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Energetics of corneal epithelial cell-ocular mucus-tear film interactions: Some surface-chemical pathways of corneal defense

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Abstract

The role of ocular mucous gel in the corneal epithelial hydration, lubrication, cleansing, wettability and defense against pathogens, is investigated based on a modified DLVO theory that accounts for the apolar, as well as polar, "acid-base" surface interactions. A strong polar repulsion keeps mucus in the form of highly hydrated "sloppy" gel, which does not adhere to the normal epithelium. Due to its strong electron donor type monopolarity, the mucus gel can form an effective barrier against contamination of the underlying epithelium by both the apolar (e.g., tear film lipids, cell debris) and the polar (e.g., hydrophilic bacteria) entities. In the absence of mucus, epithelial contamination becomes energetically favorable, which can also compromise its wettability by tears. Finally, a loss of polar surface properties can lead to adhesion of mucus to the cornea.

Keywords: Polar surface properties; Cell surface interactions; Tear film; Ocular mucus; Dry eyes; Mucus in corneal defense

1. Introduction

The corneal epithelium is continuously renewed by mitosis in basal cells [1–3]. The superficial corneal epithelial cells have a hydrophilic extracellular matrix of glycoproteins–glycocalix [4–7], but less differentiated deeper layer cells are expected to be less hydrophilic, because they lack the network of microvilli and glycocalix [4,6,8–10].

The superficial corneal cells are covered by an intensely hydrophilic [5,6,11,12] submicron sized [13] coating of hydrated mucous gel [11,14]. A 4 to 10 μm thick [11] aqueous tear film rests on the mucous coated ocular surface in normal eyes. The tear film frequently becomes unstable and breaks up rapidly in the so called "dry" eyes, which may be secondary to aqueous tear deficiency, mucous deficiency, and epitheliopathies (damage and chemical alterations of the epithelial surface) [4,9,11–16]. The outermost layer at the tear film–air interface consists of largely apolar lipids such as waxy and cholesteryl esters [11]. Relevant aspects of the epithelium and the three layered structure of the tear film are depicted in Fig. 1.

Not unlike the case of other mucosal epithelia, the ocular mucus has been implicated in roles of epithelial hydration and lubrication [11,17], in epithelial cleaning by trapping of epithelial debris and lipids of the tear film [11,13,15–18], in corneal wetting by tears [11,14,16,18], and in defense of

the ocular surface against invading pathogens [15,17,19].

Other studies [4,5,9,12,16,17] have questioned the role of mucus as a wetting agent for the normal cornea, based on the reasoning that the cell glycocalix buried under the mucous gel are also sufficiently hydrophilic [5,6,9,12,16,20] for spreading of tears. However, mucus may have a less direct role in maintaining the corneal wettability by preventing the epithelial contamination by hydrophobic cell debris and lipids [11–18]. This possibility is explored here by evaluating the free energies of adhesion.

The purpose of this paper is to study energetics (feasibility) of adhesion and binding due to physical forces, between various types of surfaces involved in issues outlined above. Recent advances [7,21–26] have shown that both the apolar and polar (acid–base) interactions leading to adhesion (or the lack of it) can be quantified by evaluating the free energy of adhesion. The free energy depends on the apolar and polar (electron donor–electron acceptor) surface parameters of the surfaces involved in adhesion, as well as the parameters of the medium in which adhesion occurs. Thus, depending on the combination of these surface properties, binding between surfaces is either encouraged or impeded [7,21–26].

In an earlier study [6,20], the apolar and polar acid–base surface properties of the ocular mucus, normal glycocalix bearing epithelial cells, and damaged cells were determined by contact angle goniometry in conjunction with a theory of polar surface properties [21–23]. Based on these data, we examine the normal and abnormal surface-chemical pathways of adhesion between the following types of surfaces: (a) Adhesion of contaminants (e.g., lipids, cell debris, pathogens, etc.) to the mucus layer; (b) Adhesion between contaminants and the epithelium, both in the presence and absence of mucus; and (c) Adhesion between epithelium and the mucous gel. An aim here is to determine whether the mucous gel can indeed impede the progression of contaminants and bacteria to the epithelium because of its surface properties. Conditions for adhesion of mucus to itself and to the epithelial cell, are examined in order to further our understanding of formation of mucous strands and corneal filaments witnessed in dry eyes (keratoconjunctivitis sicca) [11,15]. Another issue addressed is whether a loss of polar properties of the epithelium, due to damage or chemical alteration, can aid increased binding of bacteria which is known to occur in epithelial erosions, and dry eye patients [10,15,27–29]. The role of aqueous tear deficiency in binding of mucus and contaminants/bacteria to the epithelium is also explored.

In what follows, a theory of the apolar and polar surface interactions is first outlined, and

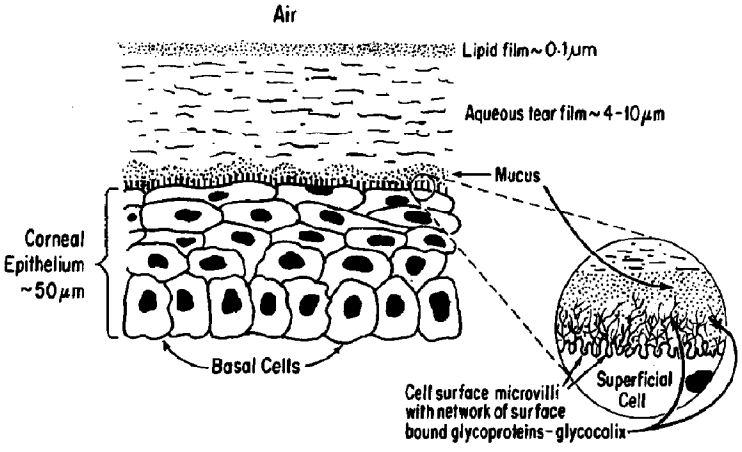

Fig. 1. Structure of the precorneal tear film and the corneal epithelium. The aqueous phase of the tear film is sandwiched between a superficial layer of apolar lipids and mucous layer of the epithelium. The basal cells multiply, differentiate, develop microvilli and glycocalix, and flatten as they move to the surface layer, where they are shed.

then applied for understanding of normal and abnormal pathways of adhesion and defense in the corneal epithelium–tear film system. The same methodology has been previously applied to understanding of physical mechanisms of adhesion for glycocalix carrying cells in the absence and presence of polymers [7,23–26]. Finally, consistency of the results with known experimental observations is examined.

