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ABSTRACT Ocular surface disorders, such as dry eye disease, ocular rosacea, and allergic conjunctivitis, are a heterogeneous group of diseases that require an interdisciplinary approach to establish underlying causes and develop effective therapeutic strategies. These diverse disorders

share a common thread in that they involve direct changes in ocular surface chemistry as well as the rheological properties of the tear film and topographical attributes of the cellular elements of the ocular surface. Knowledge of these properties is crucial to understand the formation and stability of the preocular tear film. The study of interfacial phenomena of the ocular surface flourished during the 1970s and 1980s, but after a series of lively debates in the literature concerning distinctions between the epithelial and the glandular origin of ocular surface disorders during the 1990s, research into this important topic has declined. In the meantime, new tools and techniques for the characterization and functionalization of biological surfaces have been developed. This review summarizes the available literature regarding the physicochemical attributes of the ocular surface, analyzes the role of interfacial phenomena in the pathobiology of ocular surface disease, identifies critical knowledge gaps concerning interfacial phenomena of the ocular surface, and discusses the opportunities for the exploitation of these phenomena to develop improved therapeutics for the treatment of ocular surface disorders.

KEY WORDS dry eye disease, evaporation, glycocalyx, interfacial phenomena, mucins, microvilli, rheology, surface energy, tear film, tear film lipid layer

OUTLINE

I. Introduction

II. Historical Perspective

III. Role of Surface Chemistry (Intermolecular and Surface Forces) in Ocular Surface Disorders
  A. Surface Energy and Contact Angle
  B. Characterization of the Ocular Surface Energetics
    1. Whole Tear Surface Tension
    2. Influence of Tear Components on Surface Tension
      a. Lipids
      b. Mucins
      c. Lipocalin
      d. Other Tear Constituents
    3. Cellular Contributions to Ocular Surface Energy and the Cell-Tear Film Interface
      a. Physicochemical Properties of Cellular Constituents of the Ocular Surface
      b. Role of the Glycocalyx

4. Formation and Stability of the Tear Film Lipid Layer

C. Dewetting, Evaporation and Stability/Instability of Liquid Films

1. General Concepts

2. Dewetting, Evaporation and Stability/Instability of the Tear Film

a. Nonevaporative Models

b. Evaporative Models

D. Opportunities to Exploit Surface Phenomena Related to the Ocular Surface Chemistry

IV. Physical and Chemical Heterogeneity

A. General Principles

B. Topographic and Chemical Heterogeneity of the Ocular Surface

1. Topographic Features of Ocular Surface Cells

2. Chemistry of Ocular Surface Cells

C. Opportunities to Exploit the Heterogeneity of the Ocular Surface

V. Rheology of the Tear Film

A. Rheology and Hydrodynamics

1. General Concepts

2. Hydrodynamic Models of the Tear Film

3. Rheology of Tears

B. Opportunities to Exploit the Rheology of Tears

VI. Conclusion

1.

I. INTRODUCTION

Surfaces or interfaces are the thin boundary regions separating macroscopic phases. Knowledge of the phenomena occurring at these interfaces is essential, since the properties of materials near these regions differ profoundly from those in the bulk of the substance and the interactions of matter with its environment depend on these *interfacial* characteristics.[1] Most of the reactions and interactions in biology occur at interfaces, bringing attention to the importance of interfacial science for the advancement of knowledge and the development of technology in biology and medicine.[2]

For this review of the interfacial phenomena of the ocular surface, we define the "ocular surface" as comprising all cellular constituents that cover the exposed regions of the eye (corneal epithelium, limbus, conjunctiva), as well as the lid margin and the tear film, a complex fluid phase (Figure 1). As detailed below, our use of the term "ocular surface" thus encompasses a complex mixture of interfaces possessing varying degrees of distinct borders.

The earliest written record of tears dates from the fourteenth century BC, from the Ras Shambra clay tablets found in Syria containing a poem about the response of the virgin goddess Anat to the death of her brother Baal, when she "drinks her tears like wine."[3] Among the functions of the tear film are the delivery of nutrients and control of oxygenation of the cornea, the physical protection by the trapping and removal of particles, and the antimicrobial protection by some tear components.[4] The tear film components have a glandular origin (lacrimal and meibomian glands) and a cellular origin (goblet and epithelial cells), and its main constituents are water, proteins, electrolytes, mucins, and insoluble lipids.[5-7] It is difficult to arrive at a consensus value for the thickness of the tear film for a given species and, surprisingly, no value could be located in the literature for a number of species used in ocular drug development.[8] This difficulty is in part due to the dynamic nature of its thickness profile associated with blinking and its obligatory thinning during the interblink interval. Furthermore, tear film thickness is affected by numerous other factors, including sex, age, and relative humidity.[9] Additionally, the definition of "thickness" of the tear film is complicated by a lack of consensus in the literature as to 1) the best method for determining tear film thickness (with differing approaches yielding differing values), and 2) exactly how cellular surface features such as microvilli and the glycocalyx with intrinsically associated mucin elements are accounted for in the measurement process[9]. Keeping these confounding variables in mind, for the human, there is general

agreement that the tear film ranges in thickness between 3-10 μm,[9,10] while for rabbit the range is 7-11 μm.[11-13]

This review is focused on the human tear film with the inclusion of studies involving other species limited to a very small number of commonly employed laboratory and agricultural animals. In the investigation of interfacial properties of the ocular surface, these animals have largely served as specimen donors rather than being used for in vivo investigations. It should be noted that the tear film in general and the interfacial properties of the ocular surface in particular have been markedly understudied from a comparative perspective. There are likely numerous unique adaptations in tear film biology that are yet to be discovered, given the enormous variation in evolutionary history and environmental niches populated by the >50,000 species of vertebrates with whom humans share the planet. Also, studies involving laboratory/agricultural animals are not necessarily transposable to the human condition due to inherent differences in the biology of the ocular surface (tear chemical composition, blink rates, tear film hydrodynamics, cellular elements, relative age and state of ocular surface health).[14]

As noted above, the tear film is a complex system that has been recognized since 1946 as a multi-layered structure.[15] The identity and number of layers has been largely disputed, especially the characteristics and existence of the ocular mucus layer. We note that the term *mucus* is used throughout the literature and in this work refers to the gel-forming secreted mucins that are hydrated (a more detailed description of the mucins is provided in section 3 below). The use of the term mucus predates the identification of the individual mucins that are integral to the ocular surface and tear film. Several distinct schemes for the structure of the tear film have been proposed:

1. *One-layer Model:* This simple model predates detailed information available regarding the complex nature of the composition of the tear film. Many tear film models utilize a single-layer approach that represents only the aqueous layer, which constitutes the majority of the tear film.[16-18]
2. *Two-layered model*. Since a sharp interface does not exist between the mucus and the aqueous components of the tear film, a mucoaqueous gradient layer with an insoluble lipid film on top has been proposed,[14,19] supported by the electron microscopic observation of a homogeneous structure throughout the aqueous layer in rats.[20] Additionally, in one report, the electrical potential difference measured between the tear surface and an electrode placed at

100 nm intervals across the thickness of the tear film in the mouse yielded a constant value, supporting a single-phase model (i.e., if distinct phases existed in situ, the authors suggest that differences in potential would be anticipated).[21]

3. *Three-layered model*. Composed of a mucus layer, an aqueous layer, and a thin lipid film, this was originally proposed by Wolff[15] and has been the classical model.[22-24] Although there is no sharp interface between the mucus layer and the aqueous layer, some authors still advocate for the existence of a distinct mucus layer, as recently proposed by Khanal and Millar.[25] These authors introduced and traced quantum dots in tears. The quantum dots close to the ocular surface exhibited different flow dynamics than the quantum dots in the aqueous layer, suggesting the absence of a gradient and the existence of a discrete layer (which may be a thick glycocalyx).[25]
4. *Other models:* Some authors have used alternative schemes for the modeling of the tear film, such as a two-layer model, consisting of a mucous layer and an aqueous layer,[23,26,27] or a three-layer model in which the lipids are structured in a duplex film (polar lipid monolayer and a layer of non-polar lipids) over a mucoaqueous film.[28-31]

Abnormalities in the interfacial properties of the ocular surface can result from a large group of disorders. Distinct yet not necessarily separate diagnoses that have been implicated in contributing to perturbations in the interfacial phenomena of the ocular surface include aqueous tear deficiency,[32] meibomian gland dysfunction,[33] tear hyperosmolarity,[34] unstable preocular tear film,[35] ocular rosacea,[36] exposure keratopathy,[37] microbial keratitis,[37] chemosis,[37] allergic conjunctivitis,[38] pemphigoid,[36] metaplasia,[39] inflammation,[40] and ocular irritation.[41] These require an interdisciplinary approach to better understand the causes, diagnosis, and treatment. A key aspect of the challenge arises from a complex interplay between these disorders, interfacial phenomena and the stability of the tear film (Figure 2):

1. These disorders can interfere with the production of constituents of the ocular surface, the dynamics of blinking and drainage or the rate of evaporation.
2. The disturbances modify the physical and chemical properties of the ocular surface that are essential for the formation and stability of the tear film.
3. The disruption of the tear film aggravates the conditions following multiple feedback loops.

Whereas the symptoms of ocular surface disease is usually assessed with the aid of questionnaires,[42] numerous tests have been employed to characterize ocular surface pathologies, including Schirmer's tear test,[43] lissamine green staining,[44] rose bengal staining,[45] tear osmolarity,[46] specular microscopy,[47] tear meniscus height,[48] a variety of biomarkers[49] such as inflammatory cytokines (interleukin [**IL**]-1α, IL-1β, IL-6, IL-8, tumor necrosis factor [**TNF**]-α)[50,51] or other proteins (S100A8, S100A9, α-1 antitrypsin, metalloproteinase-9, lacrimal proline-rich protein 4),[52-54] evaporation,[55] meibometry,[56] interferometry,[57] mucus ferning test,[58] mucopolysaccaride degrading enzymes,[59] and tear film breakup time (**TFBUT**)[60]. Many of these endpoints provide specific information regarding a narrowly defined attribute but do not provide an integrated assessment of the state of the ocular surface. Arriving at a definitive assessment of the ocular surface using any single endpoint is analogous to the parable of blind men examining an elephant and being asked to describe it, each with limited information based on their individual experience. It should be noted that the complexity and dynamic interdependence of the constituents of the tear film and their interaction with the cellular elements of the ocular surface *is not* reflected in the multiple diagnostic tests currently in use.

Among these endpoints, TFBUT is considered to best reflect a measure of tear film stability, although other methods have also been employed, such as Tear Film Breakup Dynamics (**TBUD**), tear film particle assessment, topographical analysis systems, interferometry of the lipid layer, confocal microscopy, visual acuity testing, functional visual acuity, wavefront aberrometry, or integrated multimodal metrology.[61] TFBUT measurement either employs fluorescein (fluorescein is instilled to show breakup under blue light)[60,62] or TFBUT is assessed noninvasively (evaluating the breakup time and the location of the defects by measuring distortions of a projected grid on the cornea).[63,64] Problems in the reproducibility of TFBUT measurement[65,66] have limited its use for the assessment of the effectiveness of treatments,[4] but some effort have been made to standardize these measurements, including the use of defined minimal amounts of fluorescein[67] and the inception of the corneal protection index (CPI = TFBUT/length of the interblink).[68]

The importance of studying interfacial phenomena of the ocular surface has been recognized since the late 1960's[69-71]; however, active investigation of the physicochemical surface attributes of the ocular surface has subsided significantly since the 1990s.[72] During these recent "quiet decades," it is noteworthy that a number of new experimental techniques for the

study of molecular interactions and surface attributes in biological materials have been developed. These techniques have been underexploited in investigations of interfacial phenomena of the ocular surface. Examples include: X-ray photoelectron spectroscopy (**XPS**, a surface-sensitive quantitative technique that measures elemental composition, chemical state and electronic state of elements on a surface)[73,74]; time-of-flight secondary ion mass spectrometry (**ToF-SIMS**, a semi-quantitative technique that provides information on single ions, individual isotopes and molecular compounds from a surface)[73,75-77]; surface-enhanced Raman spectroscopy (**SERS**, a technique that allows the fingerprinting of molecules that adsorb onto metallic surfaces or are brought to close proximity to metal nanoparticles)[78,79]; attenuated total reflectance-Fourier transform infrared spectroscopy (**ATR-FTIR**, which allows fingerprinting with infrared spectroscopy on solid samples)[73,74]; atomic force microscopy (**AFM**, which provides information on biophysical attributes, such as topography and relative stiffness)[73,80]; scanning ion-conductance microscopy (**SICM**, a technique related to AFM that allows the force-free imaging of biological samples)[81]; surface plasmon resonance (**SPR**, a technique that measures the refractive index near a sensor surface, and enhances the surface sensitivity of spectroscopic methods)[82,83]; and high throughput surface characterization techniques.[75,84,85] These tools can provide fundamental data regarding the elemental composition and changes after surface modification (e.g., XPS[86]), spatial localization of specific molecular species across the ocular surface (e.g., ToF-SIMS[87], ATR-FTIR[88]), high-sensitivity immunoassays to determine specific biomarkers in the aqueous tears (SERS using gold nanoparticles functionalized with antibodies[89] or label-free immunoassays using SPR[90]) and characterization of the nano/micron-sized topography of the ocular surface (AFM[91], SICM[92]).

