

REVIEW

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Biological and Clinical Implications of Lysozyme Deposition on Soft Contact Lenses

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ABSTRACT

Within a few minutes of wear, contact lenses become rapidly coated with a variety of tear film components, including proteins, lipids, and mucins. Tears have a rich and complex composition, allowing a wide range of interactions and competitive processes, with the first event observed at the interface between a contact lens and tear fluid being protein adsorption. Protein adsorption on hydrogel contact lenses is a complex process involving a variety of factors relating to both the protein in question and the lens material. Among tear proteins, lysozyme is a major protein that has both antibacterial and anti-inflammatory functions. Contact lens materials that have high ionicity and high water content have an increased affinity to accumulate lysozyme during wear, when compared with other soft lens materials, notably silicone hydrogel lenses. This review provides an overview of tear film proteins, with a specific focus on lysozyme, and examines various factors that influence protein deposition on contact lenses. In addition, the impact of lysozyme deposition on various ocular physiological responses and bacterial adhesion to lenses and the interaction of lysozyme with other tear proteins are reviewed. This comprehensive review suggests that deposition of lysozyme on contact lens materials may provide a number of beneficial effects during contact lens wear.

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Key Words: contact lens, deposition, lysozyme, protein, tears

Contact lenses remain the most widely prescribed and successful biomaterial, with about 38 million wearers in the United States.¹ All biomaterials rapidly accumulate a biocompatible layer upon implantation,² with protein deposition being the most rapidly measurable event.^{3,4} Likewise, as soon as contact lenses are placed onto the ocular surface, contact lenses accumulate tear components, including proteins,⁵ lipids,⁶ and mucins.⁷ Traditionally, such deposition has been viewed negatively,^{8,9} as it has been believed that these deposits are deleterious, being associated with contact lens-related discomfort¹⁰ and/or triggering conjunctival immunological responses.^{11,12} More than

50% of lens wearers experience contact lens-related discomfort, with a significant number of them discontinuing lens wear,¹³⁻¹⁶ and contact lens deposition may be one of the potential reasons behind wearers ceasing lens wear.

Tears have a complex composition and several hundred tear proteins have been identified in the tear film.¹⁷ Among these, lysozyme is a protein found at a high concentration,¹⁸ which has a number of antibacterial and anti-inflammatory functions.^{19,20} This review specifically highlights the role of lysozyme in the tears and concentrates on its interaction with soft contact lenses, because this is the major protein depositing on contact lenses. In addition, this review describes the interaction of lysozyme with other major tear proteins, examines its relationship to contact lens comfort, and investigates the potential beneficial role of having high levels of lysozyme sorbed by contact lens materials.

Tear Film Composition and Structure

The tear film comprises lipids, proteins, glycoproteins, enzymes, ions, small molecules, and metabolites and serves as the first barrier of defense in the ocular system. Proteins are a major component of the tear film, providing a wide variety of antibacterial, anti-inflammatory, and nutritional roles. Zhou et al.¹⁷

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used high-performance liquid chromatography-mass spectrometry to detect more than 1500 proteins in tears. Among these proteins, lysozyme, lactoferrin, lipocalin, secretory immunoglobulin A, and serum albumin are the most highly abundant proteins. Of these, lysozyme has been the most extensively studied, because this protein is present in high quantities (ranging from 1.2 to 4.6 mg/mL),^{18,21} has important antibacterial properties, and deposits significantly onto soft contact lens materials.