2. Theory

The relevant aspects of adhesion engendered by surface interactions are summarized here. Detailed derivations and applications of the theory to biological surfaces and macromolecules may be found elsewhere [7,21–26].

In the absence of significant electric double layer effects, the total free energy of interaction (adhesion) between materials 1 and 2 immersed in liquid 3 is

$$\Delta G_{132} = \gamma_{12} - \gamma_{13} - \gamma_{23} \tag{1}$$

where γ_{ij} is the interfacial tension at the interface between materials i and j. Surface interactions lead to adhesion at the closest distance of approach, $d_0 = 1.58$ Å, only if the total free energy, ΔG_{132} multiplied by the area of contact is negative and less than $-1.5\ kT$. However, if the free energy defined in eq. (1) is larger than $+1.5\ kT$, true adhesion cannot occur, but weak binding of surfaces at the secondary minimum of the free energy, usually at separations larger than 5 nm, may take place [23,24].

The total surface tension of a material i, as well as the total interfacial tension between materials i and j, represent the sum of their respective apolar and polar energy components:

$$\gamma_K = \gamma_K^{LW} + \gamma_K^{AB} \quad (K = i, ij) \tag{2}$$

The apolar component (γ^{LW}) is derived from Lifshitz–van der Waals (LW) forces, whereas the polar component (γ^{AB}) is due to conjugate electron donor–electron acceptor, or Lewis acid–base (AB) interactions. Hydrogen bonding engendered by proton transfer (Brønsted acid–base interactions) is an important subset of Lewis acid–base interactions.

The asymmetric, conjugate nature of acid–base interactions is incorporated by defining the polar component of surface tensions in terms of the geometric mean of the electron acceptor (proton donor) parameter, γ^+ and the electron donor (proton acceptor) parameter γ^- [21–26].

$$\gamma_i^{AB} = 2\sqrt{\gamma_i^+ \gamma_i^-} \tag{3}$$

It now becomes possible to represent the total interfacial tension between two polar materials in terms of their apolar and polar parameters as [21–23]

$$\gamma_{ij} = \left(\sqrt{\gamma_i^{LW}} - \sqrt{\gamma_j^{LW}}\right)^2 + 2\left(\sqrt{\gamma_i^+ \gamma_i^-} + \sqrt{\gamma_j^+ \gamma_j^-} - \sqrt{\gamma_i^+ \gamma_j^-} - \sqrt{\gamma_i^- \gamma_j^+}\right) \tag{4}$$

The first term in eq. (4) is γ_{ij}^{LW}, and the second term is the polar contribution, γ_{ij}^{AB}, which is the difference between energies of conjugate cohesive and adhesive acid–base interactions for like and unlike molecules. The contribution of the apolar LW interactions to interfacial tension (γ_{ij}^{LW}) is always positive, but the polar component of the interfacial tension (γ_{ij}^{AB}) becomes negative when the adhesive acid–base interactions overwhelm the cohesive polar interactions. In fact, negative interfacial tensions against water for intensely hydrophilic polymers and biosurfaces seem to be the rule, which is usually due to a strong electron donor type of monopolarity ($\gamma^+ = 0$, $\gamma_S^- > 28$ mJ/m^2) [21–26]. Adhesion of biological surfaces usually occurs in an aqueous environment and hence, negative interfacial tensions against water can render the free energy positive (eq. 1) and thereby discourage intimate binding. This polar repulsion, often dubbed as a hydration pressure, has profound implications for the cell adhesion and fusion [7,23–26]. All of the corneal constituents also display significant polar properties [6,20]. Thus a role for polar properties is anticipated in binding of mucus to the corneal epithelial cell, and in adhesion of foreign bodies (e.g., microorganisms) to the corneal layers.

In view of eqs. (1) and (4), the free energy of adhesion of a surface 1 to surface 2 in medium 3 may be written as [21–23].

$$\Delta G_{132} = 2\left(\sqrt{\gamma_1^{LW}} - \sqrt{\gamma_3^{LW}}\right)\left(\sqrt{\gamma_3^{LW}} - \sqrt{\gamma_2^{LW}}\right) + 2\left[\sqrt{\gamma_3^+}\left(\sqrt{\gamma_1^-} + \sqrt{\gamma_2^-} - \sqrt{\gamma_3^-}\right) + \sqrt{\gamma_3^-}\left(\sqrt{\gamma_1^+} + \sqrt{\gamma_2^+} - \sqrt{\gamma_3^+}\right) - \sqrt{\gamma_1^+\gamma_2^-} - \sqrt{\gamma_1^-\gamma_2^+}\right] \quad (5a)$$

$$\equiv \Delta G_{132}^{LW} + \Delta G_{132}^{AB} \quad (5b)$$

Computation of the free energy of adhesion requires determination of the apolar and polar surface tension parameters for surfaces 1 and 2 and intervening medium 3. The surface tension components of water are known ($\gamma_w^{LW} = 21.8$, $\gamma_w^+ = \gamma_w^- = 25.5$, all in mJ/m^2) [21–23] and the surface tension parameters of various types of corneal surfaces are reported later. The LW component of the free energy is almost always negative (favoring adhesion) for biological surfaces, as their apolar surface tension components (γ_1^{LW}, γ_2^{LW}) are usually larger than that of water ($\gamma_w^{LW} = 21.8\ mJ/m^2$). However, the polar component may readily become positive and dominating, thus preventing adhesion at the closest distance of approach. In such an event, the equilibrium separation between surfaces is determined by the location of the secondary minimum in the free energy vs. separation distance diagram. The variation of the total free energy with intersurface distance, d is given by a modified DLVO theory [30], which, in addition to LW and electric double layer interactions, also incorporates the acid–base (polar) interactions [21–26].