A key conclusion of this review is that these techniques should be evaluated for their potential to provide insight into ocular surface phenomena, as they may provide critical data needed for identifying optimal paths forward in the development of therapeutics for the treatment of ocular surface disorders. In the following review, we highlight knowledge gaps involving interfacial phenomena of the ocular surface and identify opportunities in research and the development of therapies for ocular surface disorders.

II. HISTORICAL PERSPECTIVE

A timeline in the study of interfacial phenomena in the eye is shown in Figure 3. Early studies on the physicochemical properties of the ocular surface characterized tear film instability as the appearance of dry spots,[93-95] which was later identified as "insufficient wetting" of the epithelial surface by Holly.[96] In 1965, Mishima recognized the presence of a substance on the ocular surface that helped retain the fluid layer.[71] This "hydrophilic" material was incorrectly proposed by Ehlers to be lipids from the meibomian glands,[70] but was later identified as mucins.[97] In 1968, Norn made a distinction between two different phenomena observed in dry eyes. 1) A "hole" in the tear film developed after the eye had been kept open for some time, which occurred at random sites and was not related to any ocular pathology[60] (which is now recognized as the time point at which TBUT is determined). 2) A permanent local dryness (or "dellen") noticed at the moment the eye is opened was attributed to local surface discontinuities or protuberances raised above the tear film thickness.[98]

During the 1970s and 1980s, Holly led the systematic investigation of the physicochemical properties of the ocular surface when his laboratory started measuring tear surface tension[99-101] and ocular surface energy[96] for different corneal layers.[102] During this period, the corneal epithelium was believed to be inherently hydrophobic, but an adsorbed layer of mucus was thought to act as a wetting agent in the interface between the epithelium and the aqueous tears.[96] The dominant model of the tear film was a three-layered model, and mucus was also regarded as a lubricant, protectant, and surfactant at the aqueous-lipid interface,[22] and the contamination of this mucus layer by lipids was believed to be the cause of tear film instability.[103,104]

The first formal mathematical analysis of tear film stability and rupture was proposed in 1974 by Berger, based on the suggestion that a gradient in surface tension is the driving force for the formation of the tear film after a blink.[105] Lin and Brenner later proposed that flow due to gradients in surface tension (Marangoni effects, see inset II) and viscosity are the origin of stabilizing stresses in the tear film,[106] while the interactions arising from van der Waals and other intermolecular forces destabilize the tear film and are responsible for the dewetting of the ocular surface.[16] Following this direction, in 1985 Sharma and Ruckenstein proposed the rupture of the mucus layer of the tear film as the mechanism leading to the exposure of the hydrophobic epithelium.[23]

During the 1980s, following electron microscopy studies of the apical surface of the ocular epithelium,[107-109] the postulated characteristics of the epithelial surface were questioned.[110,111] New measurements on the wettability of the corneal epithelium and the discovery of a hydrophilic glycocalyx demonstrated that gel-forming mucins were not needed for the spreading of tears.[112] Furthermore, the proposed role of mucins as surfactants that stabilize the spreading of the lipid layer was also debated after tear lipocalin was identified as the most surface-active molecule in this interface.[113]

During the 1990s and the first decade of the 2000s, many different thermodynamic and hydrodynamic models for the formation and destabilization of the tear film were proposed. For example, Sharma characterized the surface energy of the corneal epithelium and the mucus layer,[114] and this strategy allowed the calculation of all the surface and interfacial energies of the ocular surface, and the work of adhesion of several tear film interfaces.[115] Using these values, Sharma proposed the role of mucus as a lipid trap that collects contaminants from the tear film that are then removed from the ocular surface through blinking, rather than a surfactant for the corneal epithelium.[116]

Recent hydrodynamic models incorporate phenomena that are more realistic for the modeling of the tear film, such as shear-thinning viscosity (non-Newtonian rheology),[26] slippage (the fluid "slips" on the boundary with the epithelium),[18] 2D models of the tear film,[117,118] and the incorporation of evaporation.[17] However, despite the efforts to study the ocular surface in terms of fundamental interfacial phenomena, the functional role played by the various components of the ocular surface remains elusive.

In recent efforts to provide a consensus for the definition and treatment of the dry eye, such as the International Dry Eye Workshops[32,119], and the Delphi Panel,[120] two major categories of dry eye syndrome were defined: tear-deficient dry eye (**TDDE**) and evaporative dry eye (**EDE**). Subsequent efforts have focused on meibomian gland disorders,[28,30,33,121-134] tear film evaporation,[28,126,135-138] osmolarity,[34,139,140] inflammation,[40,141-143] and stem cell transplantation,[144,145] as highlighted in a recent citation analysis in the dry eye literature,[146] while the investigation of interfacial phenomena in the ocular surface has taken a secondary role.[147]

III. ROLE OF SURFACE CHEMISTRY (INTERMOLECULAR AND SURFACE FORCES) IN OCULAR SURFACE DISORDERS

The formation and stability of liquid thin films over a surface is highly dependent on the chemical composition and associated intermolecular forces acting between the constituents of the different phases involved.[148] As noted above, the ocular surface is a complex composite system comprised of a multilayered tear film, supported on a soft cellular substrate that is topographically and chemically heterogeneous.[107,108,149] The development of a complete understanding of thin liquid films on such complex interfaces represents a substantial challenge and requires the integration of diverse disciplines, including chemistry, physics, biology and engineering,and the knowledge of surface properties such as the energies of the interfaces that comprise the ocular surface.

A. Surface Energy and Contact Angle

At a surface of a liquid or solid exposed to a vapor, molecules experience intermolecular forces directed toward the bulk phase, drawing surface molecules toward the interior and causing the surface to seek minimum area. This contractile tendency can be quantified as force per unit length or energy per unit area, termed "surface tension" and "surface energy," respectively.[148] These cohesive forces between liquid molecules at a surface with a gas are responsible for the generation of surface energy. It is the surface energy that gives rise to the force that enables a needle to float on the surface of water in a glass or, alternatively, for a water strider to skim across the surface of a pond. Surface energy can also be interpreted as the mechanical energy that must be invested to create a unit area of a surface.[1] When two condensed phases (e.g., two liquids) are in contact, they define an "interface" and the terminology introduced above is modified accordingly (e.g., to "interfacial energy").

Examples of intermolecular forces that play an important role in determining surface/interfacial energies are 1) van der Waals (**VdW**) forces, which are attractive interactions between neutral molecules that originate from the presence of dipoles in the molecules (permanent, induced or transient),[1] and 2) coulombic forces at interfaces possessing immobilized charges (such as in the corneal and conjunctival epithelia[150]), where ions of the opposite charge (or counterions) gather in the vicinity of the interface, forming an "electrical double layer."[1]

The surface tension (or interfacial tension) of fluids can be sharply reduced by certain solutes called surfactants (or surface active agents). Generally, a solute is deemed "surface-active" if it reduces the surface tension.[1] In the respiratory system, surfactants decrease the surface tension and thus the amount of work required for expansion of alveoli. Infants born prior

to 26 weeks of gestation are typically deficient in lung surfactant, and that deficiency is associated with Infant Respiratory Distress Syndrome.[151-154] In the tear film, complexes of molecules interact to function as surfactants, such as polar lipids[113] and lipocalins[155] in combination with mucins and other proteins.[99]

A common assay used to characterize surface properties of biological molecules (e.g., lipids) is based on a Langmuir trough. A monolayer of the species of interest (often a mixture) is spread onto the surface of water and then compressed using a barrier that is moved across the surface. Measurement of the surface tension before, during, and after compression and expansion provides an assessment of the stability of the film under conditions that mimic the blink cycle. Use of these assays has been proposed as a diagnostic tool in the assessment of ocular surface disorders.[156,157]

A central property that is used to characterize the wetting of a liquid on a solid is *Young's contact angle* (the angle measured at the point of contact between the liquid and the surface).[158] Ideal surfaces (smooth, rigid, chemically homogeneous, insoluble, nonreactive surface) present a 3-phase *contact line* (solid, liquid, and vapor) with a single well-defined Young's contact angle (Figure 4).[159] The balance of forces at the contact line is described by Young's equation, which connects the surface and interfacial energies to the contact angle (Inset I). These situations are relevant to the development of topical therapeutic agents. The values of the surface/interfacial energies in Young's equation can be altered by the addition of agents that modify the interfaces of the ocular surface. For example, Young's equation predicts that decreasing the surface energy (increasing the contact angle) of the cellular constituents of the ocular surface will decrease its wettability by the tear film (which would result in dewetting of the ocular surface should the tear film break).

The contact angle provides a way to characterize the lyophilic (solvent-loving) nature of a surface. Depending on the magnitude of the contact angle, we can encounter different regimes of wetting: complete wetting (when the liquid fully covers the solid, contact angle of zero); partial wetting (contact angle between zero and 90°); partial nonwetting (contact angle between 90° and 180°); and total nonwetting (contact angle of 180°).[1] However, to fully characterize the wettability, knowledge of the specific interfacial energy components are required. Liquid surface tensions are relatively easy to determine, but the solid-liquid interfacial energy cannot be directly measured. For that purpose, other approaches to have been developed.[1,148,160]

B. Characterization of the Ocular Surface Energetics

1. Whole Tear Surface Tension

As established above, knowledge of the surface tension of tears is necessary in order to understand the wetting of tear films on the ocular surface. The measurement of the surface tension of tears, however, is challenging. The volume of tears that can be acquired from a single patient is limited, and thus tears are usually pooled from a large number of donors or from repeated sampling from a single or small number of donors. Trying to optimize the collection could induce reflex lacrimation and change the intrinsic attributes of the tears collected confounding analysis. Another problem is the structured character of the tear film, which poses difficulties for the sampling and reproduction of the native state of tears for the measurement of its surface properties. Some collection techniques include the use of Schirmer strips and other absorbent-based methods, eye washes, and glass capillary tubes.[41] It should be noted that collection of tears in general and with capillary tubes in particular is challenging in patients with a reduced tear volume. The handling and storage of the collected tears could also have an influence on the measurement of the surface tension, due to adsorption of components to the walls of the containers.[41,161]

Despite challenges in collection and analysis, there has been some success in the characterization of the aqueous tear surface properties. One of the first experimental determinations of the surface tension of tears was done on stimulated tears from calves, using a capillary rise method, where the surface tension measured was 72.3 dynes/cm (almost identical to water).[162] In 1926, Miller used a scleral contact lens carved with a trough, where tears collected from the patient were deposited and the surface tension measured with a du Nouy ring as 48 dynes/cm.[163] A method requiring a low volume of tears is the determination of surface tension by contact angle on standard polyethylene surfaces. Following this procedure, the surface tension of normal tears measured 40.1±1.5 dynes/cm.[100] Holly proposed that the surface tension of the tear film is dependent on the palpebral fissure width, because the compression of lipid layer upon blinking increases the surface concentration and reduces surface tension.[164] This dynamic character of the surface tension was demonstrated using a pulsating bubble surfactometer, which measured the dynamic surface tension of whole tears as 35 dynes/cm at minimum bubble size and 45 dynes/cm at maximum bubble size.[165] In aggregate, the modern literature suggests a value of 35-40 dynes/cm. This is approximately half the value of water and

indicates the presence of surface-active components that decrease surface tension and facilitate the wetting of tears on the ocular surface.