Lysozyme Activity and Structure

Lysozyme was first discovered by Alexander Fleming in 1922. Fleming found that lysozyme is present in many biological tissues, including nasal secretions, tears, and sputum.¹⁹ Lysozyme is a potent antimicrobial enzyme, which has been extensively studied in birds, mammals, plants, insects, and bacteria. Lysozyme was shown to have bactericidal activity against *Micrococcus lysodeikticus*,¹⁹ a fact that remains used today when examining lysozyme activity. Fleming observed the microscopic changes of *M. lysodeikticus* when exposed to tears and found that these bacteria rapidly lost their sharp outlines, became swollen, and gradually disappeared.¹⁹ Lysozyme kills bacteria by catalytic hydrolysis of their cell wall peptidoglycan, which contains sugars and amino acids. The sugar component consists of β -(1,4)-linked *N*-acetylglucosamine and *N*-acetylmuramic acid and this bond is broken by lysozyme.²² This compromises the bacterial cell wall and causes the bacterium to burst under the high internal osmotic pressure. Lysozyme has two major domains, in addition to one active site. The alpha domain of the molecule is made up primarily of alpha helices, whereas the beta domain contains the beta sheets and a few helices. In lysozyme's amino acid chain, glutamine and aspartic acid are two amino acids that are critical to the activity of this enzyme. Lysozyme is a small protein with 14.5 kDa molecular weight and a pI (isoelectric point) of 11.4 (making it highly positively charged at neutral pH), is relatively small compared with other major tear proteins (45 \times 30 \times 30 \AA), and contains 129 amino acids.²³

Lysozyme Interaction with Other Tear Proteins

Lysozyme in the tear film interacts with several other major proteins found in tears, notably lipocalin and lactoferrin.²⁴ Lipocalin can excavate lipids from the ocular surface and also transport various lipids in the tear film, including cholesterol, phospholipids, and fatty acids.²⁵ Lactoferrin interacts with lipopolysaccharide in cell membranes of Gram-negative bacteria, increasing their membrane permeability and leading to eventual death.²⁶ Leitch and Willcox²⁶ indicated that lysozyme and lactoferrin in combination demonstrates a synergic effect against *Staphylococcus epidermidis*. Subbaraman et al.²⁷ demonstrated that the adhesion of *Pseudomonas aeruginosa* or *Staphylococcus aureus* to lactoferrin-coated silicone hydrogel or etafilcon A lenses increased, with lactoferrin showing an antimicrobial effect against the attached *P. aeruginosa* strains. Lactoferrin's antimicrobial effect against *P. aeruginosa* is clinically relevant, because this type of bacteria is the predominant causative agent that can induce microbial keratitis (MK), accounting for 40 to 70% of MK cases worldwide.²⁸ Previous studies have shown that lactoferrin changes the outer membrane of Gram-negative

bacteria,²⁶ suggesting that the synergic effect of lactoferrin and lysozyme on contact lenses would be beneficial from an antimicrobial standpoint. More research is warranted to understand the interaction between lactoferrin and lysozyme and their subsequent activity against Gram-positive and Gram-negative bacteria.

Lysozyme's Interaction with Contact Lens Materials

Once a contact lens is inserted onto the ocular surface, it immediately interacts with the tear film. The interaction of the various tear film components with the lens depends on both the component in question and the characteristics of the lens material. The United States Food and Drug Administration has classified hydrogel contact lenses as ionic (groups III and IV) and nonionic (groups I and II), with groups II and IV exhibiting a higher water content ($\geq 50\%$ water) and groups I and III containing materials with lower water contents. The newer siloxane-based materials (silicone hydrogels) have, as a general rule, lower water contents but higher oxygen permeabilities.

Many studies have investigated the interaction of lysozyme with contact lens materials. Tables 1 and 2 summarize the amounts of lysozyme deposition on silicone hydrogel and hydrogel contact lenses, respectively. Examination of these tables indicates that there is several orders of magnitude difference in the amount of lysozyme deposited and the reason for this warrants further examination.