$$\phi(d) = \Delta G_{132}^{LW}(d_0^2/d^2) + \Delta G_{132}^{AB}\exp((d_0 - d)/\lambda) + (64 n_0 kT\gamma_0^2/\kappa)\exp(-\kappa d) \quad (6)$$

where λ is a decay or correlation length for water ($\lambda \cong 6$ nm) [24], κ is inverse debye length (m^{-1}), n_0 is the number concentration of ions in water, and the parameter γ_0 is defined in the Gouy–Chapman theory as [30]

$$\gamma_0 = \frac{\exp(Ze\psi_0/2kT) - 1}{\exp(Ze\psi_0/2kT) + 1} \quad (7)$$

where ψ_0 is the surface potential and Ze is the electronic charge of the counterion.

The free energy of double layer overlap in eq. (6) assumes identical potentials for surfaces, but this assumption can be easily relaxed [30]. However, it turns out that for polar surfaces considered here, electrical effects are negligible, but are retained in eq. (6) for the sake of completeness.

The location of the secondary minimum in the free energy is found by the solution of

$$(\partial\phi/\partial d) = 0 \quad (8)$$

and the magnitude of the minimum free energy is obtained by evaluating the free energy at this location.

In contrast to interactions in water (eq. 5), if interactions occur in the absence of an intervening water film, the total free energy of adhesion of materials 1 and 2 is [21–23].

$$\Delta G_{12} = -2\sqrt{\gamma_1^{LW}\gamma_2^{LW}} - 2\left(\sqrt{\gamma_1^+\gamma_2^-} + \sqrt{\gamma_1^-\gamma_2^+}\right) \quad (9a)$$

$$= \Delta G_{12}^{LW} + \Delta G_{12}^{AB} \quad (9b)$$

In this case, interactions are always attractive at attachment and LW interactions may be substantially augmented by conjugate acid–base interactions between surfaces. Equation (9) is germane to our understanding of abnormal mucus adhesion in dry eyes that are accompanied by either an almost total lack of tears, or a rapid elimination of tears from the cornea engendered by the tear film breakup immediately after blinking.

Equations (5) and (9) may be used for computing the total free energy (per unit area) when all three surface tension parameters (γ^{LW}, γ^+ and γ^-) for both the surfaces undergoing adhesion are known. These apolar and polar surface tension parameters for the corneal surfaces may be determined by contact angle goniometry on freshly enucleated corneas [6,20]. The theory for determination of surface tension parameters, and its application to biological materials including cells, have been pioneered by Chaudhury, van Oss and Good, and may be found elsewhere [7,21–26]. Briefly, the equilibrium contact angle,

Table 1

Description of the corneal surfaces in different groups of the cornea

Group	Methods of corneal processing	Description of the corneal surface
A	Corneas were gently rinsed with ice cold saline	Superficial layer of the corneal mucus
B	Corneas were irrigated with 15–20 percent *N*-acetyl cysteine solution for upto 45 min	Superficial squamous epithelial cells of the cornea complete with their glycocalix, but devoid of their mucus coating
C	Same as B, except drying of corneas by Kim® wipes after the *N*-acetyl treatment was performed	Same as in B, except that slight dehydration and damage of the cell glycocalix is anticipated
D	Saline rinsed corneas were cleansed and dried by cotton swabs for about 30 s	Relatively dehydrated and damaged superficial cells; partially exposed wing cells of the second cell layer of the epithelium (5)
E	Same as group D, except an additional pretreatment of corneas with *N*-acetylcysteine was done	Same as in group D

θ of a liquid drop (L) on a solid surface (S) is given by Young's equation, which may be written [21–23] in terms of the apolar and polar properties of the liquid and the surface.

$$(1+\cos\theta)\gamma_L = 2\left[\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^{+}\gamma_L^{-}} + \sqrt{\gamma_S^{-}\gamma_L^{+}}\right] \quad (10)$$

Thus, the three unknown surface parameters (γ_S^{LW}, γ_L^{+} and γ_S^{-}) can be evaluated by measuring advancing contact angles of at least three different well defined probe liquids, of which two must be polar. This technique has been employed previously [6,20] to determine the apolar and polar parameters for various types of corneal surfaces, by using diiodomethane, physiologic saline, glycerol and formamide as probe liquids. The surface tension parameters (γ_L^{LW}, γ_L^{+} and γ_L^{-}) for these probe liquids are known [21–23].

3. Methods

Whole eyes were rapidly enucleated from adult New Zealand albino rabbits that were killed by intravenous pentobarbital overdose. Surfaces of successively deeper corneal structures were exposed by processing corneas in five different ways. The methods of corneal treatment and the corneal surfaces thus uncovered are summarized in Table 1 for each of the five corneal groups, A to E.