In an attempt to elucidate the role of the surface tension of tears in dry eye conditions, Holly found very small differences from patients with a wide array of dry eye conditions relative to tears collected from normal subjects, and attributed the differences to changes in the components of tears, specifically the generation of inflammation-related molecules.[100] Later, Tiffany et al found a moderate correlation between TFBUT and surface tension, whereby higher surface tensions correlated with less stable films. They found the surface tension of tears to be 43.6±2.7 dynes/cm for normal eyes and 49.6±2.2 dynes/cm for dry eyes.[166] Zhao et al proposed the measurement of surface properties using a Langmuir trough as a tool to diagnose dry eye syndromes[157] and obtained significant differences in the surface tension at maximum compression (20% of the original surface area) of 46.6 ±3.8 dynes/cm for normal eyes and 52.9 ±7.4 dynes/cm for female patients with Sjögren syndrome.[156] Contrary to widely held views, Peng et al have proposed that tear films with a lower value for surface tension are more prone to disruption than films with higher surface tension.[31] In their model, the presence of lower surface tension encourages tear film breakup through evaporation-driven mechanisms. They propose that locally increased regions of evaporation associated with a thinned/absent tear film lipid layer (**TFLL**) would induce negative curvature (meniscus formation) in the tear film. At relatively low surface tensions, the curvature-driven healing flow is impaired, reducing the thickness of the tear film locally and decreasing the TFBUT value.[31] All other parameters being held constant, conditions that increase evaporative loss would decrease TFBUT values.[31]

In summary, the surface tension of tears likely plays a significant role in ocular surface disorders, such as dry eye, but, as addressed below, substantial uncertainty remains as to which components of the ocular surface are responsible for the changes in surface tension.

2. Influence of Tear Components on Surface Tension

a. Lipids

Tear lipids were the first constituents of the tear film thought to make a major contribution to tear film surface tension. The surface pressure (the reduction of surface tension) of tear lipids was estimated by Brown in 1965 to be between 15 and 33 dynes/cm.[167] However, Langmuir monolayers of meibomian gland-derived lipids showed surface pressures of only 13

dynes/cm,[168] and extracting lipids from tears only increased the surface tension of the thus modified tear film from 46.0 dynes/cm to 53.6 dynes/cm.[113] This low surface activity has been attributed to the high percentage of wax and sterol esters and the low content in polar lipids in meibomian secretions.[129,130,169] Furthermore, Nagyová et al added meibomian secretions to lipid-depleted tears and found a poorer recovery of the surface activity compared to normally resident tear lipids, suggesting that the source of lipids in tears is not solely from meibomian glands.[113] Adding model lipids to these depleted tears showed that while wax esters and sterol esters reduced the surface tension, phospholipids had the greatest impact on altering surface tension.[113]

Other studies on the influence of the composition of the lipid layer have shown that nonpolar lipids destabilize the lipid film spreading properties and polar lipids act as stabilizers.[170] While the broad class of phospholipids have been shown to have the greatest effect in lowering surface tension,[113] zwitterionic (neutral molecules with a positive and a negative charge in different locations within the same molecule) phospholipids, in particular, better increased tear film stability as measured by TFBUT in an artificial eye model.[171]

In summary, polar lipids originating from the meibomian glands and alternative sources (such as phospholipids from the cellular membrane of shed cells) appear to be primarily responsible for the low surface tension of tears.

b. Mucins

The effect of mucins in modulating tear film surface tension is complex, and the literature is populated with conflicting reports. An indication of the role of mucins in lowering the surface tension in tears was given by Lemp et al, where the surface tension of artificial tears was reduced from 71.1 dynes/cm to 43.2 dynes/cm with the addition of 0.5% bovine submaxillay gland-derived mucin (**BSM**).[97] Holly investigated mucins, albumin, globulin, and lysozyme as the surface-active components of tears and determined mucins to be the most surface active molecules, with surface pressures up to 35 dynes/cm at 1% concentration.[99]

Holly's work identifying mucins as the major contributors for the tear surface tension was done using BSM as a model, due to its accessibility. However, mucins are a heterogeneous group of glycoproteins whose properties depend on their amino acid sequence and their post-translational glycosylation,[172,173] as well as their purity,[174] concentration, pH, and electrolytes in the media.[175] Results obtained with BSM may not be directly transposable to the effect of ocular surface mucins.

To test whether ocular mucins were indeed the greatest contributors to the surface tension of tears, Pandit et al hypothesized that basal tears should have a lower surface tension than stimulated tears due to the increased contact time. They observed no significant differences (43.0±2.1 dynes/cm and 46.0±1.46 dynes/cm for basal and stimulated tears, respectively).[161] Mucins have been shown to reduce surface tension, but their surface activity is not as high as initially reported, and a significant reduction in surface tension is achieved only when mucins are present at very high concentrations[113] not found in normal tears.[174] Millar et al proved that while BSM has a surface activity dependent on the concentration, purified bovine ocular mucins had no surface activity even at concentrations 100 times higher than normal.[176] However, ocular mucins may play an indirect role in increasing the surface pressure of the lipid layer by compressing the lipids and restricting mobility. Such an effect would contribute to the rigidity and stability of the lipid film between blinks.[176] In summary, mucins from nonocular sources have been shown to affect surface tension, but ocular surface/tear mucins have not been validated to have the same magnitude of effect when employed at physiologic concentrations. It appears likely that the effect of mucins on surface tension-related phenomena in the eye occurs indirectly via their influence on the distribution of lipids.

c. Lipocalin

Until the 1990s, tear-specific prealbumin had not been studied as a factor in tear surface tension because it had not been recognized as a lipid-binding protein, or lipocalin.[177] Proteins alone and in combinations with lipids reduced surface tension; however, any combination of lipocalin with lipids reduced surface tension to tear levels, while mucins reduced surface tension, but only at very high concentrations not found in natural tears.[113] Glasgow further demonstrated the surface activity of tear lipocalin[155] and the role of tear lipocalin to bind, cover, and remove lipids from the ocular epithelial surface,[178] and tear lipocalin has been shown to be reduced in patients with Sjögren syndrome.[179] Phospholipid transfer protein has also been proposed as a scavenger of lipophilic substances from ocular mucins.[180]

d. Other Tear Constituents

The contribution of other components to the low surface tension characteristic of normal tears has also been studied. Lactoferrin and β-lactoglobulin have demonstrated surface activity

by inserting and forming complexes in the lipid film, which may contribute to its stability.[170] In Langmuir troughs, most tear protein monolayers show surface pressure hysteresis behaviors very similar to monolayers prepared with whole tears, while meibomian gland-derived lipids and mucins show distinctly different behaviors. The authors of these studies suggested that in ocular surface diseases, the profile of proteins changes (due to production of interleukins or antibodies), which might disrupt the tear stability.[181] Certain polysaccharides (xanthan gum and chondroitin sulfate C) did not alter the lipid film surface pressure, but sodium hyaluronate (which is not surface-active by itself) promoted a marked reduction of the surface area of lipid films at constant surface pressure, suggesting the interaction of the sodium hyaluronate with the lipids (possibly with phospholipids).[170] In a study by Mudgil and Millar, the concentration or type of divalent electrolytes in the subphase did not show an effect on the surface tension of meibomian gland derived lipids.[182]

In summary, controversy exists with regard to the contribution and role of specific constituents of tears in determining surface tension. As detailed above, tears represent a complex milieu, and the individual constituents never occur or act in isolation. Rather, surface tension can be viewed as an “area-under-the-curve” outcome that integrates the individual contributions and differing mechanisms, as well as the impact of interactions between constituents. Changes in tear surface tension relevant to ocular surface disease remain markedly underexplored. Lipid and/or mucin deficiencies as a consequence of meibomian gland dysfunction or cicatrization of the ocular surface may produce changes in tear surface tension that are quantifiable. If this is proven to be true, alterations in surface tension could assist in diagnosing qualitative tear film disorders and could prove useful in monitoring response to therapy. By understanding the role of surface tension in tears, it may also be possible to predict their spreading behavior and optimally design artificial tear formulations that best interact with the ocular surface.

3. Cellular Contributions to Ocular Surface Energy and the Cell-Tear Film Interface

The surface energy of the cellular elements of the ocular surface is a major determinant of the behavior of the tear film and can affect the spreading of the tear film across its surface, as well as tear film stability and kinetics of dewetting. Here, we review the literature that provides conflicting reports with regard to the relative surface energy/hydrophilicity of the cellular surface, as well as the impact of exogenous lipids, mucins, and cell injury.

a. Physicochemical Properties of Cellular Constituents of the Ocular Surface

The first formal attempt to characterize the physicochemical properties of the cellular constituents of the ocular surface was made by Lemp et al in 1970.[97] The maximum surface tension needed for a liquid to wet the surface (critical surface tension) of wiped corneas from rabbits was estimated to be 31 dynes/cm with the Zisman method using solutions of electrolytes containing variable concentrations of proteins and mucins. Applying meibomian gland-derived lipids to the surface of rabbit wiped corneas did not alter the contact angle; however, by rubbing BSM over the surface, complete wetting was achieved. The authors suggested a significant role of mucins for the alteration of interfacial energetics and the promotion tear film coverage of the cellular surface.[97] They later hypothesized that the spreading and maintenance of the tear film required the production of mucus by goblet cells and the redistribution of the mucus layer across the cornea by blinking.[62] We note, however, that the caveats previously mentioned relating to the use of BSM for study apply here also and results obtained may not be directly transposed to the effect of native tear film mucins.

Of equal interest to the effect of adding extrinsic mucins is the study of the effect of depleting mucins from the cell/tear film interface. Holly and Lemp measured contact angles of hydrophobic liquids on corneas depleted of mucus and established the effects of mucin solutions on contact angle.[96] Following this characterization, they suggested that mucins play the role of surfactants for both the epithelial-aqueous and the aqueous-lipid interfaces besides working as lubricants and protectants.[96] Their proposed mechanism for achieving tear film stability implicates the interaction of mucins with lipids to decrease the surface tension of tears, and the spreading and adsorption of mucins over the epithelium by blinking, increasing the surface energy of the cellular component.[96]

The model advanced by Holly and Lemp is supported by the observation of decreased goblet cell population in certain ocular surface diseases, such as ocular pemphigoid and Stevens-Johnson syndrome.[183] In contrast, however, no significant decrease in mucus production was observed by other investigators for the pathologies mentioned above.[184] Furthermore, Cope et al suggested that confounding issues were present in the methodologies employed by Holly et al. The epithelial surface appeared to be damaged by drying, by wiping to remove surface mucus, and by the “inert” liquids used.[110] Liotet and colleagues disagreed on the role of mucus as a surfactant, because conjunctival mucoproteins polymerize and form a high molecular weight

insoluble gel, incapable of interacting with the lipid layer.[111] However, mucus may play an important role in the formation and maintenance of the tear film through a "lipid trap" mechanism, as proposed by Sharma (described below).

b. Role of the Glycocalyx

The presence of a glycocalyx (an intrinsic surface-associated cellular coating rich in polysaccharides) on the surface of epithelial cells has been recognized since the 1960s.[185] However, the studies proposing the dewetting of the tear film due to the development of a relatively more hydrophobic corneal epithelium in disease states failed to consider this intrinsic membrane-associated hydrophilic coating as part of the cellular surface. Blumcke and Morgenroth provided an early report of the ultrastructural characteristics of the corneal epithelial surface in 1967,[186] and in 1981, Dilly and Mackie identified the presence of surface glycoproteins that are heavily glycosylated on the surface of conjunctival epithelial cells.[107] The scanning electron microscopy studies of the ocular surface by Nichols et al revealed a thick glycocalyx, on the order of 300 nm,[109] and the layer of secreted mucins associated with the cell surface was found to be thicker than previously thought (1-7 microns).[187]