Protein deposition on contact lenses is a complex process that is influenced by a wide variety of factors, including lens water content,^{38–40} protein and material charge,^{38,41} polymer type,^{30,42} protein and pore size,^{39,43} surface modification,^{30,34,35} and the type of care regimen used.^{42,44}

Water Content, Surface Charge, and Contact Lens Pore Size

Protein uptake is affected by the water content of the lens material and the interaction between the surface charge of the contact lens material and the charge of the tear film proteins under investigation. Negatively charged lens materials exhibit a specific affinity for positively charged proteins.^{38,45,46} Several researchers have shown that total protein deposition on group IV hydrogel lenses (high water content, ionic) is higher than other materials (typically $>1500 \mu\text{g}/\text{lens}$), with group I hydrogels (low water content, nonionic) depositing the least (10 to 20 $\mu\text{g}/\text{lens}$).^{5,46–48} Jones et al.⁶ studied the degree of protein deposition on *N*-vinyl-2-pyrrolidone-containing group II and group IV hydrogel contact lens materials and reported that protein sorption onto group IV lenses was higher compared with that adsorbed onto group II lenses. Sack et al.⁴⁵ reported that ionic lens materials could adsorb a thicker layer of protein compared with nonionic lenses, with the former sorbing primarily lysozyme. Baines et al.⁴⁹ showed that hydrogel lenses with a higher water content had higher levels of protein sorbed to them.

Contact lens materials have pore sizes that vary between 4 and 1700 \AA ,^{39,43,50} with higher water content materials having larger pore sizes.^{39,51} Low-water content, conventional hydrogel materials based on poly(2-hydroxyethyl methacrylate) (pHEMA; Food and Drug Administration group I) have a relatively small pore size,

TABLE 1.

Amount of lysozyme deposition on silicone hydrogel contact lens materials

| Lens type | Lysozyme $\mu\text{g}/\text{lens}$ | <i>Ex vivo/In vitro</i> | Care solution | Days worn or <i>in vitro</i> incubation time | Reference |
|---------------|------------------------------------|-------------------------|-------------------|---|-----------|
| Balafilcon A | 10.9 ± 2.9 | <i>Ex vivo</i> | Not reported | 14 | 29 |
| Balafilcon A | 10 ± 3 | <i>Ex vivo</i> | — | 30 | 30 |
| Balafilcon A | 10 ± 5 | <i>Ex vivo</i> | Opti-Free Express | 30 | 31 |
| Balafilcon A | 10 ± 3.5 | <i>Ex vivo</i> | ReNu | 30 | 31 |
| Balafilcon A | 13.3 ± 9 | <i>Ex vivo</i> | ClearCare | 14 | 32 |
| Balafilcon A | 17 ± 1.4 | <i>In vitro</i> | — | 4 h | 33 |
| Balafilcon A | 10.6 ± 1.6 | <i>In vitro</i> | — | 14 | 34 |
| Balafilcon A | 19.4 ± 2.9 | <i>In vitro</i> | — | 28 | 34 |
| Balafilcon A | 44 ± 10 | <i>In vitro</i> | — | 17 | 35 |
| Balafilcon A | 50 ± 0.1 | <i>In vitro</i> | — | 14 | 36 |
| Lotrafilcon A | 0.7 ± 0.5 | <i>Ex vivo</i> | Not reported | 14 | 29 |
| Lotrafilcon A | 3 ± 1 | <i>Ex vivo</i> | — | 30 | 30 |
| Lotrafilcon A | 2.7 ± 0.7 | <i>In vivo</i> | — | 14 | 34 |
| Lotrafilcon A | 4.2 ± 0.9 | <i>In vitro</i> | — | 28 | 34 |
| Lotrafilcon A | 2 ± 1 | <i>In vitro</i> | — | 17 | 35 |
| Lotrafilcon B | 3.7 ± 0.6 | <i>In vitro</i> | — | 14 | 34 |
| Lotrafilcon B | 6.1 ± 1.3 | <i>In vitro</i> | — | 28 | 34 |
| Lotrafilcon B | 6 ± 3 | <i>In vitro</i> | — | 17 | 35 |
| Lotrafilcon B | 9.7 ± 1.5 | <i>In vitro</i> | — | 14 | 36 |
| Sifilcon A | 2.4 ± 1.2 | <i>Ex vivo</i> | ClearCare | 90 | 37 |
| Galyfilcon A | 1.6 ± 0.8 | <i>Ex vivo</i> | Not reported | 14 | 29 |
| Galyfilcon A | 8 ± 3.4 | <i>In vitro</i> | — | 14 | 34 |
| Galyfilcon A | 16.8 ± 4 | <i>In vitro</i> | — | 28 | 34 |
| Galyfilcon A | 9 ± 2 | <i>In vitro</i> | — | 17 | 35 |
| Senofilcon A | 1.4 ± 1.4 | <i>Ex vivo</i> | ClearCare | 14 | 29 |
| Senofilcon A | 2.3 ± 2.5 | <i>Ex vivo</i> | Opti-Free Express | 14 | 29 |
| Senofilcon A | 1.5 ± 1.0 | <i>Ex vivo</i> | ReNu | 14 | 29 |
| Senofilcon A | 6.1 ± 3.2 | <i>In vitro</i> | — | 14 | 34 |
| Senofilcon A | 13.4 ± 4.1 | <i>In vitro</i> | — | 14 | 34 |
| Senofilcon A | 6 ± 5 | <i>In vitro</i> | — | 28 | 35 |