Table 2

Values for the apolar and polar components of the surface energy of the corneal surfaces as determined in refs. [6,20] from contact angle measurements (reported in mJ/m^2)

Group [a]	γ_S^{LW} [b]	γ_S^{+}	γ_S^{-}	γ_S^{AB}	γ_S	γ_{SW}	ΔG_{SW} [c]
A	40.1	0.35	63.5	9.4	49.5	−23.3	−145.6
		0.56	56.7	11.3	51.4	−18.6	−142.8
B	27.4	3.2	56.3	27.0	54.4	−15.6	−142.8
		3.4	53.3	27.0	54.4	−14.1	−141.3
C	26.8	5.7	49	33.3	60.1	−10.6	−143.5
		6.6	40.2	32.5	59.3	−5.7	−137.8
D/E	28.5	4.7	32.5	24.7	53.2	−1.4	−127.4
		4.9	17.7	18.6	47.1	+5.2	−114.7

[a] Description of groups is found in Table 1. Values given in the first row for each group are calculated based on initial contact angles for saline; those in the second row are based on saline contact angles after about 45 min of corneal drying.

[b] Values of γ_s^{LW} are averages over the entire period of drying, as the contact angle of diiodomethane showed little variation with drying, which was, furthermore, not systematic.

[c] The hydrophilicity of a material relative to water is defined as the ratio $\Delta G_{sw}/\Delta G_{ww}$, where $\Delta G_{ww} = -145.6$ mJ/m^2.

Contact angles of small drops of probe liquids —diiodomethane, physiologic saline and glycerol —were measured on the corneal surfaces of groups A to E by a goniometer. The structure and surface properties of biological materials are easily altered by their interactions with probe liquids, which is amply documented for fragile epithelial cells of the cornea [5]. The artificial changes in the cornea were minimized by recording *advancing* contact angles within one minute of depositing the test drop and by choosing a fresh corneal site for each measurement. Variations in the contact angles were also recorded with room temperature drying of the corneal surfaces for a period of 45 minutes.

Contact angles of probe liquids thus determined on different groups (Table 1) of the cornea are reported elsewhere [6,20]. The three surface tension parameters (γ_L^{LW}, γ_L^+ and γ_L^-) of the probe liquids used are known [21–23]. Based on these data, the apolar and polar surface properties (γ_S^{LW}, γ_S^+ and γ_S^-) of different corneal layers are easily determined from eq. (10). The polar component of the corneal surface tension (γ_S^{AB}), the total surface tension (γ_S), the interfacial tension against water (γ_{SW}), and the free energy of adhesion to water (hydrophilicity; ΔG_{SW}) are then obtained from eqs. (3), (2), (4) and (9), respectively. these surface properties are summarized in Table 2 for different types of corneal surfaces investigated [6,20].

Interestingly, the apolar component (γ_S^{LW}) for all corneal structures other than the mucus are similar (in the range of 26–29 mJ/m^2), but the apolar component of surface tension for mucus is substantially higher (about 40 mJ/m^2). These values of the apolar components of surface tensions agree with the "critical" surface tensions obtained by Zisman's method in earlier studies of the corneal wettability [31]. However, all of the corneal surfaces studied also have significant polar (acid–base) properties, which largely determine their overall surface tensions, interfacial tensions and free energies of adhesion to other materials.

The electron donor parameter of the corneal surface decreased upon drying, and also declined with damage and with progression to deeper corneal structures from group A to E (Table 2). Surfaces other than mucus (which displays electron donor type monopolarity) also had moderate electron acceptor (base) parameters, which, however, did not display systematic variations with corneal damage. With progressive drying and damage to the surface, the hydrophilicity of the corneal surface (as measured by the negative free energy of binding to water, ΔG_{SW}) declined and the interfacial tension against water increased (Table 2).

An interesting finding to be noted is that the corneal mucus (group A) and healthy epithelial cell with associated glycocalix (group B) are about equally hydrophilic, and indeed, both should be completely wetted by the overlying aqueous tear film of surface tension less than about 50 mJ/m^2 [5,12,16]. This seemingly reasonable line of argument has called into question [4,5,9,12,16,17] the postulated role of the ocular mucus as a wetting

Table 3

Free energies of adhesion (in mJ/m^2) of uncontaminated mucus to various types of corneal surfaces in water

Surfaces involved in adhesion (surface 1–water–surface 2)	ΔG_{1W2}^{LW}	ΔG_{1W2}^{AB}	$\Delta G_{1W2} = \Delta G^{LW} + \Delta G^{AB}$
1. Hydrated mucus (group A)–hydrated normal cell with glycocalix (group B)	−1.9	+40.9	+39.0
2. Dehydrated mucus (group A)–dehydrated normal cell (group B)	−1.9	+35.3	+33.4
3. Hydrated mucus (group A)–damaged cell (groups D/E)	−2.2	+22.6	+20.4
4. Dehydrated mucus (group A)–hydrated and damaged cell (groups D/E)	−2.2	+8.4	+6.2
5. Hydrated mucus (group A)–severely damaged/abnormal apolar [a] epithelium [31]	−2.2	−15.6	−17.8
6. Mucus–mucus (group A)	−5.5	+43	+37.5 [b]

[a] Actually, attachment is favored even for the epithelium with small monopolarity ($\gamma_S^- < 3.9$ mJ/m^2), as ΔG_{1W2} from eq. (5) becomes negative.

[b] Minimum value for relatively dehydrated mucus. Value for hydrate mucus is even larger ($\cong +42$ mJ/m^2).

agent in the eye [11,14,18,32]. However, based on the energetics of binding of contaminants to the epithelium, it will be shown that the mucus, among other things, may also have a role in the corneal wetting by tears, because it can indeed prevent contamination of the epithelium by hydrophobic cell debris and lipids, as has been postulated previously [11,18,32].