We note here that mucins can be categorized as membrane-associated mucins (e.g., MUC1, MUC4 and MUC16), which are an intrinsic constituent of the glycocalyx, and secreted mucins. which can be subdivided into gel-forming (e.g., MUC5AC) and soluble (e.g., MUC7). Although the gel-forming mucin MUC5AC may be proteolytically degraded on the ocular surface[188,189] and not form a true gel layer,[190] it likely interacts loosely with the cell surface (and associated glycocalyx),[115,191] and it may also interact with microorganisms present in the tear film.[192] Smaller fractions of MUC5AC have also been found dispersed in the aqueous phase.[193] MUC7 is a smaller molecule, found predominantly in human saliva, and it is widely believed that it does not form gels.[191] While MUC7 is also expressed by acinar cells of lacrimal glands,[194] its role in the ocular surface is largely unknown. This is because, to our knowledge, it has not been detected in the aqueous component of tears.[193,195,196]

Many functions were initially attributed to the glycocalyx of the ocular surface, including maintenance of a negative surface charge, masking of surface antigenicity, cellular recognition, pinocytosis, organ differentiation, and the regulation of cellular adhesion.[197] In 1992, Gipson et al developed the H185 antibody that binds sugar epitopes in highly glycosylated glycoproteins,

demonstrating that all the apical cells of the ocular surface possess a glycocalyx in rat[198] and human.[199] The membrane-associated MUC1 was the first mucin to be identified as an important component of the ocular glycocalyx[200] (it is expressed by both corneal and conjunctival epithelial cells), but the presence of the membrane-associated mucins MUC4[191,201] and MUC16 was later determined.[202] The expression pattern of membrane-associated mucins depends on its location on the ocular surface (cornea or conjunctiva) and in the epithelial layer.[203] A disrupted glycocalyx can be rapidly regenerated,[204] and if the tight junction of apical epithelial cells are disrupted, underlying cells differentiate into superficial cells and start secreting glycocalyx components.[205]

Royle et al partially analyzed the polysaccharide components in ocular mucins of human, dog, and rabbit, and found simple tetra- tri- or disaccharide structures.[150] In their studies, they lysed the entire epithelium; thus, their results analyzed the effects of mucins *en toto*, not distinguishing between mucins of differing origin. In humans, the majority of saccharides were negatively charged (terminated in sialic acid), while in dog and rabbit they were neutral (terminated in α 1-2 fucose and/or 1-3 N-acetylgalactosamine).[150] Guzman-Aranguez et al confirmed this finding by determining that 66% of the glycan pool in humans consisted in mono-sialyl O-glycans.[173] Alterations in the mucin distribution or mucin glycosylation have been extensively observed in dry eye pathologies.[196,206-209].

Subsequent work done by Tiffany in 1990 recognized the corneal and conjunctival epithelial glycocalyx as responsible for the hydrophilic properties of the ocular surface.[112] Tiffany measured the wettability of epithelium using different methods and concluded that the surface energy of the intact epithelium (68.3 ± 0.8 dynes/cm) and the epithelium with mucus removed (67.5 ± 0.6 dynes/cm) were not significantly different.[112] When drying was allowed to occur, the contact angle increased, suggesting a change in structure and denaturation of the components of the cell membrane.[112] Although wiping the epithelium disrupts the structure, complicating the measurements, Tiffany determined a surface energy of wiped corneas of 40 dynes/cm, attributing the low surface energy to the release of cytosolic proteins and lipids, which may have a role in the rupture of the tear film.[112]

Sharma performed a more complete characterization of the various contributions to the ocular surface energy.[114,210,211] Measurements of the surface energy of rabbit corneas with mucus coating were 49.5 dynes/cm, while for corneas with mucus removed were 54.4 dynes/cm, confirming that mucus is not needed to increase the surface energy of the epithelial surface.[114]

He also determined that damaging and drying of the epithelium reduced the polar component of the surface energy.[114] The measurement of the polar component of the surface energy allowed the calculation of the interfacial energy between the corneal surfaces and the tears, making it possible to evaluate the energy of adhesion of different substrates. The energy of adhesion of gel-forming mucins-glycocalyx in water is 39.0 dynes/cm; the value for mucus adhesion to damaged cells in water is -17.8 dynes/cm, and for mucus adhesion to mucus in water it is 42 dynes/cm. The positive values of adhesion imply that the gel-forming mucus does not strongly adhere to the glycocalyx or to itself, instead forming a very hydrated loose glycopolymer that is mobile on the ocular surface.[115] However, when cells are damaged, the adhesion of mucus to the ocular surface is greater (indicated by the negative value of the energy of adhesion), lowering lubrication and leading to more cell damage and promoting desquamation by the shear forces acting on the adhered mucus.[115]

Sharma also proposed a “lipid-trap” role for mucus, hypothesizing that nonpolar particles adhering to mucus make the mucus more cohesive. Mucins are negatively charged and repel themselves. The integration of lipids is suggested to oppose this tendency, allowing the creation of larger aggregates/threads of mucus and subsequently promoting removal from the corneal surface by blinking. Furthermore, although nonpolar particles can adhere to the glycocalyx in an aqueous media, the adhesion of nonpolar particles in mucus is not thermodynamically favorable.[115] In later papers, Sharma expanded his theory and concluded that the corneal epithelium can be rendered hydrophobic by lipids in the tear fluid attaching directly to the epithelium whenever there is absence of mucus. These hydrophobic areas could lead to dewetting, even in cases where the patches are micron-sized (the size of a cell).[116] Some experimental evidence of the adhesive/anti-adhesive characteristics of mucins was provided by Berry et al, when AFM tip-tethered mucins showed little/no adherence to mucins deposited on mica[212]; and by Sumiyoshi, et al, who demonstrated increased adhesion between epithelial cells when the glycosylation of mucins was disrupted.[213] The adhesive properties of the ocular surface are also important for the modulation and selectivity of the bacterial strains that compose the ocular microbiome.[192]

In summary, the glycocalyx of the corneal and conjunctival surface epithelial cells contributes to the wettability of the cellular surface as well as to the relative adherence/non-adherence properties of the ocular surface. The glycocalyx plays a role in the removal of

particulate contaminants (through promotion of appropriate mucin dynamics across the ocular surface), and, by resisting adhesion of microorganisms, serves as an innate barrier to infection. The impact of diverse ocular surface diseases on the glycocalyx and concomitantly on the interfacial properties of the ocular surface remains under-investigated. Knowledge of ocular surface energetics may contribute to the development of a new set of diagnostics, as well as therapeutic strategies whereby the ocular surface is optimally engineered to render it pathogen-resistant and promote stability of the native tears.

4. Formation and Stability of the Tear Film Lipid Layer

The TFLL is mainly constituted by meibomian gland-derived lipids,[125] with an approximate composition of 60-70% nonpolar lipids (wax esters, cholesterol, and cholesterol esters) and 15% polar lipids (phospholipids and glycolipids).[214] Meibomian gland-derived lipids exhibit a melting range of 19.5-32.9±0.9 °C[215] and a high viscosity of 9.7-19.5 Pa sec, with non-Newtonian behavior (viscosity increases with applied stress). TFLL thicknesses of 32-200 nm have been reported.[124,216,217] Thickness depends on many factors, such as the stability of the lipid layer, the dynamics of blinking (the TFLL thickens and thins throughout the cycle), or meibomian gland health.[124] We agree with the suggestion of Nagyová et al[113] and Sharma[116] that another source of tear film lipids is from the epithelial constituents of the ocular surface. The ocular surface is constantly renewed with frank epithelial cell sloughing, as well as continuous microtrauma associated with blinking and rubbing.[218,219] Additionally, a varying population of inflammatory cells is present in the tear film.[220]

Reported functions of the TFLL include: the maintenance of the lid margins in a hydrophobic state helping to prevent overflow of tears,[124] the lowering of the surface tension of tears (acting as a surfactant) to impart stability,[22] and the retardation **of** evaporation.[221] Since the TFLL is compressed by blinking with the lid not traveling over the lipid surface, it likely does not play a role in lubrication.

To investigate the role of the TFLL on the formation and stability of the tear film, Brown and Dervichian performed qualitative in vitro experiments simulating blinking, and proposed a two-step process for the formation of the tear film.[222] In the first step, the opening upper lid pulls water by capillary action, wetting the ocular surface. In the second step, the TFLL spreads over the aqueous layer, thickening the film by Marangoni flow (see inset II).[222] When the eye opens,

the lipid first spreads as a monolayer against the upper eyelid. Excess lipid subsequently flows across the ocular surface and a multimolecular lipid film is formed dragging aqueous fluid with it, thickening the tear film.[104] Berger and Corsin provided evidence for this mechanism by tracking particles in the tear film following an upward movement.[105]

A duplex film structure for the TFLL was introduced by McCulley and Shine,[30] with a monolayer of polar lipids between the mucoaqueous and a thick nonpolar phase (Figure 5). The polar phase is made from sphingolipids and phospholipids (phosphatidylethanolamine, phosphatidylcholine, sphingomyelin), and the stability of this phase depends on the balance and type of phospholipids, fatty acids, ions, and pH.[30] Recently, Rosenfeld et al advanced the duplex model of the tear film by proposing a viscoelastic suspension with lipid lamellar-quasicrystals imparting mechanical structure.[223] Low levels of phospholipids in meibomian gland secretion has been associated with dry eye syndrome.[171] The nonpolar phase forms the bulk of the lipid layer and is dominated by long-chain fatty acids, fatty alcohols, and hydrocarbons. Although the primary function of the nonpolar phase is thought to control the transmission rate of gases, a secondary function is to act as reservoir of triglycerides, wax esters, and other lipids to maintain the stability of the polar phase.[224] TFLL abnormalities have been demonstrated to correlate with evaporative dry eye disease.[125]

In summary, the TFLL contributes to the formation and stability of the tear film and provides an important barrier to evaporative loss of the aqueous component of the tear film. The spreading and quality of the TFLL depends on the surface properties of this thin film, thus those properties are critical for the stability of the tear film overall.

C. Dewetting, Evaporation and Stability/Instability of Liquid Films

1. General Concepts

Liquid films are formed when two interfaces of a liquid are at close proximity. The spreading coefficient (see inset III) determines whether or not a liquid spreads on a surface. The stability of a thin film depends on parameters such as the spatial variation of temperature or surfactant (Marangoni effects, see inset II), the chemical heterogeneity of the surface, the evaporation of the liquid, the segregation and adsorption of liquid film constituents, and the intermolecular forces acting in the system.[225] Thin films are often unstable, tending to thin or to thicken spontaneously due to the interactions of the differing phases (solid/liquid/gas).

An important consideration for the stability of thin films of volatile liquids is the rate of evaporation, which is dependent on the chemical potential and the transport resistance between the liquid surface and the surrounding gas.[135] The transport resistance can be increased (decreasing evaporation) by lipid monolayers, a phenomenon that has been recognized since the early 1920s.[226] Suppression of evaporation is achieved through inhibition of diffusion of aqueous elements across the lipid layer residing at the air-tear film interface.[227] Diffusion across the thin lipid layer is modulated by a variety of factors, including temperature and surface pressure, as well as the carbon chain length and saturation level of the lipids.[228] In biological systems, such as the respiratory tract, the skin, and the ocular surface, lipid layers serve to impede water loss from the thin fluid films intimately associated with mucous membranes and, in turn, from the underlying cellular constituents.[229]

The lipid layer of the tear film, rather than being a monolayer, is duplex. In duplex lipid films, a polar monolayer is intercalated between a thicker external nonpolar layer (at the air/lipid interface) and the internally located aqueous interface. In considering the thickness of lipid films in isolation, duplex lipid films (which are thicker and contain nonpolar lipids as well as insoluble polar lipids acting as surfactants) theoretically should provide more resistance to evaporation than monolayers. This is not the case, however, as duplex films are usually less stable than monolayers, and they dewet relatively rapidly into lenses (discrete islands of continuous lipid separated by lipid-free zones in the fluid film). When islands form, the regions devoid of a lipid layer experience much higher rates of evaporation loss.[135]

2. Dewetting, Evaporation, and Stability/Instability of the Tear Film

a. Nonevaporative Models

Holly proposed a model in which the lipids from the superficial layer of the tear film migrate to the epithelial surface, contaminating the mucin layer and transforming it into a hydrophobic surface resulting in tear film breakup.[22] In this model, the thinner the starting thickness of the tear film at the end of a blink, the faster the tear film will be destabilized and the smaller the value for TFBUT. Lin and Brenner considered this explanation to be physically inconsistent, because Marangoni flow in the tear film due to surfactant concentration gradients during the migration of lipids will oppose, at least in part, the proposed diffusion of lipids and will assist to stabilize the film.[106] Instead, they proposed a mechanism in which the VdW forces

are responsible for the instability of the tear film and calculated, using a static modeling paradigm, that it is theoretically possible to have unstable films on the order of microns in thickness, depending on the specific strength of these VdW forces.[16]