“—” denotes where no solution was used.

which should not be as favorable for proteins to penetrate into their matrix. Small proteins such as lysozyme (14.5 kDa) would be predicted to penetrate to a greater degree than larger proteins such as lactoferrin (82 kDa) and albumin (66 kDa). Relatively few studies investigating protein penetration into soft lens materials have been published. Refojo and Leong⁴³ used light microscopy to visualize the penetration of lysozyme into hydrogels and reported that lysozyme could penetrate slightly deeper into the more hydrated solution-polymerized pHEMA hydrogels than into the more compact bulk-polymerized gel. Garrett et al.³⁸ used confocal scanning microscopy to demonstrate the degree to which the small, positively charged protein lysozyme and the larger, more neutral charge protein albumin could penetrate into the matrix of a range of model soft lens materials comprising varying ionicities. Their results showed that for lysozyme, increased uptake was observed at higher degrees of ionicity, owing to a combination of electrostatic attraction between the negative material and positive protein, and increasing ease of penetration of the protein into the more porous hydrogel matrix. In contrast, albumin uptake was primarily by surface adsorption, with little diffusive penetration into the matrix. This was attributed to the larger molecular weight and lack of electrostatic attraction. They also demonstrated that the introduction of carboxymethyl charged

groups into pHEMA-based materials increased the water content of the material, resulting in a larger pore size.³⁸ Further work by Garrett et al.⁴⁶ compared lysozyme penetration into two commercially available group IV materials, etafilcon A (pHEMA and methacrylic acid) and vifilcon A (pHEMA, methacrylic acid, and polyvinyl pyrrolidone). They showed that lysozyme penetration was a direct function of charge density of the lenses, with a higher degree of penetration of lysozyme into etafilcon A than in vifilcon A lenses. They concluded that the properties of the lens materials, particularly charge density (ionicity) and porosity (water content), determine the type and rate of protein penetration. Using a protein dye (Coomassie Brilliant Blue) and lens sectioning, Okada et al.⁵² examined the penetration of lysozyme into etafilcon A lenses. Their results demonstrated that group IV lenses accumulated a considerably greater amount of lysozyme than did lenses of other groups and that lysozyme accumulated not only on the surface but also throughout the matrix of group IV lenses. Most recently, Luensmann et al.⁵³ used confocal microscopy to examine lysozyme penetration into the matrix of group IV hydrogels (etafilcon A) and a variety of silicone hydrogel materials. They showed that all pHEMA-based materials, notably etafilcon A, and the negatively charged silicone hydrogel material balafilcon A, accumulated lysozyme throughout the entire

TABLE 2.