4. Results

4.1. Adhesion of mucus to normal and abnormal epithelia

The mucous gel over the corneal epithelium appears to be 0.4 to 1 μm thick in freeze fracture TEM studies [13]. The overlying aqueous tear film maintains its integrity in normal eyes, and therefore, normal patterns of mucus adhesion must be studied in the presence of water. In this case, eq. (5) together with the data for surface parameters (Table 2), determine the total free energy of adhesion of normal (uncontaminated) mucus to various types of corneal cells. The apolar and polar components of the free energy, as well as the total free energy of adhesion, are summarized in Table 3. The apolar component of the free energy due to LW interactions is always negative, which favors adhesion. However, there is a strong polar repulsion between mucus and the corneal cells, which decreases with increased dehydration and cell damage (see first four entries in Table 3). Cases 2 and 4 in Table 3 correspond to adhesion of somewhat dehydrated mucus to moderately damaged and dehydrated epithelium, which may occur due to frequent tear breakup on the cornea, coupled possibly with a mild epithelial cell disorder. Even in a normal cornea, an ongoing maintenance of the epithelium results in continuous maturation and desquamation of surface cells [1–3]. The sites of recent cell desquamation may be viewed as transient micro-erosions, or locally damaged surfaces of about 100 μm^2 area. Therefore, the four cases of Table 3 show that an intimate binding of mucus to the normal epithelial cell, as well as to moderately damaged and dehydrated cells, is not possible. However, for these cases, secondary minima of attraction in the total free energy do exist at separation distances ranging from 6.2 nm (case 4) to 7.7 nm (case 1) (obtained from eqs. 6–8). The corresponding free energy of attraction at the secondary minima ranges from -1.15×10^{-3} mJ/m^2 (case 4) to a low of -6.52×10^{-4} mJ/m^2 (case 1). At physiologic saline concentration of the tear film, and moderate surface potentials of mucus and cell glycocalix ($|\psi_0| < 30$ mV) [7,23], the electric double layer contribution (last term of eq. 6) to the free energy is minimal.

Further, the highly hydrated, "fluid" like nature of mucous gel is a direct consequence of a strong polar repulsion between mucus molecules at their closest distance of approach (entry 6 in Table 3).

The picture which emerges from these calculations is of a mucous layer which is separated from the cell glycocalix at equilibrium distances exceeding 5 nm. The intervening spaces are filled by an electrolyte–water mix of the tear film. Indeed, in high magnification TEM photographs [13], clear spaces of about 20 nm are visible between the cell microvilli and the ocular mucus. A part of this space is occupied by the length of glycocalix strands.

Finally, in contrast to normal and moderately damaged cells, an intimate binding of mucus to the cell becomes possible if the cell loses almost all of its polar properties (case 5 in Table 3). In this case, the free energy of binding of even hydrated mucus to the epithelium becomes strongly negative, signifying a substantial hydrophobic attraction. Indeed, on heavily wiped and dried corneas, initial contact angles of glycerol ($\gamma_L^{LW} = 34$, $\gamma_L^+ = 3.92$ and $\gamma_L^- = 57.4$; in mJ/m^2) and formamide ($\gamma_L^{LW} = 39$, $\gamma_L^+ = 2.28$ and $\gamma_L^- = 39.6$; in mJ/m^2) [21–23] were found to be 90 and 80 degrees, respectively [31]. These values of contact angles are entirely consistent with those computed from eq. (10) for an apolar epithelium with $\gamma_S^{LW} = 28.5$ mJ/m^2 (from Table 2). Further, it is easily shown from eq. (5) that adhesion of hydrated mucus can occur even if the epithelium is not completely apolar, but has a small residual monopolarity with $\gamma_S^- < 3.9$ mJ/m^2.

All of the above results pertain to adhesion of

normal uncontaminated mucus to the epithelium in the presence of adequate amount of aqueous tears. However, in order to examine other pathological pathways of mucus adhesion, two additional situations must be considered; (a) decreased polar properties of the mucus due to increased contamination (discussed later) with hydrophobic cell debris and superficial lipids, as witnessed frequently in dry eye disorders [11,15,16], and (b) lack of aqueous tears engendered either by deficiencies of secretion or by a short lived stability of the tear film after blinking [11,15,16].

Equation [5] predicts that adhesion of monopolar mucus to normal epithelium becomes possible in the event the electron donor parameter (γ^-) of mucus declines to 15 mJ/m^2 or less. If the epithelium is damaged, or mucus also acquires some electron acceptor polarity (γ^+) due to contamination, attachment is possible even for higher values of mucus electron donating capacity, i.e., for smaller degree of mucus contamination. Further, for such heavily contaminated mucus, the free energy of adhesion of mucus to itself in water also becomes negative (as discussed later), which should lead to collapse of highly expanded "sloppy" mucus gel into hydrophobic aggregates and clumps.

Finally, in the event of severe aqueous tear film deficiency, a very tenacious attachment of mucus to the epithelium results due to LW, as well as AB interactions (eq. 9). The free energy of adhesion in this case is always negative; of the order of -100 mJ/m^2.

Implications of the above results in various roles of the ocular mucus, and in formation of corneal filaments are discussed later.

4.2. Adhesion of foreign bodies and bacteria to ocular surface

The corneal epithelium, aqueous tear film and mucus are continuously renewed. Contamination of the tear film, therefore, occurs because of the internal sources (e.g., relatively hydrophobic cell debris, and apolar lipids of the superficial lipid layer of the tear film [11,18,32]), as well as external sources (e.g., bacteria and foreign particles).