Subsequently, Sharma and Ruckenstein proposed (on the basis of calculation) that the dewetting process advanced by Lin and Brenner would require months to take place. Using the same values for VdW forces as Lin and Brenner, but applied to a dynamically thinning mucus layer, they proposed that an increase in the relative hydrophobicity of the cell/tear film interface as the mucus layer thins to be the underlying destabilizing mechanism that results in breakup of the tear film.[23,230] This model considered a starting mucus layer thickness of 20-50 nm, although it was later recognized that the mucus forms a much thicker film, between 1.0 and 7.0 μm.[187] Subsequently, Sharma was unable to generate empirical evidence in support of the proposed theory that thinning of the mucus layer was responsible for tear film destabilization.[231]

Other models that have been proposed to explain the development of tear film instability include the exposure of cells with a relatively immature glycocalyx immediately after desquamation of the apical layer[111] and rupture due to changes in the mechanical properties of the tear film.[232] Given that a variety of models have been proposed, the majority of more modern publications have focused on tear film instability arising from events related to evaporation.[137,233,234]

b. Evaporative Models

In 1961, Mishima and Maurice discovered that upon the removal of the TFLL by washing or the destruction of the meibomian glands, the rabbit corneal surface would dry 17 times more rapidly.[221] Iwata et al determined a 20-fold increase in evaporation rate when the lipid layer was removed.[235] There has been extensive research on the measurement of evaporation in dry eye disorders, which has been recently reviewed in a meta-analysis by Tomlinson et al.[236] The evaporation in aqueous-deficient type of dry eye disease increased 30% with respect to normal eyes, whereas in the evaporative type of dry eye it increased by 87%.[236]

Some studies tried to measure the specific influence of the lipid layer on evaporation. Craig and Tomlinson found a 4-fold increase of evaporation when the lipid layer was absent or abnormal,[237] and King-Smith et al found weak correlations between the TFLL thickness and the rate of evaporation.[217] However, most in vitro studies show only a moderate reduction of

evaporation by meibomian gland-derived lipids, a finding that does not account for the impressive results observed in vivo.[126,135,238,239] In aggregate, these findings suggest that the composition and the structuring of the TFLL are extremely important in the inhibition of evaporation.[126,217]

Recent work by Rantamaki et al suggests that the evaporation-retarding effects depend on the physical properties of the wax esters of the TFLL; specifically their melting point.[240] For the retardation effect to occur, the temperature of the lipid film has to be very close to the melting point of these materials. If the wax esters are too solid, a large area of the interface is not covered. If the wax esters are too fluid, the intrinsic motion of the lipid is proposed to provide random, transient generation of minute, spatially discrete lipid-free zones in this highly dynamic film allowing the passage of water molecules.[240]

Most evaporation models considered the thinning of the tear film and subsequent thermodynamic instability to be responsible for the breakup of the tear film.[17] However, Peng et al recently proposed a breakup mechanism based on locally elevated evaporation (i.e., spatially discrete regions of increased evaporative loss occurring in spatially discrete regions of a thinner lipid layer).[31] In this model, the TFLL retards evaporation and is very thin, unstable, and imperfect, as described in a number of reports.[127,135,217,241] At discrete regions of thinning and/or discontinuities in the TFLL, the rate of evaporation is increased, and if it is high enough, it overpowers the stabilizing forces arising from surface tension and osmotic-driven flow (concentration of salts increases locally in regions of evaporation).[31]

Formation of spatially discrete dry spots is also influenced by wind speed, relative humidity, shape of the TFLL defect, and VdW forces. If the spatially discrete discontinuities in the lipid layer of the tear film did not form, and an adequate tear film thickness (of about 7 microns) could be maintained, then a steady state would be achieved (evaporative loss through the TFLL compensated by tear production and conditions of flow), and disruption of the tear film would not occur.[31] King-Smith et al provided experimental evidence supporting this hypothesis by comparing fluorescein images with interferometric TFLL images and showing a degree of correspondence between areas of tear film thinning and abnormalities in the lipid layer.[29]

In summary, the exact processes leading to the breakup of the tear film remains controversial, with two main concepts having adherents in the literature: 1) Tear breakup is incited by instability and dewetting of the tear film due to changes in the energy of the ocular

surface, and 2) tear breakup is incited by abnormalities/defects/thinning of the TFLL, which, in turn, promotes evaporative processes. Both mechanisms may be important for the stability/instability of the tear film, and both mechanisms are highly influenced by surface properties.

D. Opportunities to Exploit Surface Phenomena Related to the Ocular Surface Chemistry

Studies characterizing the intrinsic surface energy of the epithelium have not been reported for the human cornea, and only limited studies are available for other species.[96,112,114] This introduces the opportunity to further our knowledge of the interfacial properties of the ocular surface of humans. Comparing results of studies from different species could provide the key to understanding the differences known to exist in tear film stability.[242-250] The dynamic nature of the tear film presents significant challenges for readily translating findings obtained using in vitro and ex vivo models to the in vivo condition. However, better understanding of the nature of the interfacial phenomena of the ocular surface may enable the development of more relevant in vitro and ex vivo models for investigating the spreading, stability/instability, and evaporation of tear fluid. No mathematical, in vitro, or ex vivo tear film breakup model developed to date appears adequate, due to the complexity of the systems, lack of consensus with regard to the underlying mechanisms, and the need for integration of the different elements involved in the formation and maintenance of the tear film. The creation of more relevant models will likely promote the development of novel therapeutics to improve the stability of the tear film.

IV. PHYSICAL AND CHEMICAL HETEROGENEITY

A. General Principles

In the previous section, we discussed the wetting phenomena, using models in which the interfaces are idealized as planar, chemically homogeneous, isotropic, and nonreactive surfaces. In reality, biological surfaces are topographically patterned, chemically heterogeneous, and can interchange solutes/ions and present reactive moieties. Ideal surfaces can be characterized by a single value for the contact angle, but in situ, the heterogeneity of the ocular surface can lead to spatial variation in contact angle. Additionally, the contact line can be pinned by

heterogeneities.[251] This effect produces hysteresis between an advancing and a receding contact line,[252] and Young's contact angle is no longer applicable.[158]

Topographic features of surfaces can have a significant impact on the wetting phenomena. The Wenzel model (inset IV) provides a good model for characterizing the influence of topographic features on the contact angle when the liquid follows the surface topography (Figure 6a).[253] This model implies that if the surface has hydrophobic properties, the apparent contact angle is higher for rough surfaces, making the surface more hydrophobic. This underlies, in part, the idea of superhydrophobicity, documented for several materials.[254] The Wenzel model also implies that if a surface has hydrophilic properties, the apparent contact angle of water is lower for rough surfaces, making the surface more hydrophilic (superhydrophilicity or hemi-wicking surfaces).[252]

Chemically heterogeneous surfaces, such as the cornea, behave such that the contact angle of liquids on the surface represents an average of the distinct spatially discrete regions (Cassie-Baxter model, inset IV, Figure 6b).[158] In short, topographic attributes of the ocular surface will interact with the intrinsic surface chemistry to determine the interfacial properties.

Chemical heterogeneity of thin film systems can also cause spontaneous instability and dewetting, accelerating the breakup of the tear film, depending on the sharpness of the heterogeneity.[255] Chemically heterogeneous substrates are more sensitive to changes in the rate of evaporation and humidity: evaporation can enhance the time of rupture on chemically heterogeneous surfaces by an order of magnitude and a chemical heterogeneity can induce faster rupture at higher thicknesses than on homogeneous substrates.[256] Interestingly, both less wettable (more hydrophobic) and more wettable (more hydrophilic) chemical heterogeneities can engender rupture.[257] It is the spatial differences that induce instabilities in the contact angle and promote tear film rupture.[257] Chemical heterogeneities can destabilize otherwise stable films, reduce the breakup time for thicker films, and produce complex geometries for the defects formed in the film.[258]

B. Topographic and Chemical Heterogeneity of the Ocular Surface

1. Topographic Features of Ocular Surface Cells

The apical surface of the ocular epithelia presents a rich topography formed by microvilli and microplicae that has been characterized by scanning electron microscopy (**SEM**)[107,109,186]

and atomic force microscopy (**AFM**)[91] (Figure 7). Hoffman and Schweichel distinguished a population of corneal epithelial cells rich in microvilli, and a population of smooth surface cells with defective membranes.[259] After UV radiation, while smooth cells were shed, the cells rich in these topographic features regenerated their microvilli, suggesting a connection between cell functionality and surface features.[259] On SEM studies, a marked difference in the brightness of the corneal cells can be observed, which is related to the density and morphology of the microplicae and microvilli. The more pronounced the topographic features, the lighter the cell appears when viewed by SEM.[108] It has been proposed that this difference is correlated with different stages of maturation of the cells, the light cells being those most recently exposed to the surface.[260]

The classically attributed roles of these cellular topographic features include: increasing surface area exposure for molecular transport; serving as a membrane reservoir for endo/exocytosis; and regulating the cell volume in response to osmotic exposure. More recently suggested roles include:

1. Serving as a diffusion barrier (transport membrane proteins are located just at the top of the microvilli, the organized microfilaments that compose the core structure of the microvilli proposed to control the influx of solutes and molecules to the bulk cytoplasm);

2. Active transport of membrane components through myosin motors;

3. Ca^{2+} release and influx. Ca^{2+} is tightly bound to the F-actin in the microfilaments contained within the topographic features, serving as a reservoir. Upon receptor stimulation, the F-actin is disassembled and Ca^{2+} is liberated; and

4. Surface cleaning. Cytotoxic lipophilic substances are trapped on the surface of microvilli, and while the microvilli elongate, the substances are then shed by vesiculation.[261]

Recent studies have correlated a reduction of size and density of the corneal epithelial microvilli to tear film abnormalities[262] and dry eye syndromes.[263,264] However, to date, no studies have been done on the role of the topography of the ocular surface on the wettability and contact line pinning during tear film formation and dewetting. The role of microvilli and microplicae in the hysteresis of the contact angle was actively downplayed by Holly in 1978, who instead attributed it to conformational changes of cell surface molecules from hydrophobic to

hydrophilic.[102] In the context of interfacial phenomena, we feel it is likely that the presence of an amplified surface area for interaction with the tear film may promote its stabilization depending on the relative hydrophilicity/hydrophobicity of the surface itself. In other words, if the intrinsic nature of the surface is relatively hydrophilic, increasing the surface area through the introduction of microplicae will accentuate the hydrophilic nature increasing the likelihood that thin aqueous films will wet the surface. This effect has been examined experimentally using demixed polymer brushes grafted onto microstructured substrates. By exposing the surface to selective solvents, the surface properties are reversibly tuned, and the surface structure amplifies the response, enabling the switching between superhydrophilicity and superhydrophobicity.[265] Importantly, the presence of surface topographic features will slow the dewetting process once rupture of the tear film has occurred. Topographic features function as "kinetic barriers" and can induce "pinning" of the receding contact line, reducing its velocity[266].

2. Chemistry of Ocular Surface Cells

The cell surface is generally recognized as highly heterogeneous, composed of thousands of different lipids, proteins, and carbohydrates that depend on cell type, life stage in the cycle of the cell, and state of disease (Figure 8).[267] The differential expression of cell surface components in the different layers of the corneal epithelium,[268] along with its high rate of renewal,[269] also support a high degree of chemical heterogeneity present within the population. Sharma further suggested that heterogeneities in ocular surface chemistry encompassing the size of just one epithelial cell could trigger the rupture of the tear film.[116,231] Despite the known impact of chemical heterogeneity of surfaces on the stability/instability of thin films, there is a paucity of information regarding differences in chemical composition across the ocular surface.

C. Opportunities to Exploit the Heterogeneity of the Ocular Surface

There is a knowledge gap and studies are lacking relating the topographic features of the ocular surface cells with the stability of the tear film. Importantly, differences in topographic features have been noted in association with ocular surface disease. These intrinsic biophysical attributes may be important for determining the stability of the intact tear film and dynamics of dewetting once the integrity of the tear film has been compromised. Similarly, the chemical characteristics and heterogeneity of the corneal and conjunctival epithelium across the ocular

surface need to be further defined, especially the protein and lipid components of the cell membranes.