Amount of lysozyme deposition on conventional hydrogel contact lens materials

| Group | Lens type | Lysozyme $\mu\text{g}/\text{lens}$ | <i>Ex vivo/In vitro</i> | Care solution | Days worn or <i>in vitro</i> incubation time | Reference |
|-------|---------------|------------------------------------|-------------------------|-------------------|---|-----------|
| I | Polymacon | 16 \pm 8 | <i>In vitro</i> | — | 14 | 34 |
| I | Polymacon | 23.2 \pm 9 | <i>In vitro</i> | — | 28 | 34 |
| II | Alphafilcon A | 44.5 \pm 13 | <i>In vitro</i> | — | 14 | 34 |
| II | Alphafilcon A | 53.3 \pm 11 | <i>In vitro</i> | — | 28 | 34 |
| II | Omafilcon A | 35.3 \pm 8 | <i>In vitro</i> | — | 14 | 34 |
| II | Omafilcon A | 43.8 \pm 13 | <i>In vitro</i> | — | 28 | 34 |
| II | Omafilcon A | 68 \pm 28 | <i>In vitro</i> | — | 17 | 35 |
| IV | Etafilcon A | 985 \pm 241 | <i>Ex vivo</i> | Opti-Free Express | 14 | 30 |
| IV | Etafilcon A | 935 \pm 271 | <i>Ex vivo</i> | Opti-Free Express | 30 | 31 |
| IV | Etafilcon A | 1551 \pm 371 | <i>Ex vivo</i> | ReNu | 30 | 31 |
| IV | Etafilcon A | 1433.5 \pm 76 | <i>In vitro</i> | — | 14 | 34 |
| IV | Etafilcon A | 1434.5 \pm 56 | <i>In vitro</i> | — | 28 | 34 |
| IV | Etafilcon A | 427.5 \pm 6.4 | <i>In vitro</i> | — | 1 | 33 |
| IV | Etafilcon A | 1800 \pm 600 | <i>In vitro</i> | — | 17 | 35 |
| IV | Etafilcon A | 2200.3 \pm 15.6 | <i>In vitro</i> | — | 14 | 36 |
| IV | Etafilcon A | 1852.1 \pm 19.16 | <i>In vitro</i> | Complete-No rub | 14 | 36 |
| IV | Etafilcon A | 1841.5 \pm 10.38 | <i>In vitro</i> | Complete-Rub | 14 | 36 |
| IV | Etafilcon A | 1666.1 \pm 15.83 | <i>In vitro</i> | ClearCare | 14 | 36 |
| IV | Vifilcon A | 356 \pm 48 | <i>In vitro</i> | — | 14 | 34 |
| IV | Vifilcon A | 512.3 \pm 51 | <i>In vitro</i> | — | 28 | 34 |

“—” denotes where no solution was used.

lens material. In contrast, the plasma-coated silicone hydrogel materials lotrafilcon A and lotrafilcon B only deposited lysozyme on the lens surface.⁵³

In summary, these studies demonstrate that high-water content, negatively charged materials such as etafilcon A rapidly adsorb and absorb high quantities of small, positively charged proteins such as lysozyme, with the protein penetration occurring throughout the lens material, even after only short periods of exposure. Furthermore, it has been shown that lysozyme that is adsorbed by the etafilcon A material could easily transport through the lens matrix and will be released onto the surface reversibly,⁴⁶ and it is speculated that this mobile lysozyme would be free to interact with bacteria or other tear components that are adhered to the lens surface.

Silicone Hydrogel Surface Modification and Protein Deposition

Previous studies have shown that most silicone hydrogel lens materials deposit 5 to 20 μg per lens of total lysozyme (Table 1). Certain silicone hydrogel materials undergo surface modification to improve their wettability and this surface modification influences the amount of lysozyme that deposits on them.^{30,34,35} Balafilcon A has a surface that undergoes plasma oxidation and lotrafilcon A and lotrafilcon B materials have a 25-nm-thick plasma coating, whereas senofilcon A and galyfilcon A have no surface modification but contain internal wetting agents to assist with wettability.^{54,55} Analysis of the total protein and total lysozyme extracted from silicone hydrogel lenses indicates that the amount of protein deposited on the lenses was affected by the presence or absence of a surface charge and the absence or type of surface treatment.²⁹ Deposition amounts on plasma-coated lotrafilcon A or lotrafilcon B are similar to those seen with poly(methyl methacrylate) (PMMA) lenses,³⁴ demonstrating