Once a particle gains access to the aqueous tear film, its further progression to the cornea depends on its adhesion to mucous layer, which may be determined by computation of its free energy of binding to mucus in water (from eq. (5) and surface tension parameters reported in Table 2). A wide variety of substances (including polymeric and biological entities) characterized thus far have all shown either predominantly electron donor properties (very low γ^+) or largely apolar "hydrophobic" behavior (low γ^- and very low γ^+) [7,21–26]. Damaged or degenerating epithelial cells (groups D/E of Table 2) appear to be the only contaminants that can impart weak electron acceptor properties to mucus. As is shown in the first row of Table 4, only the adhesion of weak electron donor (and the apolar) surfaces to the mucus is energetically preferred in water. Therefore, based on surface forces, binding of bacteria, with hydrophilic "slimy" walls of peptidoglycan, to the mucus may be ruled out, since much bacterial surfaces are likely to be strongly electron donor with γ^- well in excess of 10 mJ/m^2 and γ^+ very low [6,7,21–26]. Thus, only the apolar foreign bodies (e.g., apolar lipids of the tear film), as well as the gram-negative species with a lipid-rich outer coat of hydrophobic lipopolysacharides (e.g., *E. coli*) may gain entry into the mucus layer if their electron donor parameter is small (Table 4). However, once such particles are imbedded in the mucus, they are denied further adhesion with the normal, as well as with damaged and dehydrated epithelial cells (see second and third rows of Table 4). Thus, in the absence of specific receptors, bacteria with strong electron donor monopolarity are selectively retained in the aqueous tears, whereas weakly polar and apolar foreign bodies are concentrated in the mucus phase, without gaining further adherence to the underlying corneal epithelium The contaminants thus accumulated are easily pushed out of the eye due to an ongoing turnover of aqueous and mucus phases. Finally, the fate of cells desquamating from the superficial cell layer is not unlike that of other foreign bodies, because their polar properties are likely to be similar to properties of damaged cells of groups D and E (Table 2). In any event, the cell

debris is not expected to be more polar than slightly damaged cells of group C. Based on eq. (5), it is easily shown that the free energies of adhesion of such cells to normal cells (group B), and even to damaged cells (group D/E), are positive in the presence of mucus. Therefore, degenerating and desquamating cells and their debris cannot contaminate the epithelial surface, but remain trapped in mucous and/or aqueous layers, depending on their surface properties.

However, in the event of severe mucus deficiency, the normal pathways of mucus adhesion (presented earlier) and of contamination disposal are likely to be affected adversely in the following two different ways. (a) A decreased rate of mucus secretion (and removal) would engender an increased degree of contamination of mucus with largely hydrophobic apolar debris, thereby reducing its electron donor properties, and facilitating its adhesion, both to itself and to the epithelial cell (see results presented above in Table 3). Any hindrance in free movement and removal of mucus would provide further opportunity for contamination thus triggering a vicious cycle, which may end in the accumulation of heavily contaminated and aggregated mucus and formation of mucus filaments adherent to the epithelium. (b) Deficiency of mucus, as well as its aggregation in clumps due to contamination, may leave parts of the epithelium devoid of its mucous covering. The adhesion of contaminant to the epithelium now occurs in water, rather than in mucus. As is shown by the fourth and fifth entries in Table 4, contamination of the epithelium can now readily occur by the apolar and moderately electron donor polar materials, which is not possible in the presence of mucus.

Finally, the last entry in Table 4 shows that in the event of severe deficiency of aqueous tears, adhesion of any type of contaminants to all types of ocular surfaces is strongly favoured due to LW, as well as to the polar AB interactions.

5. Discussion

5.1. Role of ocular mucus in hydration, cleaning and mechanical protection (lubrication) of the epithelium

The relative hydrophilicity (capability to bind and retain water) of a material is indicated by the

Table 4

Conditions for adhesion of foreign particles/bacteria to various types of ocular surfaces (surface parameters are in mJ/m^2)

No.	Surfaces involved in adhesion in a medium 3 (surface 1–medium 3–surface 2)	Condition for adhesion $\Delta G_{132} < 0$
1.	Polar particle/bacteria [a]–water–hydrated mucus (group A)	Adhesion is possible only for $\gamma_1^- < 5$, if $\gamma_1^+ = 0$, for $\gamma_1^- \leq 10$, if $\gamma_1^+ \leq 2$
2.	Particle [b]–mucus–hydrated normal epithelium (group B)	Strong repulsion between surfaces; adhesion not possible unless $\gamma_1^- > 48$ [c]
3.	Particle [b]–mucus–damaged and dehydrated epithelium (group D/E)	Adhesion not possible unless $\gamma_1^- > 36$ [c]
4.	Particle [a]–water [d]–normal epithelium (group B)	Adhesion possible if $\gamma_1^- < 2.5$ [c]
5.	Particle [a]–water–damaged hydrated epithelium (group D/E)	Adhesion possible if $\gamma^- < 12$ [e]
6.	Particle [a]–air–epithelium (in deficiency of aqueous tears)	Strong adhesion possible for all types of particles and corneal surfaces (see eq. 9)

[a] Assuming $\gamma_1^{LW} = 40\ mJ/m^2$, which in this case gives the maximum negative value of ΔG_{132}^{LW}.

[b] Assuming $\gamma_1^{LW} = 30\ mJ/m^2$, which in this cases gives the maximum negative value of ΔG_{132}^{LW}.

[c] Assuming particle to be monopolar ($\gamma_1^+ = 0$). If particle has electron acceptor type polarity, adhesion becomes even less likely (eq. 5).

[d] Adhesion to the epithelium occurs in water in deficiency of the ocular mucus.