V. RHEOLOGY OF THE TEAR FILM

A. Rheology and Hydrodynamics

1. General Concepts

Rheology is the study of the flow and deformation of viscoelastic fluids.[270] The viscosity of Newtonian fluids is not affected by the application of external forces (they lack viscoelastic properties). In contrast, the application of external forces to non-Newtonian fluids results in the modulation of their viscosity (they possess viscoelastic properties). Non-Newtonian fluids can be further classified as pseudoplastic (shear-thinning), or dilatant (shear-thickening).[271] It should be noted that *viscosity* is a term that is distinct from *viscoelasticity* (see inset V).

To fully understand the formation and stability of thin liquid films, knowledge of the viscoelastic properties of fluids in the vicinity of a contact line is essential. This is because the hydrodynamics of wetting/dewetting are controlled in part by the rheological properties of the fluid. The viscoelastic properties of the components of the fluid strongly affect thin film formation, kinetics of renewal, stability, kinetics of dewetting phenomena, and resistance to shear.[272] In the case of multilayered films, it has been demonstrated that monolayers of viscoelastic surfactants stabilize thin films and decrease the critical thickness for dewetting, allowing the formation of a thinner subphase.[273] In other words, thin multilayered films will intrinsically be more resistant to dewetting if the outermost constituent exposed to air has increased viscosity.

2. Hydrodynamic Models of the Tear Film

Hydrodynamics refers broadly to the formation, maintenance, flow, and subsequent removal of the tear film on the ocular surface. It includes consideration of tear film viscosity, as well as viscoelastic behavior. A detailed account of general tear hydrodynamics can be found in the recent review by Braun.[274] A variety of mathematical models have been subsequently developed,[105,222] incorporating differing variables to account for factors that influence thin film kinetics, including intermolecular forces,[16] time-dependent factors,[23] and perturbations on the thickness of the tear film.[275,276] However, all those models have the intrinsic flaw of considering the tear film to behave like water rather than having viscoelastic properties.

3. Rheology of Tears

Human tears exhibit a shear-thinning viscoelastic (non-Newtonian) behavior that prolongs the contact time on open eyes but protects the ocular surface through decreasing viscosity during blinking.[277] The shearing forces during blinking increase proportionally to the thinning of the film.[278] These forces may damage the epithelial cell surface and cause painful sensations.[279] In 1991, Tiffany characterized the viscosity of human tears and found a significant difference in the rheological properties between normal and dry eyes.[277]

Mucus was initially regarded as the component responsible for the shear-thinning behavior of tears[280]; however, the levels of gel-forming mucins in tear fluid was found to be too low,[174] and no significant difference in the rheological properties of stimulated and unstimulated tears was found, although the amount of mucus was expected to be greater in the unstimulated tears.[161] Subsequently, other tear constituents have been evaluated as potential candidates for the shear-thinning behavior. Single proteins (lysozyme, lactoferrin and sIgA) were found to be nonviscoeleastic, whereas mixtures of proteins (lysozyme+lactoferrin and lysozyme+sIgA) possessed viscoelastic properties.[161] Additional combinations of proteins and peptides have been demonstrated to contribute to the viscoelastic properties of tears.[281]

Lipids have been identified as the most important determinant of viscoelastic properties of the tear film. When lipids were extracted from tears, the viscoelasticity was lost, but returned upon reintroduction.[282] Lipocalins have also been implicated in contributing to viscoelasticity.[283] This viscoelastic behavior of the TFLL has been attributed to the structuring of the duplex film.[223]

The first hydrodynamic model evaluating the influence of viscoelasticity on tear film rupture was proposed in 2003 by Zhang et al, who observed a stabilizing influence on the rupture of the tear film by the increase of viscosity of the tears during the interblink.[26] This result was corroborated by Gorla and Gorla in a later publication.[284] In 2003 Sharma proposed the presence of a nonadherent mucus layer in the tear film and suggested that this mucus layer induces slippage (the tear film moves at the boundary in contact with the cell surface) during breakup of the tear film.[257] Zhang et al. modeled the influence of slip on the rupture of the tear film and determined a very significant reduction of the breakup time.[18]

In summary, the viscoelastic properties of tears contribute to the stabilization of the tear film by altering the hydrodynamics of the rupture of the tear film.

B. Opportunities to Exploit the Rheology of Tears

More research on the interfacial rheology of the tear film, specifically the viscoelastic properties of the mucus and the TFLL, may provide advances in the development of materials to enhance the spreading and stability of the tear film. Although significant efforts have been made in the use of more biomimetic parameters for the modeling of the formation, maintenance, and breakup of the tear film, no mathematical model integrates all the features that are involved in such events. Models incorporating intermolecular interactions, interfacial rheology, slippage, duplex lipid films, evaporation, osmotic flow, and lid motion may significantly contribute to our understanding of the tear film. Additionally, the impact of ocular surface disease on viscosity, viscoelasticity, and the consequences to tear film formation, stability/instability, and dewetting phenomena have been minimally investigated.

VI. CONCLUDING REMARKS

We have presented a review of the literature concerning the characterization of the interfacial phenomena of the ocular surface and its implications for formation and the stability of the tear film and ocular health. We have emphasized the knowledge gaps concerning the physicochemical attributes of the ocular surface and identified controversies regarding the elements and events involved in the formation and breakup of the tear film. This review identifies a need for further investigations of interfacial phenomena with the possibility that such investigations will point to the development of novel endpoints in the assessment of ocular surface health, as well as therapeutics for the treatment of ocular surface disorders.

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LEGENDS

Figure 1. The ocular surface is a complex system that includes a series of interfaces. It is comprised of the superficial cells that line the exposed regions of the eye (corneal epithelium, limbus, and conjunctival epithelium), as well as the lid margin and the tear film. The tear film is a complex multilayered fluid phase. This figure represents the classical three-layered model, composed of a mucin-gel layer adjacent to the epithelial surface, an aqueous layer containing mucin, and other soluble proteins and a thin lipid film on the outermost surface.

Figure 2. A large group of ocular surface disorders can lead to abnormalities in the interfacial properties of the ocular surface that interact with each other through multiple feedback loops. Ocular surface disorders interfere with the production of constituents of the tear film, as well as the blinking, dynamics of tear drainage, and/or the rate of tear evaporation. In turn, these disturbances modify the interfacial properties of the ocular surface that are essential for the formation and stability of the tear film. The disruption of the tear film can aggravate a given ocular surface disorder.

Figure 3. The active study of interfacial phenomena in the ocular surface started in the late 1960s, and developed during the 1970s and 1980s, but has subsided significantly since the 1990s.

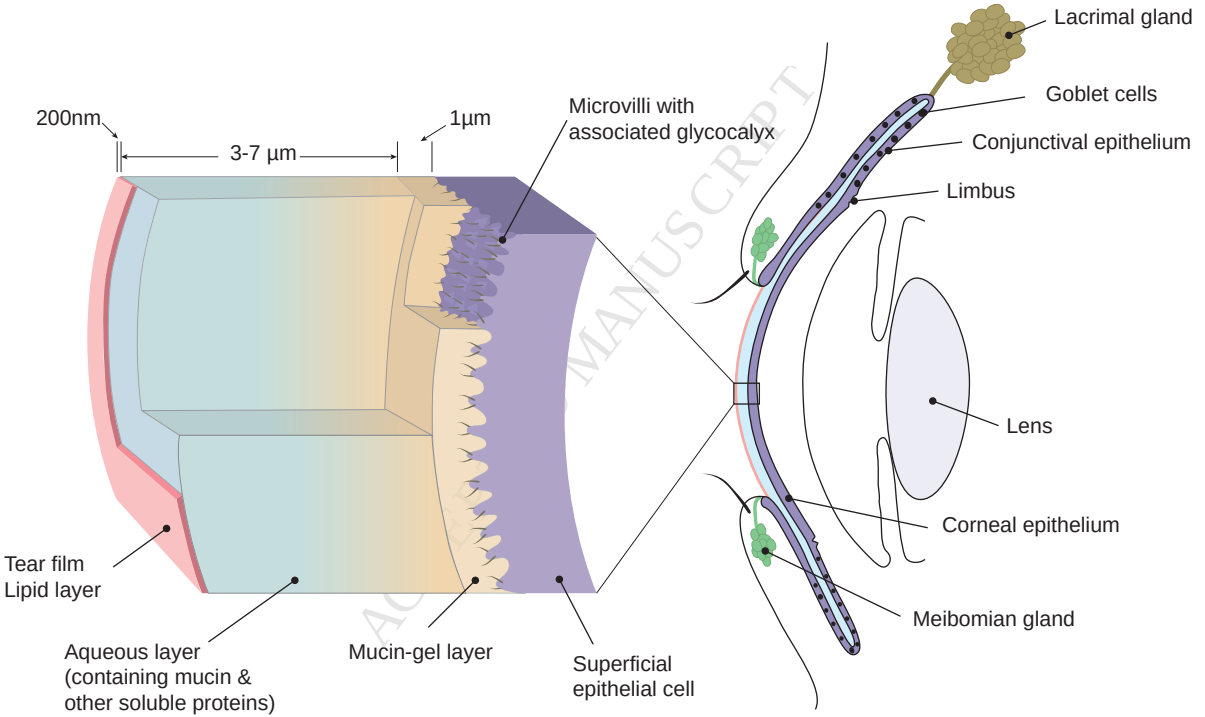
Figure 4. A property used to characterize the wetting of a liquid on a solid is the *contact angle*, measured at the point of contact (called the contact line) between a liquid and the surface. a) The value of the contact angle is a consequence of the equilibrium of surface forces at the contact line, and provides a way to characterize the wettability of a surface. b) If the liquid used is water, and the contact angle is high, the surface has hydrophobic characteristics. c) If the measured contact angle is low, the surface has hydrophilic characteristics. Θ= contact angle; σ_L=surface tension of the liquid; $\sigma_{S=}$ surface energy of the solid; $\sigma_{SL=}$ interfacial energy between the liquid and the solid.

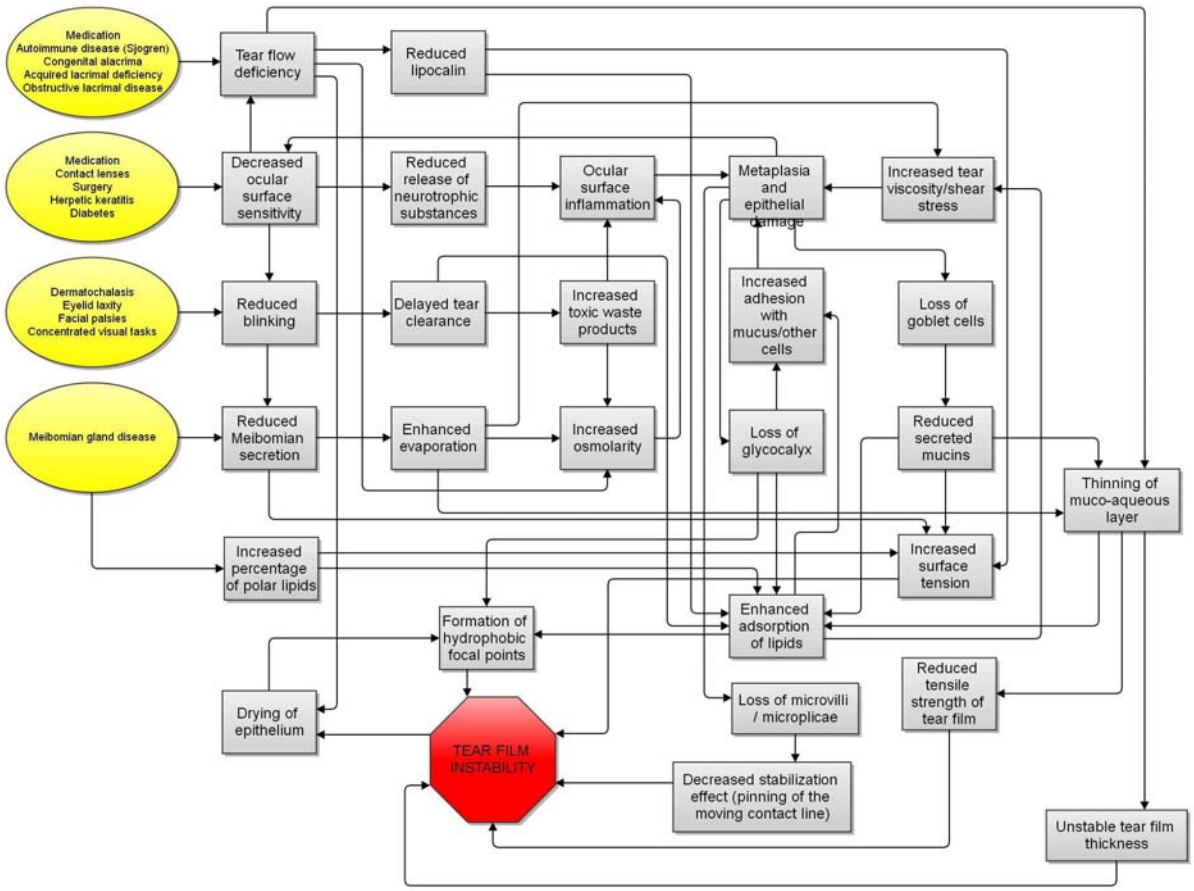
Figure 5. Model of the tear film lipid layer (TFLL). McCulley and Shine introduced a duplex film structure for the tear film lipid layer, with a monolayer of polar lipids (polar phase) between the mucoaqueous and a thick nonpolar phase. P= phospholipids; TG= triglycerides; WE= waxy esters; C= cerebrosides; HC= hydrocarbons; F= free fatty acids; CE= cholesteryl esters. Modified from McCulley JP, Shine W. *Trans Am Ophthalmol Soc* 1997;95:79, and printed with permission of the American Ophthalmological Society (reference 30).