the low values of lysozyme deposited at such interfaces. Balafilcon A silicone hydrogel lenses deposited the highest amount of lysozyme, with galyfilcon A and senofilcon A materials depositing lysozyme at an intermediate level.³⁴ Jones et al.,³⁰ using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting, indicated that lysozyme was sorbed to etafilcon A at higher levels than a first-generation silicone hydrogel material (balafilcon A). Other investigators reported similar results.^{31,33,34}

In summary, published studies to date indicate that compared with conventional hydrogels, silicone hydrogel contact lenses deposit significantly lower levels of lysozyme. Among conventional hydrogel lenses, lysozyme deposition is lowest on group I lenses (low water content, nonionic), followed by group II (high water content, nonionic polymers), with the highest amounts of lysozyme being deposited on group IV (high water content, ionic) materials.

Lysozyme Denaturation on Contact Lenses

Proteins have a three-dimensional structure, which is controlled by a number of highly specific interactions, including hydrophobic forces, hydrogen bonds, and van der Waals forces. Once a biomaterial comes in contact with a biological fluid, such as blood or tears, proteins begin to adsorb to the surface.⁵⁶ Higher-affinity proteins adsorb to the surface in greater quantities than lower-affinity proteins, which could facilitate further protein adsorption.⁵⁷ Smaller proteins can move faster than larger proteins, and thus they adsorb to the surface of the biomaterial before the larger proteins.⁵⁷ This results in the surface being covered by a monolayer of protein before the arrival of host inflammatory cells. By the time cells arrive, they confront a monolayer of protein rather than the biomaterial surface itself.⁵⁷

Once proteins are adsorbed, they undergo conformational changes that depend on the hydrophobicity of the surface and occur to minimize Gibbs free energy.⁵⁶ Any change in the structure of a protein is termed *protein denaturation*, in which the protein is changed in some way that influences its function.⁵⁷ Protein denaturation is affected by a host of factors, including contact time of the protein with the substrate, chemical composition of the substrate, environmental pH, and temperature.^{56–59} It is well established that when proteins are exposed to a hydrophobic surface, they are more likely to denature than when exposed to more hydrophilic surfaces.^{46,58,60–62} Once lysozyme becomes bound to the contact lens surface, it undergoes conformational changes that result in the protein becoming denatured.^{29–31,35,37,63} Previous studies have suggested that denatured protein on contact lenses can be associated with palpebral lid changes.^{11,64,65}

To date, relatively few studies have examined protein denaturation, particularly on clinically sourced samples. Sack et al.⁴⁵ reported that most of the lysozyme that primarily adsorbed to ionic materials retained its enzymatic activity. Jones et al.³⁰ determined the activity of lysozyme deposited on group IV ionic hydrogel lens materials (etafilcon A) and silicone hydrogel contact lenses. They reported that etafilcon A lenses adsorbed the greatest amount of lysozyme compared with a variety of other lenses and that most of the deposited lysozyme retained its activity, with only 5 to 10% being denatured. In the same study, they suggested that although silicone hydrogels sorb a lower amount of lysozyme compared with conventional hydrogels, the level of lysozyme denaturation was higher in silicone hydrogels. Subsequent studies by other investigators, examining both *ex vivo* lenses and *in vitro* spoiled samples, reported similar results,^{31,35,66} with ionic materials consistently exhibiting higher levels of active lysozyme than nonionic materials. In a more recent study, researchers investigated the effect of lysozyme activity in the presence of a variety of tear film components using an *in vitro* model.⁶⁶ They found that in the presence of a major tear protein such as lactoferrin, or lipids, the activity of lysozyme deposited on conventional hydrogels such as etafilcon A was not affected.⁶⁶ These results suggest that there could be a synergistic effect between various tear film components, which could enhance the activity of tear proteins such as lysozyme and limit its denaturation. Clinically, these analytical results appear to make sense, because if these high levels of lysozyme denaturation were relevant, then it is expected that many subjects would exhibit inflammatory lid changes such as giant papillary conjunctivitis. However, etafilcon A has a significant market share among hydrogel lenses and previous studies have shown that patients can use etafilcon A for many years without such clinical problems being manifest.⁶⁷ Thus, it can be assumed that lysozyme, despite being present in large quantities, is active and does not induce inflammatory changes in the palpebral conjunctiva of patients using etafilcon A–based lenses.

