozyme (FDA Groups I and IV; Figures 2 and 5, respectively), the concentration of lysozyme in the incubation vials decreased significantly (data not shown). In other experiments where the lenses absorbed less lysozyme (FDA Groups II and III; Figures 3 and 4, respectively), the concentration of lysozyme in the vials either did not decrease, or actually seemed to increase (Figure 1). The authors’ data are consistent with previous studies showing that FDA Group IV lenses adsorb more protein than other types of lenses,23 or when compared only to FDA Group II lenses.24 It is interesting that the pattern of lysozyme adhesion to the different types of contact lens materials is similar, regardless of the FDA group designation. In all cases, lenses adsorbed some lysozyme after one day of incubation, and then appeared to lose lysozyme after another day of incubation. The lenses then appeared to adsorb more lysozyme by the third day, only to lose some again by the fourth day. In some cases, these changes were statistically significant; in others they were not, but the trend is evident in all experiments. The mechanism whereby the lenses could adsorb and then deadsorb lysozyme remains a mystery, especially since the concentration of lysozyme in the incubation solution remained relatively constant. However, in some experiments, the concentration of lysozyme in the vials themselves appeared to change, indicating that lysozyme was adsorbing and then deadsorbing to the walls of the vials as well.

There is much about the dynamic interaction between tear proteins and contact lenses and vials that is not understood at this time. The authors have previously demonstrated significant differences in tear protein adhesion between individuals and among the two eyes of the same individual,13,14 as well as differences between contact lenses of differing composition.15,16 The in vitro experiments reported here were conducted in an attempt to minimize the impact of these other independent variables on protein deposition on lenses. However, the high degree of variability found in this study clearly indicates the necessity for further studies of the complex interactions between tear proteins and contact lenses and vials. References