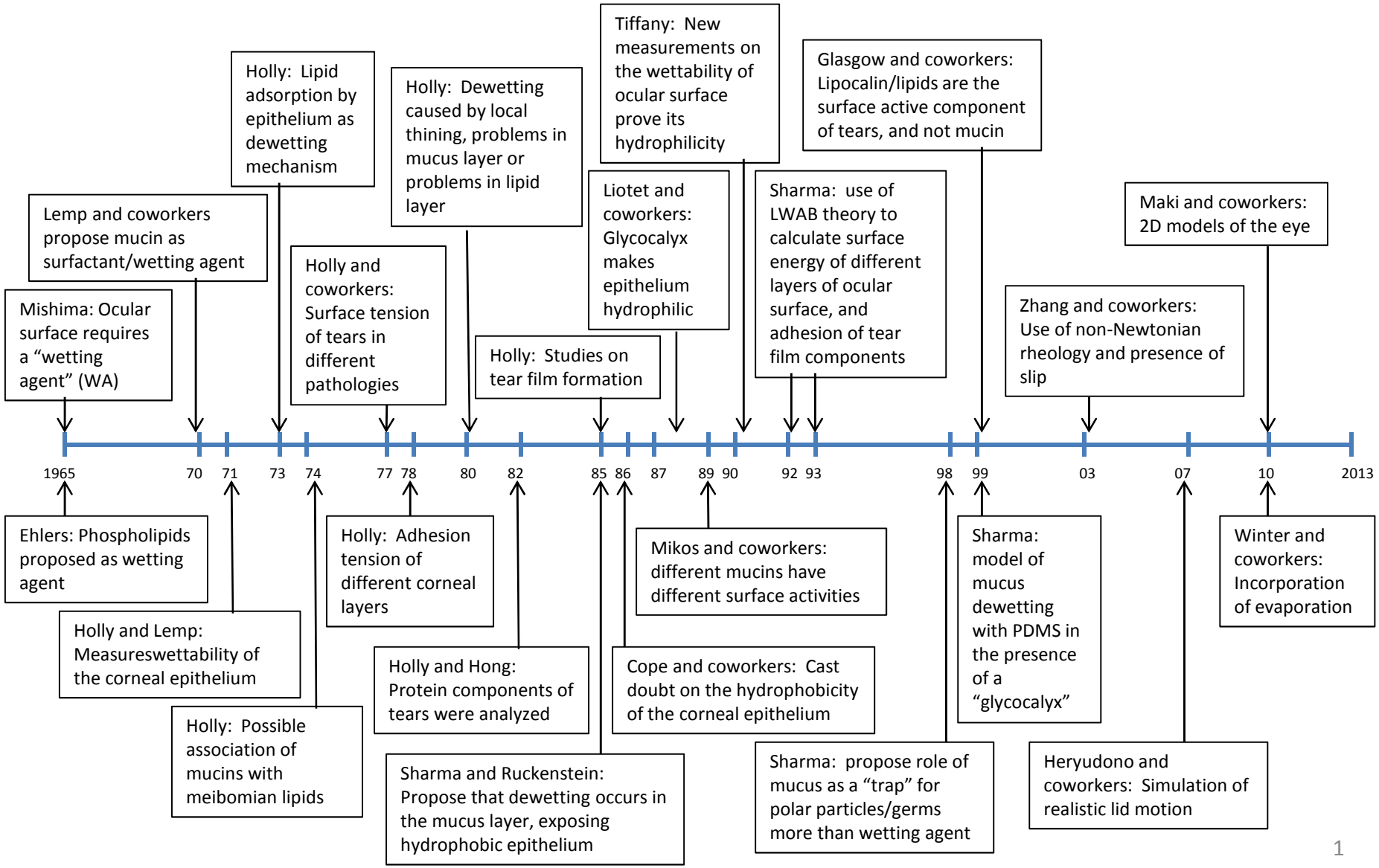
Figure 6. The interaction between fluid films and heterogeneous surfaces. a) For wetting systems, the presence of topography and roughness can alter the value of contact angle. In the Wenzel model, the liquid follows the surface topography, and the contact angle depends on the "roughness" (surface area/projected area), increasing for intrinsically hydrophobic substrates and decreasing for intrinsically hydrophilic substrates. b) In the Cassie-Baxter state, the presence of chemical heterogeneities (pictured in the figure as different colors) alters the value of the contact angle, representing an average of the distinct spatially discrete regions. Θ= contact angle; $\sigma_{L=}$ surface tension of the liquid; $\sigma_{S=}$ surface energy of the solid; $\sigma_{SL=}$ interfacial energy between the liquid and the solid.

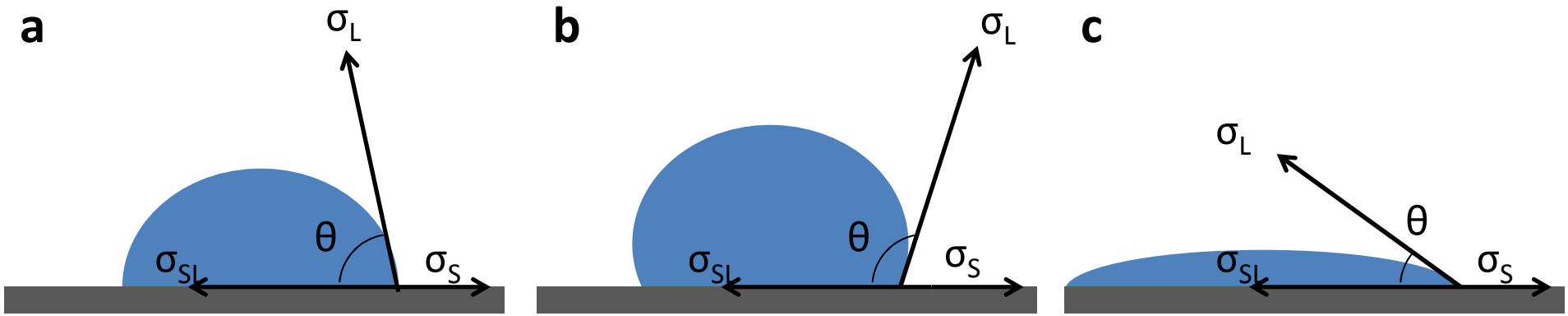
Figure 7. The apical surface of the ocular epithelia presents a rich topography. a) Transmission electron micrograph (TEM) section of normal conjunctiva, showing the surface (*S*) covered with microvilli. The image shows the presence of goblet cells (*g*) and nuclei of epithelial cells (*n*). b) Details of microvilli (*mv*) from the surface of a normal conjunctiva. The hair-like glycocalyx (*h*) extending from their surfaces are shown. Reproduced from Dilly PN, Mackie IA.*Br J Ophthalmol* 1981;65(12):833-842, copyright 1981, with permission from BMJ Publishing Group Ltd (reference 107).

Figure 8. The cell surface is highly heterogeneous, composed of different lipids, proteins, and carbohydrates. The section of the cell surface shows lipids (pink), major proteins (blue), and carbohydrates (orange). The heterogeneity of the lipid component of the cell membrane is omitted for simplicity. Adapted by permission from Macmillan Publishers Ltd from: Mager MD, LaPointe V, Stevens MM. *Nature Chemistry* 2011;3(8):582-589. Copyright 2011 (reference 267).





