Tear Film Proteins: Examining Production, Role and Interaction With Contact Lenses

The accumulation of tear film proteins historically has been viewed negatively, but instead of working to remove these proteins from contact lenses, perhaps we should consider maintaining them in their natural state.

By Philip Morgan, PhD

Hydrogel contact lenses have been used by millions of patients since their introduction nearly 40 years ago. Over time, multiple improvements in lens design have enabled more types of vision disorders to be corrected, and the development of new materials has led to increased comfort, which in turn allows patients to wear their lenses for longer periods of time. But the relationship between the eyes, the tear film and contact lenses and lens care solutions has not always been a harmonious one. Tear film proteins accumulate on contact lenses; historically, this has been viewed negatively, and lens care solutions have been designed to neutralize and remove proteins. Newer research has begun to view the purpose of these accumulated proteins from another angle. Instead of working to remove these proteins, what if they were maintained in their natural (and not denatured) state? Would that maintain a healthy ocular environment while still providing consistent vision correction? Let’s examine the three players in this relationship: the cornea, tear film and the contact lens and lens solutions.

Cornea
The cornea helps shield the rest of the eye from bacteria, dust and other foreign materials. It is also the eye’s outermost refracting surface, contributing between 65% and 75% of the eye’s total focusing power. The cornea is a highly organized substance consisting of cells and proteins. There are no blood vessels in this part of the eye, so it is reliant on

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tears and the aqueous humor that fills the chamber behind it to provide nourishment and protect it from infection. In order to refract light properly, the cornea must remain transparent. Vision may be adversely affected by cloudy or opaque areas that can exist in any of the five corneal layers (Figure 1).1

The five layers that make up the cornea are:

1. **Epithelium:** This is the cornea’s outermost region and constitutes approximately one-tenth of the cornea’s thickness. Its main functions are to: block the passage of environmental debris, bacteria, and other foreign materials into the eye and other corneal layers; provide a smooth surface that promotes the absorption of oxygen and cell nutrients from tears; and serve as a distributor of nutrients to the rest of the corneal layers. This outermost region of the cornea contains thousands of nerve endings that make the cornea extremely sensitive to pain when rubbed or scratched.1

2. **Bowman’s Layer:** This transparent layer of the cornea is comprised of collagen and lies directly below the basement membrane of the epithelium. If this layer is injured, resultant scars, if large enough and centrally located, can result in partial vision loss.1

3. **Stroma:** The next corneal layer is the stroma, which makes up almost 90% of the cornea’s total thickness, and is made up almost entirely of water (78%) and collagen (16%). The collagen content of the stroma provides the cornea with its strength and elasticity. The shape, arrangement and spacing of the collagen are the prime components of the cornea’s light-conducting transparency.1

4. **Descemet’s Membrane:** Beneath the stroma layer lies Descemet’s Membrane, which is a protective barrier against infection. This thin but strong sheet of tissue is made up of collagen fibers (different from those of the stroma) that are produced by the endothelial cells that lie below it.1

5. **Endothelium:** This innermost layer of the cornea is extremely thin and is essential for keeping the cornea clear. As a normal part of corneal function, fluid slowly leaks from inside the eye and into the stroma. However, if too much fluid collects in the stroma, it swells with water and becomes clouded. The endothelium’s primary function is to remove excess fluid from the stroma. Healthy eyes have a balance between the fluid entering the cornea and that which is pumped out. Endothelial cells are important, not only for the function they serve, but also because once they are lost, the body cannot replace them. If too many of these cells are destroyed, corneal edema and blindness may occur.1

**Tear Film**
The tear film has several functions: it works to maintain the health of the ocular surface, protects it against harmful agents, repairs damage, and creates a clear anterior refracting surface for clear vision.2 The structure of the tear film consists of three components: an innermost mucin layer; an aqueous component; and a lipid layer (Figure 2).

**The Mucin Layer**
The mucins are the product of the corneal and conjunctival cells and they contribute to the epithelial cell surface structure and anchor the overlying aqueous component. The mucins are primarily produced by goblet cells of the conjunctiva. This gel helps cleanse the eye surface by removing debris and bacteria, and by exfoliating cells.2

Figure 1. The five layers of the cornea.