Ultimately, of greatest interest is the role of these protein deposits on comfort and overall clinical performance. A recent study has demonstrated that there is a strong association between the activity of lysozyme and subjective comfort, during 1 day of wear of etafilcon A lens material.³² This study showed that there was no association between total lysozyme and total protein deposited on the etafilcon material and any other clinical signs and symptoms. These results suggest that the conformational state of the deposited protein has a greater influence on comfort than the amount of protein deposited, among the lens types tested.

Competitive Adsorption of Proteins onto Contact Lenses

The size and charge of proteins, along with the characteristics of the lens material, control the degree to which certain tear proteins deposit onto lenses. In addition, the initial deposition pattern that occurs may impact subsequent deposition of other tear components. This competitive adsorption profile is of significant relevance and importance to understanding contact lens deposition. In one of the earliest studies examining this, Bontempo and Rapp conducted a study that examined whether the presence of lipid or protein impacted the subsequent deposition of either component on a variety of soft lens materials.⁶⁸ They showed that the presence of lipid deposits on group IV lenses decreased the adsorption of lysozyme, whereas the presence of protein deposits reduced the amount of total lipid adhering to a group II lens. The sequential and competitive adsorption of some proteins onto hydrogel contact lenses was also examined by Sariri and Sabbaghzadeh.⁶⁹ They reported that the chemical composition of the lens and the charge of the previously adsorbed protein affected the sequential and competitive adsorption of proteins on a contact lens surface. They further suggested that if the electrostatic interaction is more favorable for the second protein, sequential adsorption can result in total replacement of the preadsorbed protein.⁶⁹ Based on these results, it could be speculated that the etafilcon A lens material, which deposits significantly high amounts of lysozyme compared with other lens materials, could potentially repel the deposition of other “unwanted” components from the tear film. However, work by Carney et al.⁷⁰ would refute this and further evidence is needed to understand this concept.

Impact of Lysozyme on Contact Lens Wettability

Contact lens wettability could be impacted by deposition. In an *in vivo* study, Tonge et al. showed that the wettability of etafilcon A lenses, as determined by contact angle assessment postwear, was not modified by up to 8 hours of eye wear.⁷¹ In an *in vitro* study, Cheng et al.⁷² used a captive-bubble technique to measure the advancing and receding contact angles of soft contact lenses and indicated that tear proteins did not impact the wettability of etafilcon A lens materials. Ketelson et al.⁷³ used a sessile drop method in another study investigating the impact of lysozyme on *in vitro* wettability. They also showed that adsorbed lysozyme on etafilcon A hydrogels had no influence on their wetting properties. Thus, to date, it appears that lysozyme deposition on etafilcon A has no impact on the wettability of these lens materials.

The Impact of Lysozyme on Bacterial Adhesion to Contact Lenses

The first step in the development of contact lens–related microbial keratitis is the exposure of the contact lens to pathogens.⁷⁴ Once exposed, bacterial colonization of any biomaterial occurs because of the engagement of bacterial adhesins on their surface with the biomaterial surface.⁷⁵ Once adhered, the bacteria can replicate on the lens to form microcolonies or biofilms.⁷⁶ Bacterial accumulation on contact lens surfaces has been associated with MK, contact lens acute red eye,⁷⁷ contact lens peripheral ulcers, and certain inflammatory keratitis events.⁷⁸ This is believed to occur because of the

adhered bacteria eventually binding to the corneal epithelium, followed by bacterial invasion into the corneal stroma, releasing inflammatory agents and initiating infection and inflammation.⁷⁴