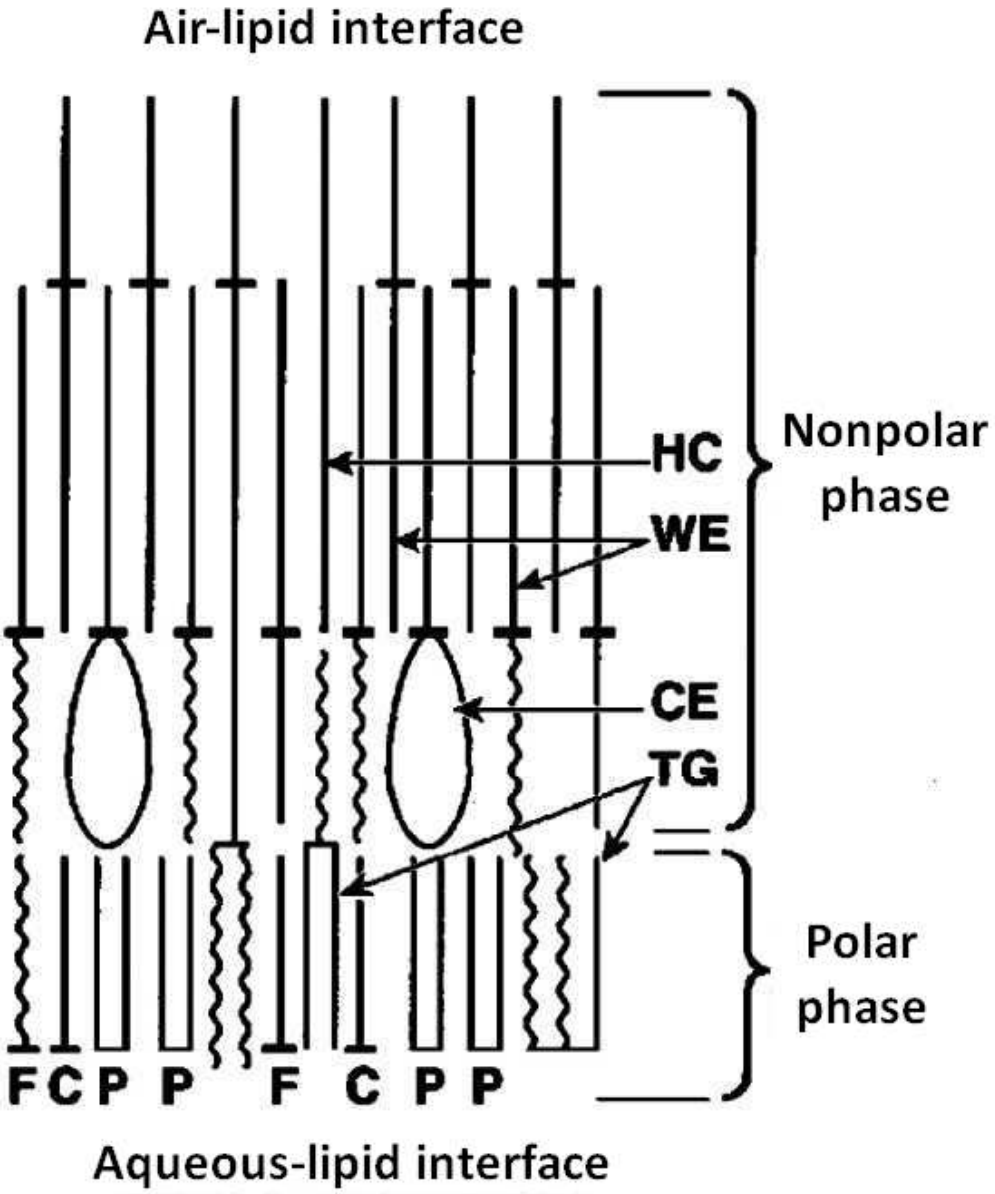

1965
Ehlers: Phospholipids proposed as wetting agent
Mishima: Ocular surface requires a "wetting agent" (WA)
70
Lemp and coworkers propose mucin as surfactant/wetting agent
71
Holly and Lemp: Measureswettability of the corneal epithelium
73
Holly: Lipid adsorption by epithelium as dewetting mechanism
74
Holly: Possible association of mucins with meibomian lipids
77
Holly and coworkers: Surface tension of tears in different pathologies
78
Holly: Adhesion tension of different corneal layers
80
Holly: Dewetting caused by local thining, problems in mucus layer or problems in lipid layer
82
Holly and Hong: Protein components of tears were analyzed
85
Holly: Studies on tear film formation
Sharma and Ruckenstein: Propose that dewetting occurs in the mucus layer, exposing hydrophobic epithelium
86
Tiffany: New measurements on the wettability of ocular surface prove its hydrophilicity
Cope and coworkers: Cast doubt on the hydrophobicity of the corneal epithelium
87
Liotet and coworkers: Glycocalyx makes epithelium hydrophilic
89
Mikos and coworkers: different mucins have different surface activities
90
92
93
Sharma: use of LWAB theory to calculate surface energy of different layers of ocular surface, and adhesion of tear film components
98
Sharma: propose role of mucus as a "trap" for polar particles/germs more than wetting agent
99
Glasgow and coworkers: Lipocalin/lipids are the surface active component of tears, and not mucin
Sharma: model of mucus dewetting with PDMS in the presence of a "glycocalyx"
03
Zhang and coworkers: Use of non-Newtonian rheology and presence of slip
07
Heryudono and coworkers: Simulation of realistic lid motion
10
Maki and coworkers: 2D models of the eye
Winter and coworkers: Incorporation of evaporation
2013

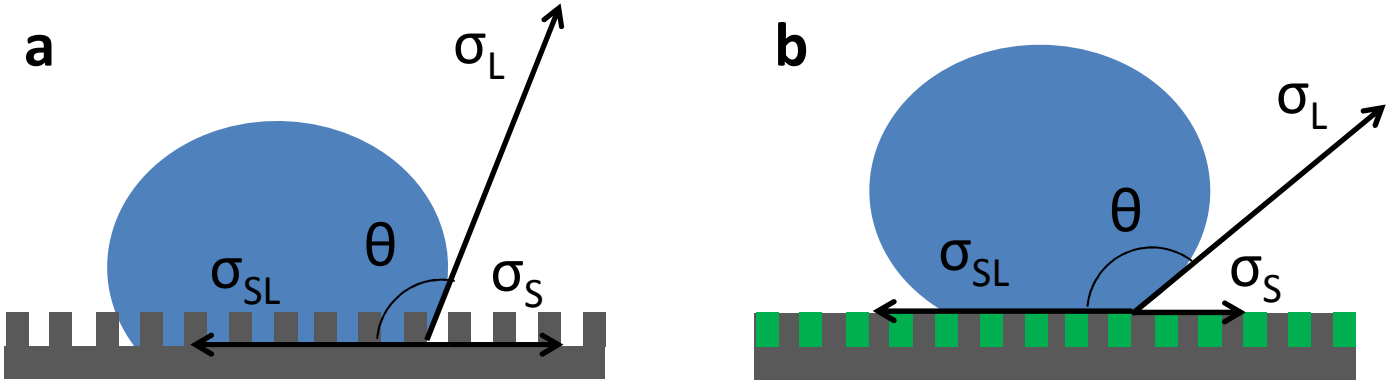


$$\sigma_L \cos\theta = \sigma_S - \sigma_{SL}$$

Hydrophobic surface

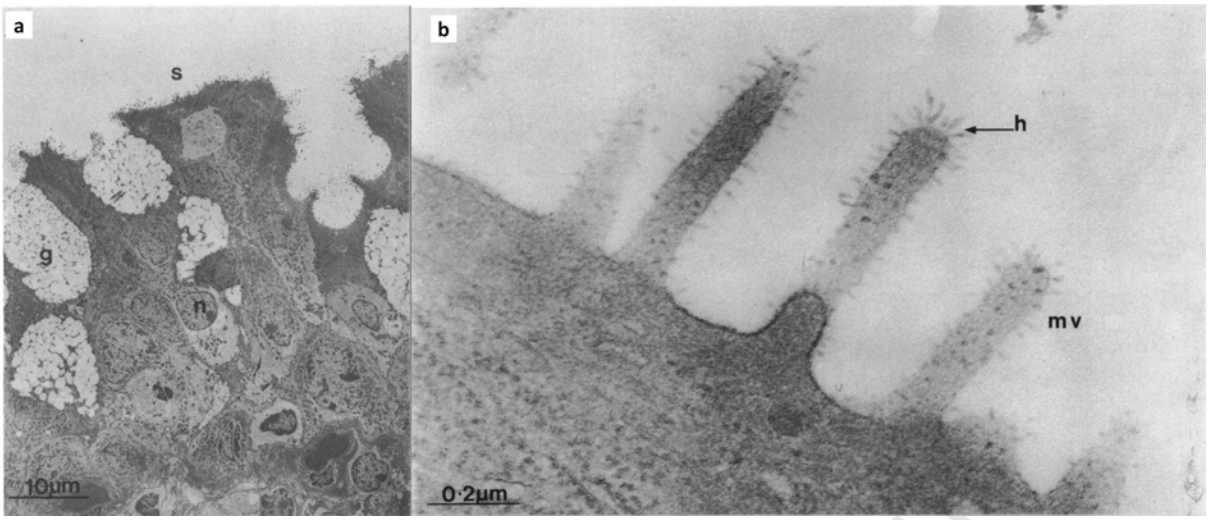
Hydrophilic surface

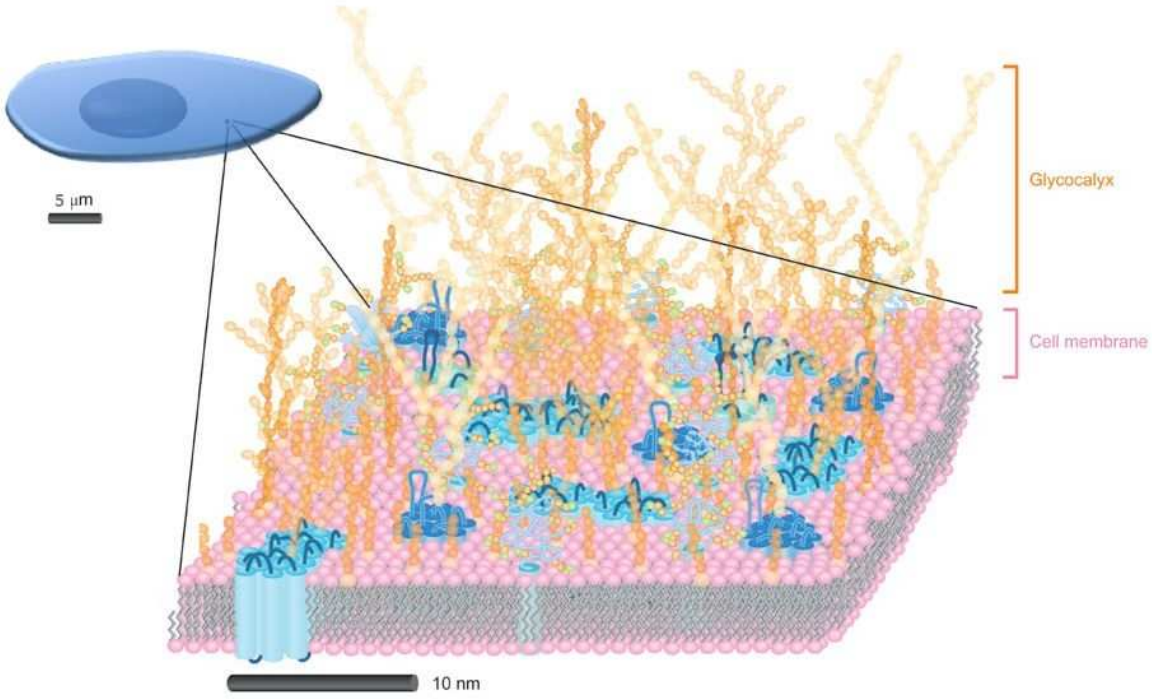

Air-lipid interface
HC
WE
CE
TG
Nonpolar phase
Polar phase
F C P P F C P P
Aqueous-lipid interface



Wenzel state

Cassie-Baxter state





INSET I: Young's equation:

$$\gamma_{SL} = \gamma_{SG} - \gamma_{LG}\cos\theta$$

θ: Young's contact angle
γ_{SL}: Interfacial energy between the solid and the liquid
γ_{SG}: Surface energy of the solid
γ_{LG}: Surface energy of the liquid.

INSET II: Marangoni Flow:

Whenever there are spatially discrete differences in surfactant concentrations and/or temperature there are associated spatially discrete differences in surface tension. These differences induce fluid flow from lowest surface tension to highest surface tension regions. This is what happens in creation of "legs (or tears) of wine" that is observed after swirling a glass of red wine. In this case alcohol reduces the surface tension is first evaporated from the thinnest aspect of a continuous film nearest the rim of the glass. The evaporation decreases the alcohol content near the rim and is at a relatively higher concentration in the lower (bulk) of the film adjacent to the now settled wine. Fluid therefore flows up to the regions located closer to the rim of the glass that contain less alcohol on a per volume basis (cause of evaporation). When there is enough fluid present for gravity to overcome Marangoni flow, the drop flows down creating the "legs". This same phenomenon occurs on the ocular surface in cases where there are spatial discontinuities present as regards surface chemistry, topography and temperature. When the lids are open, surfactant is greater in the lower dependent portion of the tear film, Marangoni flow therefore occurs upward.

INSET III: Spreading coefficient.

A liquid B over a solid A in a medium C has a spreading coefficient:

$$S_{B/AC} = \gamma_{AC} - \gamma_{AB} - \gamma_{BC}$$

$S_{B/AC}$ = Spreading coefficient
γ_{AC} = Surface energy of solid A
γ_{AB} = Interfacial energy between A and B
γ_{BC} = Surface tension of liquid B

If $S_{B/AC} \geq 0$, the liquid spreads spontaneously over the solid
If $S_{B/AC} < 0$, the liquid does not completely wet the solid

INSET IV: Wenzel and Cassie-Baxter models

Wenzel equation:

$$\cos\theta_W = r\cos\theta_Y$$

θ_W: Apparent contact angle
θ_Y: Young's contact angle
r: Roughness ratio. Total area of the surface divided by the projected area (r > 1)

Cassie-Baxter equation

$$\cos\theta_C = \sum_i x_i \cos\theta_{Yi}$$

θ_C: Apparent contact angle
θ_{Yi}: Young's contact angle of chemistry i
x_i: Surface fraction of chemistry i

INSET V: Viscosity and viscoelasticity

Viscosity: Term that refers to the resistance to flow. It is a measure of the internal friction of a fluid. This friction becomes apparent when a layer of fluid is made to move in relation to another layer. The greater the friction, the greater the amount of force (shear) required to cause this movement. Highly viscous fluids, therefore, require more force to move than less viscous materials.

Viscoelasticity: Property of materials that exhibit both viscous and elastic characteristics when undergoing deformation. A force must be applied to a fluid to demonstrate viscoelasticity and the viscosity of changes as a result of this. As paint is applied or ketchup is squeezed out of a bottle, its viscosity decreases (shear-thinning). Concentrated suspensions of corn starch present a resistance to passage that increases proportionally with the speed of finger movement (shear-thickening).