Figure 2. The three components of tear film.
**The Aqueous Component** The most significant portion of the tear film by volume, the aqueous component is produced by the lacrimal glands and contains electrolytes and hundreds of proteins and peptides. To date, up to 491 proteins have been detected in human tear film, and four (secretory immunoglobulin A, lipocalin, lysozyme and lactoferrin) are thought to be present at high concentrations (Table 1). Many of the remaining proteins are present in smaller quantities or may be completely absent and only become present in response to disease, environmental stress, or injury.2

**The Lipid Layer** This layer is produced by the meibomian glands that are located in the eyelids and is responsible for impeding tear loss through evaporation.2 The lipid layer also works with the mucins to provide lubrication between the surface of the eye and the eyelids.2

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molar mass (daltons)</th>
<th>Concentration (mg • ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme</td>
<td>14,000</td>
<td>2.07</td>
</tr>
<tr>
<td>Lipocalin</td>
<td>17,500</td>
<td>1.55</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>90,000</td>
<td>1.65</td>
</tr>
<tr>
<td>Secretory IgA</td>
<td>385,000</td>
<td>1.93</td>
</tr>
</tbody>
</table>

The four proteins that are most abundant in the tear film have different roles. Lysozyme, which was discovered by Alexander Fleming in 1922, is a potent antibacterial enzyme that hydrolyzes the bonds in the outer walls of bacteria, particularly those that are Gram positive. It is also thought that lysozyme may directly permeate bacterial cell membranes.7 The principal bacteria in the tear film that are attacked by lysozyme are species of *Streptococcus* and *Staphylococcus*, which can cause conjunctivitis.

Lipocalin was first described in 1956, but its full structure was not discerned until almost 40 years later. It appears to play a lipid-binding role within the tear film and has a strong affinity to fatty acids.6 This role has a two-fold purpose: binding between lipocalin and lipids determines the surface tension of tears, and long-chain fatty acids can inactivate lysozyme, so binding between these fatty acids and lipocalin prevents this, thus resulting in an indirect enhancement of the tear film’s antimicrobial actions.7

Lactoferrin, first reported in 1966, can bind to both Gram positive and Gram negative bacterial membranes and has been reported to inhibit the growth of various bacteria including *Escherichia coli*, *Haemophilus influenza*, and species of *Streptococcus*, *Staphylococcus* and *Pseudomonas*. In addition, there is some evidence there may be a synergistic connection between lactoferrin and lysozyme. For example, *Staphylococcus epidermidis* is only susceptible to lactoferrin in the presence of lysozyme.9 Lactoferrin’s antimicrobial action is enhanced by its ability to bind to free iron in the tear film, thus denying bacteria the use of this for its growth.10

Secretory immunoglobulin A’s role in tear film is to prevent bacteria from gaining a foothold on the ocular surface and making them targets for phagocytosis.11 Its function is part of an adaptive response system, in contrast to lysozyme, lipocalin and lactoferrin, which are part of the innate defense mechanisms of the tear film.12

**Contact Lenses and Contact Lens Solutions** A contact lens, while made specifically for use in the eye, is still considered by the eye to be a foreign body. Contact lenses have shown to have several effects on the ocular surface,13 including hypoxia, a slight elevation in corneal temperature, and increased epithelial fragility. Despite these changes, most patients with a normal ocular surface and tear
film easily adapt to the presence of a contact lens. They disrupt it and increase the rate of tear evaporation. In patients with adequate tear volume, the impact of contact lenses on tear film is tolerable, but in those with inadequate tear volume, dry eye can result, which is estimated to occur in up to 30% of those who wear soft contact lenses and more than 80% of those wearing rigid contact lenses.

Contact lenses deposited on the surfaces of contact lenses are thought to become denatured over time, but a number of studies have demonstrated that some materials may promote denaturization more than others. Senchyna and colleagues reported higher levels of lysozyme on Etalflon lenses after wear (1 mg/lens) compared with Balafilcon (10 µg/lens) lenses, and significant differences were also found in the level of denaturation of each lens type, with the Balafilcon material demonstrating a greater percentage of lysozyme denaturation than the Etalflon material. Figures 3 and 4 show the amounts of lysozyme and measures of lysozyme denaturation on a range of contact lens materials from a 2007 study. Studies of silicone hydrogel and hydrogel lens materials have found the addition of hyaluronic acid to these materials results in less adsorption of proteins into the lens than materials not containing hyaluronic acid. This reduced adsorption ability could be important as denatured proteins themselves are thought to increase patient susceptibility to the development of papillary conjunctivitis.