[e] Assuming particle to be monopolar ($\gamma_1^+ = 0$). If particle has electron acceptor polarity also, adhesion is even more likely and would occur for increasing value of γ_1^-.

ratio of its free energy of adhesion to water (ΔG_{SW}) and the energy of cohesion of water ΔG_{WW}, which is -145.6 mJ/m^2. As is shown in Table 2, the ocular mucus, as well as the cell glycocalix are almost as hydrophilic as water itself and are thus able to retain their moisture even after prolonged exposure to air. Indeed, visual observations [6,12,20] show that surfaces of mucus covered corneas (group A) and of corneas with intact glycocalyx (group B) remain smooth and glossy even upto 45 min of exposure, whereas damaged corneas of group C to E lose their luster and become dull. This observation correlates well with a decrease in polar properties and hydrophilicity (ΔG_{SW}) of damaged surfaces upon drying (Table 2).

Due to an intense electron donor type monopolarity of the mucus, the free energy of binding of mucus to itself in water is positive ($\Delta G_{MWM} \cong +42$ mJ/m^2), on account of which uncontaminated mucus forms a highly expanded and hydrated "sloppy" gel [13,17], which cannot adhere to the underlying epithelium in the presence of aqueous tears (see entries 1–4 and 6 in Table 3). These qualities may assist in the spreading of mucus by mechanical action of blinking [11,14], as well as in the removal of cell debris by mucus [33]. It is interesting to note that normal mucus cannot adhere even to relatively hydrophobic damaged cells (Table 3), which rules out the possibility of transient attachments at sites of recent cell desquamation. The situation thus seems similar to that in the respiratory tract, where the mucous gel is not adsorbed on the cell *in vivo*, but floats on top of ciliated epithelium and pericilliary fluid [17]. It is precisely the lack of an intimate binding between the ocular mucus gel and the epithelium which allows mucus to maintain its often posulated [11,13–18,32,33] dual roles of epithelial lubrication and cleaning, both of which depend on the free movement of mucus "blankets" [33] on the surface. If mucus were to adsorb on the cell, a part of the shear produced by rapidly moving lids during blinking would be transmitted to the epithelium, causing damage to fragile cells, ocular discomfort and pain. As is discussed later, this is indeed what happens when the corneal filaments form due to adhesion of contaminated mucus with compromised epithelium [11,15]. Moreover, immobilization of mucous gel in the vicinity of the epithelium would interfere with renewal of mucus during blinks, resulting in excessive build-up of contaminants.

5.2. Surface chemical pathways of ocular defense by the tear film

Based on Table 4 and the results presented earlier, the hydrophilic bacteria enclosed in "slimy" sheaths, which have strong electron donor type monopolarity [6,7,21–26], should not adhere to the mucus layer in water, but be retained in aqueous tear film. In contrast, the apolar and weakly polar bacteria and other contaminants should preferentially adsorb and absorb in the mucous gel. However, the important thing is that all such contaminants trapped in mucus are denied further entry to deeper corneal structures, because their adhesion to superficial cells is energetically unfavorable in the presence of mucus (Table 4). The bacteria and foreign bodies thus trapped in the tear film may then be neutralized by the known immunologic defense systems of mucosal surfaces [19] and gradually pushed out of the eye by the ongoing renewal of the aqueous and mucous phases. The two-tier system of defense offered by the bipolar aqueous and electron donor monopolar mucous layer appears to be the optimal one, and works much like a sieve that sorts out foreign bodies according to their surface properties. Indeed, were it not for the mucous phase, binding of the apolar, electron acceptor and even moderately strong electron donor particles to the epithelium should occur readily, especially on sites of recent cell desquamation (Table 4). Finally, in dry eyes secondary to the aqueous deficiency [11,15], or to the short-lived stability of the tear film, a strong attachment of any type of foreign body to the ocular surface becomes possible (last entry in Table 4).

In summary, energetic considerations show that a loss of polar surface epithelial properties (increased hydrophobicity), lack of mucus "blanket" or its increased contamination, and dry eyes, all favor increased binding of bacteria, cell debris, tear film lipids and foreign bodies to the ocular

surface. These surface energetic considerations correlate well with the observation [29] that *in vivo* adherence of *Pseudomonas aeruginosa* to the ocular surface increased more than 20 times after creation of corneal abrasions that remove mucus and damage epithelial cell layers. A rapid decline in the number of adhering bacteria was also noted after six hours of the corneal injury, when healing of the cell injury and mucous layer are expected. Other studies show that pathogens such as *Pseudomonas* do not bind to the mature surfaces of superficial cells *in vivo* [10,27], but they attach in increasing numbers to deeper layer cells that are devoid of microvilli and glycocalyx [10], and are therefore expected to be weak electron donors (less polar). It is also known that epithelial damage, keratinization of superficial cells, mucus-deficiency (as in vitamin A deficiency) and dry eyes, all put the eye at increased risk of infection [11,13,15,17,27–29,34–36]. Examples of other mucosal epithelia where surface properties of mucus coating may be instrumental in defense are the lung, and the buccal epithelial cell, where salivary glycoproteins similar to mucus have been known to prevent attachment of microorganisms [37].

In conclusion, our results argue that the aqueous and mucous layers not only serve a passive role in ocular defense (as mere anatomical barriers), but perhaps a more important and active role in screening, and preventing adhesion of pathogens based on their polar surface properties.

5.3. Role of mucus in trapping of cell debris and superficial lipids of the tear film

An inspection of Table 4 shows that the apolar lipids of the superficial oily layer of the tear film are preferentially absorbed in the mucous gel upon their diffusion, and further, they do not adsorb on the epithelial cell in the presence of mucus. A direct attachment to the epithelium becomes possible only in the absence of the mucous covering. Such adsorption can mask the polar nature of the glycocalyx and make the epithelium non wettable, which can trigger the tear film breakup. Indeed, a lipid trapping role for the ocular mucus has been postulated in many studies [11,13,14–18,31–33]. In addition to diffusion, Marangoni flows in the tear film may also enhance the migration of lipids to the corneal surface [38].