The introduction of silicone hydrogel lens materials has significantly reduced hypoxia and hypoxia-related complications, owing to the dramatic increase in their ability to transmit oxygen.⁷⁹ However, the incidence of MK is unchanged compared with hydrogels,⁸⁰ and infiltrative events with silicone hydrogels appear to be higher than those seen with hydrogel materials.^{81,82} It is perplexing that the benefits of oxygen have not reduced the incidence of MK and may have actually increased inflammatory events. Apart from oxygen transport, another fundamental difference between hydrogels and silicone hydrogels relates to their deposition profile. As described previously, silicone hydrogel materials deposit substantially less protein, particularly lysozyme, than group IV materials such as etafilcon A.^{30,34} Dart et al.⁸³ investigated the risks of MK associated with daily disposable (DD) soft contact lenses and showed that patients who used the etafilcon A material had the lowest risk for MK when compared with other DD lenses. In addition, etafilcon A has been shown to significantly decrease the rate of sterile keratitis compared with other DD contact lenses.⁸⁴ Diec et al.⁸⁵ also reported the lowest rate of adverse events with an etafilcon A-based DD lens, when compared with other DD soft lenses.

It is interesting to consider whether a possible reason for the reduced rate of infiltrative events with conventional hydrogel lens materials, particularly ionic group IV lenses, is that these materials accumulate high levels of the antibacterial proteins lysozyme and lactoferrin. If these proteins remain active, then they may have the ability to reduce the viability of adherent Gram-positive and Gram-negative bacteria, resulting in reduced rates of infiltrative events and possibly MK. A recent study by Wang et al.⁸⁶ indicated that when PMMA intraocular lenses were coated with a hyaluronic acid-lysozyme (HA-lysozyme) composite, adherence of *S. aureus* and human lens epithelial cells on PMMA with HA- or HA-lysozyme-coated lenses was significantly reduced because of the hydrophilic property of HA. They also used a LIVE/DEAD bacterial viability kit and showed that the HA-lysozyme coating had bactericidal activity against *S. aureus*, which they attributed to the lysozyme coating. These results warrant further investigation with regard to the potential role of active proteins on soft lens materials and their protective effects on inflammatory responses.

CONCLUSIONS

Lysozyme is an antibacterial protein that is found in a relatively high concentration in the human tear film. The positive charge and small size of lysozyme result in it having a great affinity for negatively charged, group IV hydrogel lenses, in particular those with relatively high amounts of acidic groups, such as etafilcon A. Ionic hydrogel materials accumulate significantly more lysozyme than silicone hydrogel materials, with some ionic hydrogel materials accumulating upward of 100 times more lysozyme than certain plasma-coated silicone hydrogels. When deposited on etafilcon A, lysozyme retains most of its activity and is primarily located within the bulk of the lens rather than on the surface. This lysozyme can also freely move through the lens matrix, diffuse to the lens surface, and then interact with any adhered bacteria or

other tear contaminants on the surface of the lens material. Lysozyme deposition does not increase bacterial adhesion to lenses and does not reduce contact lens wettability, and it appears that lysozyme deposition only negatively impacts contact lens comfort after its denaturation. Moreover, other proteins such as lactoferrin are synergistic with lysozyme and have the potential to reduce the viability of Gram-negative and Gram-positive bacteria, which are involved in the pathogenesis of contact lens-related MK and inflammation.

Although deposition on contact lenses has traditionally been believed to be deleterious, a comprehensive review of the literature seems to suggest that for modern materials that are replaced in 4 weeks or less, the deposition of certain tear components such as lysozyme and lactoferrin on contact lenses may actually be beneficial to lens wear. This concept warrants further investigation, to determine what other components from the tear film are either beneficial or problematic for soft lens wearers.

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