There are little data available on the effect of contact lens solutions on the denaturation of tear film proteins. Instead, studies have focused on: how much protein has been removed, especially in rub versus no-rub formulas; what proteins are collected from contact lens wearers using a variety of contact lens solutions; and the differences in protein removal depending on contact lens solution and contact lens material combinations. However, in a recent study by Burke and colleagues of five multipurpose lens care solutions, it was shown that there were significant differences among the solutions in their ability to maintain lysozyme in its natural state. Additional studies should be conducted to ascertain the effect that different contact lens solutions, in combination with different contact lens materials, have on tear film proteins.

**Contact Lenses and Tear Film: Are Proteins the Problem or the Answer?**

As mentioned earlier, tear film proteins that accumulate on contact lenses have traditionally been viewed as requiring removal. Now that the roles of the cornea, tear film and contact lenses have been examined, let’s examine this relationship.

**Protein Build Up**

The amount of protein deposits that accumulate on a lens is closely related to the water content and the ionicity of the lens material. Ionic lenses that contain methacrylic acid attract much higher levels of protein compared with other materials, such as non-ionic lenses that contain...
n-vinyl pyrrolidone. The process of protein build-up can begin almost immediately after insertion, as protein deposits have been detected on lenses made of ionic, high-water content (FDA Group IV) that have been worn for as little as 1 minute. Lysozyme, in particular, is relatively small and carries a high positive charge, attracting it to the negative charge of some contact lens materials. This protein has been the focus of attention in the literature regarding the relationship between tear film proteins and contact lens materials.

**Protein Denaturation** Proteins are usually described in their ‘native’ state, but many can adopt multiple conformations in vivo, which are changes to the protein’s non-primary (i.e., amino acid sequence) structure. The denaturation of a protein occurs when its secondary and tertiary structures are disrupted or destroyed, usually as a result of temperature changes, pH, radiation, surface hydrophobicity and peroxidizing lipids. When proteins have been denatured, they usually lose their biological function, but this may be reversible.

**Tear Film Protein: Friend or Foe?** As mentioned earlier, the attraction and accumulation of tear film proteins onto contact lenses has been regarded as a negative development. However, an argument could be made that, in light of the antimicrobial characteristics of these proteins, their accumulation on a contact lens might be beneficial. This hypothesis is supported by a study conducted by Williams and colleagues in 2003 that compared the amount of bacteria found on worn versus unworn contact lenses. In this study, there were greater numbers of viable Gram-negative bacteria found on new lenses compared with that found on worn lenses.

Denaturation of proteins causes a reduction in their protective benefits, for example, denaturation of lysozyme results in it having less bactericidal action. In addition to having fewer protective benefits once denatured, the presence of denatured proteins may be related to the development of papillary conjunctivitis, which is a common reason patients give for discontinuing their lens use. The presence of denatured proteins may also lead to reductions in vision correction and lens comfort.

**Conclusions** The tear film has several functions: keeping the eye moist, creating a smooth surface for light to pass through, nourishing the eye and providing protection from injury and infection. Denaturation of tear film proteins as a result of contact lens wear, either through length of time deposited on the lens or in reaction to the contact lens material itself, could potentially lead to an increased susceptibility to inflammation and infection, and to reduced vision and comfort. Lens care solutions also can have an effect on tear film proteins but, to date, there is little data on how they affect the structure of these proteins.

The development of lens materials that limit the denaturation of proteins or the adsorption of proteins, and of lens care solutions that maintain the antibacterial nature of tear film proteins, may help to promote ocular health while still providing the patient with optimal vision correction. Additional studies should be conducted to further elucidate the relationship between the antimicrobial properties of tear film proteins and of contact lenses and contact lens solutions.


34. Leake L, Karel M. Polymerization and denaturation of lysozyme exposed to peroxidizing lipids. *J Food Sci.* 1982;47;737-743.