Another major source of hydrophobic contaminants to the tear film is the epithelium itself; a few hundreds of degenerated squamous cells are lost every minute from the corneal epithelium alone [1–3]. While a reattachment of these cells and cellular debris to the epithelium is ruled out (see Section 4.2) in the presence of mucus, their accumulation on the epithelium becomes likely in mucus deficient eyes.

In conclusion, factors such as reduced volume of mucus, decreased turnover of mucus and increased cell loss can increase the degree of mucus contamination, and thereby adversely affect normal pathways of mucus adhesion, hydration, epithelial cleaning, bacterial defense and tear film stability.

5.4. Abnormal adhesion of mucus: Formation of corneal filaments and mucus strands

From Table 3 and the results presented earlier, a strong adhesion of mucus to itself and to the epithelium is possible in the following cases and their combinations: (a) lack of aqueous tears, (b) damage or chemical alteration (e.g., keratinization) of the epithelial cells resulting in significant loss of polar surface properties, and (c) a significant loss of electron donating capacity of the ocular mucus aided possibly by a concurrent increase in its electron accepting property, both of which may result from excessive contamination of the mucus by lipids and desquamating cells. In events (a) and (c), the normal monopolar repulsion between mucus molecules turns into a hydrophobic attraction, which can cause a collapse of the expanded gel and aggregate it into strands of contaminated mucus. These results are in accord with a hypothesis on the formation of mucous strands in mucus and aqueous deficient eyes [11,15,32].

A strong attachment of these contaminated mucous strings to the damaged epithelium results

in the corneal filaments on which epithelial cells grow. The picture which emerges based on energetic arguments is suprisingly close to what has been theorized earlier regarding the birth of a corneal filament [15]. In addition to supporting the hypothesis, our results quantify the parameters of filament formation, and show that while the aqueous deficiency helps, it is neither a prerequisite, nor the sole cause of corneal filaments.

5.5. Keratoconjunctivitis sicca (dry eyes)

The roles of aqueous and mucus deficiencies in KCS are clinically well established [11,14–16,32], and are consistent with mechanisms proposed here. Based on energetics of adhesion, it seems that an increased rate of cell loss, and substantially decreased electron donating capacity of the cell surface are also sufficient to trigger a vicious cycle leading to increased mucus adherence and contamination, collapse of hydrated mucus gel, increased epithelial contamination and damage, and finally, decreased wettability and tear film breakup time. Indeed, in keratoconjunctivitis sicca, the following abnormalities of the epithelial surface have been noted: greatly increased exfoliation of superficial corneal cells followed by premature uncovering of smooth cells, abnormalities of glycocalyx synthesis and/or their extracellular attachment [4,9,16,39,40], degeneration of cells, and keratinization (skin like change due to expression of hydrophobic, water insoluble keratins) of conjunctival cells [11,15,16]. All of the above alterations should promote a loss of epithelial polar surface properties and increased levels of mucus and epithelial contamination. In summary, initial abnormalities in any of the three components [11]—aqueous tears, mucus and epithelium—can progressively compromise functioning of other components and lead to dry eyes.

Interestingly, it does not seem to be a mere coincidence that some of the polymeric ingredients (e.g., Dextran and polyvinyl alcohol) of artificial tear substitutes have surface properties [7,21] remarkably similar to that of mucus (group A in Table 2]. These materials, like mucus, display electron donor monopolarity with $\gamma^- > 50$ mJ/m^2 and $\gamma^{LW} = 42$ mJ/m^2 [7,21].

6. Summary

The role of the ocular mucus in the corneal protection, contamination, wetting and defense are investigated based on the contributions of the apolar and polar surface forces to the free energy of adhesion. The following conclusions emerge from the energetics of surface interactions.

(a) There is a strong polar repulsion between extremely hydrophilic uncontaminated mucus molecules, and also between the superficial epithelial cell and the mucous gel. These factors explain the highly hydrated "sloppy" form of the mucous gel and its lack of tenacious attachment to the epithelium in normal eyes—factors that should promote epithelial hydration, lubrication and cleansing of contaminants.

(b) Hydrophilic bacteria with predominant electron donor surfaces are rejected by mucus, whereas contaminants/bacteria with apolar and electron acceptor properties are absorbed. However, all types of contaminants trapped in the mucous gel experience repulsion to the epithelium, and hence, the epithelium remains uncontaminated by the apolar lipids of the tear film and by debris of desquamating cells. In the absence of the mucous covering, however, direct adsorption of the apolar, electron acceptor, and even moderately electron donor surfaces to the epithelium is energetically favored. These results argue for an active role of mucus in the corneal defense, cleaning and maintenance of the corneal wettability.

(c) Increased contamination of mucus by apolar and weakly polar substances reduces its electron donor properties, thereby encouraging attraction of mucus to itself and to the epithelium. Attachment of the contaminated mucus is further aided by damage/chemical alteration of the epithelium. Thus, excessive contamination engendered by mucus deficiency, slow mucus turnover or increased level of contaminants can lead to formation of corneal filaments and mucous strands.

The surface energetic considerations quantified here seem to be in accord with earlier hypotheses on the role of the ocular mucus based on experimental and clinical findings.

Clearly, the factors mentioned in (a) to (c) are not truly independent of each other, and several possibilities exist for formation of vicious cycles leading to compromised corneal cleaning, wettability and defense. Finally, some of the surface interactions noted here may also be germane to our understanding of the role of mucus at other mucosal epithelia.

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